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

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

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

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

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

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

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

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

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

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

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

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

Keywords

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

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Table 1. Manuscript length at a glance

Article type	Abstract Length	Manuscript Word Count*	Maximum Number of Authors	Maximum Number of References$^{\Phi}$
Original Research	250 words	,500 words (~22 pages)$^{\Psi}$	NA	30
Case report	150 words	,000 words (~8 pages)	4	8
Systematic review	300 words	6,250 words (~25 pages)	4	60
Current commentary	250 words	,000 words (~12 pages)	4	12
Procedure and Instruments	200 words	,000 words (~8 pages)	4	10
Letters	NA	350 words	4	5

*Manuscript length includes all pages in a manuscript (ie, title page, abstract, text, references, tables, boxes, figure legends, and appendixes). $^{\Phi}$Suggested limit. $^{\Psi}$The Introduction should not exceed 250 words. ~approximately; NA, not applicable.

Original researches should have the following sections;

Introduction

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

Materials and Methods

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

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

Results

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

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

Discussion

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

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

Conclusion

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

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

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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

We are to meet with you, our esteemed colleagues, again with the June 2022 issue of the Turkish Journal of Gynecology and Obstetrics. In this issue, we presented to you studies with high-quality scientific content that were sent to our journal from Turkey and other countries and evaluated with great dedication.

At the Turkish Gynecology and Obstetrics Association Congress held in Antalya between 18-22 May 2022, we updated our knowledge with topics of high scientific content and held a separate session for our journal. In this session chairmanship, which I shared with our chief editor Prof. Dr. Eray Çalışkan, we shared the topics of "How should scientific journal editorship be?", "Refereeing system in scientific article evaluation", "Statistical review of research articles", "Evaluation of research literature" and "Evaluation of review and meta-analysis articles" with both our referees working in our journal and our colleagues who will take a new step into the role of referee.

It's all about bringing the most scientifically powerful articles together with our colleagues. We hope to meet in our future issues

Ercan Yilmaz, MD
Co-Editor in Chief

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2022;19:88-97

COVID-19 related maternal mortality cases in associated with Delta and Omicron waves and the role of lung ultrasound

COVID-19'a bağlı anne ölümü olgularının Delta ve Omikron dalgaları ile ilişkisi ve akciğer ultrasonunun rolü

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Abstract

Objective: To present coronavirus disease-2019 (COVID-19) related maternal mortality in relation to Delta and Omicron waves and to investigate the role of lung ultrasound (LUS) in estimating mortality.

Materials and Methods: This retrospective cohort study was conducted in the obstetrics and gynecology clinic of a tertiary pandemic hospital between March 2020 and January 2022. The hospitalized pregnant women with COVID-19 diagnosis and maternal deaths were studied in relation with Delta and Omicron waves. The relationship between LUS scores of hospitalized patients and maternal mortality was explored.

Results: Thousand and sixty-five pregnant women were hospitalized because of COVID-19 infection. Fifty-one (4.79%) of these patients had critical sickness, 96 (9.01%) of them had severe illness, 62 (5.82%) of them were admitted to the intensive care unit and 28 (2.63%) of all hospitalized pregnant women had died. Of the 1.065 patients, 783 (73.5%) were hospitalized before the Delta wave and the maternal mortality rate was 1.28% (10/783), 243 (22.8%) were hospitalized during the Delta wave and the maternal mortality rate was 7% (17/243) [relative risk (RR)=5.478, 95% confidence interval (CI) (2.54-11.8), z=4.342, p<0.001]. During the Omicron wave 39 (3.66%) patients were hospitalized and the maternal mortality rate was 2.56% (1/39). Maternal mortality rates, according to LUS scores, were 0.37% (1/273) for LUS 0, 0.72% (2/277) for LUS 1, 2.58% (10/387) for LUS 2 and 11.72% (15/128) for LUS 3 respectively (LUS 3 vs. others; maternal mortality: RR=8.447, 95% CI (4.11-17.34), z=5.814, p<0.0001). There were no vaccinated patients in the study cohort.

Conclusion: The maternal mortality rate was relatively high, particularly during the Delta wave at our referral center. The Delta wave, delayed vaccination and vaccine hesitancy of pregnant women might have important roles in maternal mortality. Higher LUS scores should warn clinicians of an increased risk of maternal death.

Keywords: COVID-19, Delta, lung ultrasound, maternal mortality, Omicron

Öz

Amaç: Delta ve Omikron dalgaları ile ilişkili olarak koronavirüs hastalığı-2019 (COVID-19) ile ilişkili anne ölümlerini sunmak ve akciğer ultrasonunun (AUS) hastalık şiddeti-mortalite tahminindeki rolünü araştırmaktır.

Gereç ve Yöntemler: Bu retrospektif kohort çalışması, üçüncü basamak bir pandemi hastanesinin kadın hastalıkları ve doğum kliniğinde Mart 2020 ile Ocak 2022 tarihleri arasında yapılmıştır. Hastanede yatan COVID-19 tanılı gebeler ve anne ölümü olguları Delta ve Omikron dalgaları ile ilişkili olarak incelenmiştir. Hastanede yatan hastaların AUS skorları ile anne ölümleri arasındaki ilişki araştırılmıştır.

PRECIS: Among pregnant women infected with COVID-19, the highest mortality rate was observed in the Delta wave and higher lung ultrasound scores should warn clinicians of an increased risk of maternal death.

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Bulgular: Bin altmış beş hamile kadın COVID-19 enfeksiyonu nedeniyle hastaneye yatırıldı. Bu hastaların 51'inde (%4,79) kritik hastalığa, 96'sında (%9,01) ağır bir hastalık mevcuttu, 62'si (%5,82) yoğun bakıma yatırıldı ve hastanede yatan tüm gebe hastaların 28'inde (%2,63) ölüm gerçekleşti. Delta dalgası öncesinde 783 (%73,5) hasta hastaneye yatırıldı, anne ölüm oranı %1,28 (10/783), Delta dalgası sırasında 243 (%22,8) COVID-19 hasta hastaneye yatırıldı ve anne ölüm oranı %7'di (17/243) [göreceli risk (RR)=5,478, %95 güven aralığı (GA) (2,54-11,8), z=4,342, p<0,001]. Omikron dalgası sırasında 39 (%3,66) hasta hastaneye yatırıldı ve anne ölüm oranı %2,56 (1/39) idi. AUS skorlarına göre anne ölüm oranları, AUS 0 için %0,37 (1/273), AUS 1 için %0,72 (2/277), AUS 2 için %2,58 (10/387) ve AUS için %11,72 (15/128) idi (AUS 3 ve diğerleri; anne ölüm oranı: RR=8,447, %95 GA (4,11-17,34), z=5,814, p<0,0001). Çalışma grubunda aşılı hasta bulunmamaktaydı.

Sonuç: Anne ölüm oranı, özellikle sevk merkezimizde Delta dalgası sırasında nispeten yüksekti. Gebelerin Delta dalgası, aşı gecikmesi ve aşı tereddütlerinin anne ölümlerinde önemli rolleri olabilir. Daha yüksek akciğer ultrason skorları, klinisyenleri artan anne ölümü riski konusunda uyarmalıdır.

Anahtar Kelimeler: COVID-19, Delta, akciğer ultrasonu, anne ölümü, Omikron

Introduction

Recent studies and reviews indicate that pregnant women are more likely to are affected by severe illness and intensive care and mechanical ventilation[1-4]. Coronavirus disease-2019 (COVID-19) was associated with a substantial increase in maternal morbidity and mortality[5]. The maternal mortality rates varied widely from 1.35% to 12.3%[6-8]. Variants of concern have begun to be reported by the World Health Organization (WHO)[9] and the pace of the pandemic has increased. Data on maternal mortality associated with the increasing waves of the pandemic have been accumulated recently[7,10-12]. As the pandemic progressed, new genetic variants of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) were identified in the second half of the 2020[5]. In May 2021, the Delta variant was identified as a variant of concern[5], with research indicating that it was more transmissible and that patients with the Delta variant became ill faster and showed higher viral loads in the respiratory tract compared to the other variants[13]. Recently, Delta wave was related to a high maternal mortality rate, particularly for unvaccinated pregnant women[7]. Nowadays, the Omicron variant has taken the place of the Delta variant and it is identified as a more transmissible variant with decreased severity[14].

Diagnosis of COVID-19 is mainly based on the symptoms of the patient, nasopharyngeal polymerase chain reaction (PCR) test results, and chest imaging tests[9,15]. For obstetricians, the lung ultrasound (LUS) is a rapid and safe technique for the triage, diagnosis and follow-up of pregnant women with COVID-19[16,17]. Performing LUS right after the fetal assessment for screening of COVID-19 is feasible until PCR results are acquired. It has the advantage of eliminating the ionizing radiation exposure from chest computed tomography (CT) and pregnant women's concerns about ionizing radiation. LUS is a new thoracic imaging method that is becoming more widely used than other thoracic imaging modalities. Its usage to determine the pulmonary involvement in COVID-19 has increased recently[18]. Abnormal LUS findings were found relevant to early admission into intensive care units (ICU) or ICU and mortality in the pandemic[16,18].

The data on maternal mortality rates during the latest variants of the COVID-19 pandemic needs to be updated. In this study, we aimed to investigate the COVID-19 related maternal mortality in connection to Delta and Omicron waves and the impact of LUS in determining disease severity-mortality in one of Turkey's largest pandemic centers.

Materials and Methods

This retrospective cohort study was conducted in the obstetrics and gynecology clinic of a tertiary pandemic hospital specialized in the care of pregnant women infected with SARS-CoV-2. At this center, approximately 4.000 women give birth annually and during the pandemic maternal healthcare continued along with the management of pregnant women with COVID-19. The diagnosis and management of the patients with COVID-19 were applied in line with WHO and, the Turkey Ministry of Health recommendations as well as the local protocol of the obstetrics clinic.

All pregnant women who were hospitalized and diagnosed with COVID-19 between March 2020 and January 2022 were included in this study. There were no further exclusion criteria. The patients were divided into three groups: those who were hospitalized before the Delta wave, those who were hospitalized during the Delta wave, and those who were hospitalized during the Omicron wave. The maternal deaths were classified and further analyzed in detail regarding the period of the pandemic. The data of pregnant women who were admitted to the hospital and confirmed as COVID-19 by nasopharyngeal swab reverse transcription-PCR test and LUS or chest-CT were analyzed retrospectively using the hospital electronic health records. The primary outcome of the study was to evaluate the change in the maternal mortality rate associated with the Delta and Omicron waves and to investigate the maternal mortality cases in detail. The study's secondary outcome was to look at the link between initial LUS scores upon diagnosis and maternal mortality.

Electronic health records were used to collect information on the demographic and clinical characteristics, obstetric outcomes, and the laboratory results of COVID-19-diagnosed mothers. Some patients in the study cohort had been included in the previous publications[19-23]. The study was approved by the local ethics committee (University of Health Sciences Turkey, Şehit Prof. Dr. İlhan Varank Sancaktepe Training and Research Hospital - no: 2021/218) and by the Ministry of Health COVID-19 Scientific Research Evaluation Commission. The research was conducted ethically in accordance with the guidelines for human studies and World Medical Association Declaration of Helsinki.

Hospitalization Criteria

At the time of admission to the hospital and during follow-up in the hospital, pregnant women with COVID-19 were classified as having mild, moderate, severe and critical illness. Mild illness was defined for the pregnant or postpartum cases of COVID-19 who were symptomatic but without lower respiratory tract symptoms (shortness of breath) or abnormal chest imaging (i.e., mainly LUS or occasionally tomography, chest X-ray). Moderate illness was defined for the pregnant or postpartum cases of COVID-19 who were symptomatic with lower respiratory tract symptoms without significant hypoxia (pulse oximetry saturation ≥94% on room air). Severe illness was defined for the pregnant or postpartum cases of COVID-19 who were symptomatic with oxygen saturation <94%, respiratory rate more than 30 breaths per minute, Po2 to the fraction of inspired oxygen <300 mmHg, or lung infiltrates >50%. Critical illness was defined for the pregnant or postpartum cases of COVID-19 who were symptomatic with respiratory failure, septic shock, hyper-inflammatory syndrome, or other organ system dysfunction[24]. Pregnant women who were diagnosed with moderate, severe and critical COVID-19 were hospitalized. Patients with mild disease were hospitalized, if their LUS score 2 or 3 or, there were difficulties in follow-up from home and some of the mild cases were hospitalized because for obstetric reasons. Additionally, asymptomatic pregnant women with positive nasopharyngeal PCR tests who were admitted to the hospital for obstetric reasons were hospitalized.

Effects of Delta and Omicron Wave

The impact of the Delta and Omicron waves was evaluated based on the Turkey Ministry of Health declarations. According to these declarations, the Delta wave was effective between August 1, 2021 to December 27, 2022 and after that Omicron wave has been effective[25-27].

Lung Ultrasound

The lung involvement of the patients was scored between 0 and 3 with LUS at the time of the hospital admission as a routine local protocol. LUS scores 0, 1, 2, and 3 were defined as normal, mild lung involvement, moderate lung involvement, and severe lung involvement, respectively[16,17]. LUS scores of hospitalized patients in relation to the clinical severity of all hospitalized patients and with maternal mortality were investigated.

Statistical Analysis

The data of this study were analyzed with IBM SPSS Statistics version 22.0 (IBM Corporation, Armonk, NY). The normality assumption of the variables was checked using the Shapiro-Wilk test and skewness/kurtosis values. Descriptive statistics were used as mean, standard deviation, median, number, and frequency. The chi-square test and Fisher's Exact test were used to compare categorical variables. Wilcoxon signed ranks tests Mann-Whitney U test and independent samples t-test were used to compare continuous variables according to normality assumptions All tests were 2-sided and the p-value <0.05 was set as statistically significant.

Results

Overall, there were 1.065 pregnant women hospitalized due to the COVID-19 infection between March 2020 and January 2022. Fifty-one (4.79%) of these patients had critical sickness, 96 (9.01 percent) of them had a severe illness, 62 (5.82 percent) of them were admitted to the ICU and 28 (2.63 percent) of the hospitalized pregnant women had died. Monthly data for the number of hospitalized patients and the maternal deaths between March 2020 and January 2022 are presented in Figure 1.

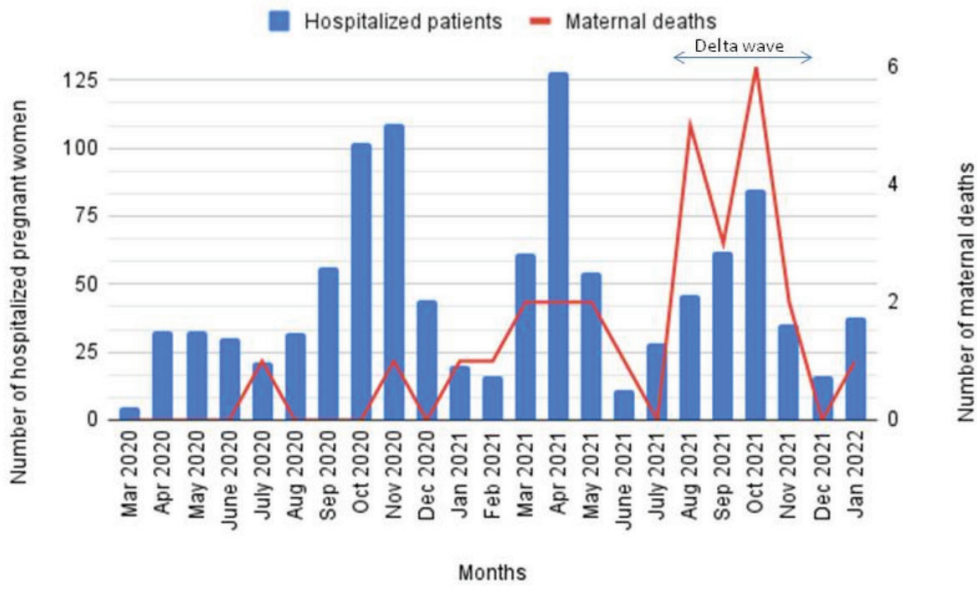

Figure 1. Monthly data for the number of hospitalized patients and maternal deaths

Of the 1,065 patients, 783 (73.52%) were hospitalized before the Delta wave with a maternal mortality rate of 1.28% (10/783), 243 were hospitalized during the Delta wave with a maternal mortality rate of 7% (17/243), and 39 (3.66%) patients were hospitalized during the Omicron wave with a maternal mortality rate of 2.56% (1/39) (p<0.001, chi-square=23.71532). There was a significant increase in maternal death with the impact of the Delta wave compared with pre-Delta period [relative risk (RR)=5.478, 95% confidence interval (CI) (2.54-11.8), z=4.342, p<0.001]. The count and the rates of maternal deaths, patients with ICU admission and with severe-critical disease during pre-Delta, Delta, and Omicron waves are summarized in Table 1. There were not any difference between demographic and obstetric characteristics of maternal deaths before and during the Delta wave and these are presented with characteristics of all maternal deaths in Table 2.

Table 1. The count and the rates of maternal deaths, ICU admission and severe-critical disease during pre-Delta, Delta and Omicron waves

	Pre-Delta (n=783)	Delta wave (n=243)	Omicron wave (n=39)	p
Patients admitted to ICU	32 (4.1%)	28 (11.5%)	2 (5.1%)	**<0.0001**
Patients with severe-critical disease	102 (13%)	43 (17.7%)	2 (5.1%)	**<0.0001**
Maternal deaths	10 (1.28)	17 (7%)	1 (2.56%)	**<0.0001**

ICU: Intensive care unit

Table 2. Demographic, obstetric characteristics of the maternal deaths in relation with Delta wave

	Values[a]			p
	All (n=28)	Pre-Delta (n=10)	Delta (n=17)	
Age (year)	31.19±5.41	29.5±5.7	31.35±5.05	0.505
BMI (kg/m²)	26.6±3.49	25.97±3.5	27.18±3.68	0.443
Gestational age at birth or abortus (week)	30.36±5.06	29.3±5.52	30.94±5	0.505
Fetal birth weight (gr)	1967.46±792.70	1898.78±751.26	1989.44±852.34	0.714
Nulliparity	11 (39.3%)	4 (40%)	7 (41.2%)	
Primiparity	9 (32.1%)	4 (40%)	5 (29.4%)	0.807
Multiparity	8 (28.6%)	2 (20%)	5 (29.4%)	
Second trimester (14-28 week)	7 (25%)	3 (30%)	4 (23.5%)	
Third trimester (>28 week)	21 (75%)	7 (70%)	13 (76.5%)	0.711
Multiple gestation				
Singleton	26 (96.4%)	10 (100%)	16 (94.12%)	
Triplet	1 (3.6%)		1 (5.88%)	
Pregnancy outcome[b]				
Cesarean section	24 (88.9%)	9 (90%)	14 (82.4%)	
Vaginal delivery	2 (7.4%)		2 (11.8%)	
Miscarriage	1 (3.7%)	1 (10%)		
Preterm				
34-37 week	5 (17.9%)	6 (66.67%)	2 (11.11%)	
<34 week	20 (71.4%)	3 (33.33%)	13 (72.22%)	
Term	3 (10.7%)		3 (16.67%)	
Positive PCR results at the end of the pregnancy[c]	27 (96.4%)	10 (100%)	16 (94.12%)	

Data presented as mean ± standard deviation or n (%).
BMI: Body-mass index, PCR: Polymerase chain reaction, [a]: Only one mother was died during the Omicron wave. Omicron column was removed due to statistical difficulties, [b]: All of the cesarean sections and one labor induction were done due to a rapid deterioration in maternal clinical status. One of the vaginal deliveries was preterm labor. Miscarriage was spontaneous at 18th week. One of the pregnant women died at 23th gestational week under medical induction (due to *in utero mort fetus*), [c]: At the cesarean section, vaginal delivery and abortus

Twenty-seven maternal deaths occurred mainly due to respiratory failure in the ICU and one patient died from acute pulmonary embolism in the inpatient clinic, 5 days after her discharge from the ICU. One of the pregnant women had an intrauterine fetal death during follow-up in the ICU and medical induction for abortion was applied, but, the patient died before the abortion. All the other maternal deaths occurred postpartum. demographic characteristics, obstetric characteristics and outcomes of maternal deaths are presented in Table 2. Twenty-eight babies were born and the 5th minute APGAR score of 7 babies was below 7. Twenty-one babies were followed up in the neonatal ICU primarily due to prematurity. There was no proven vertical transmission to babies according to nasopharyngeal PCR in the first 12 h of babies.

Clinical characteristics of maternal deaths in relation with Delta wave are summarized in Table 3. COVID-19 related imaging techniques, interventions during the follow-up of the disease in relation with Delta wave are presented in Table 4. Twenty-three pregnant women (23/28, 82.14%) admitted to the hospital with severe and two pregnant women (2/28, 7.14%) admitted with critical disease. Three of the pregnant women (3/28, 10.71%) had moderate disease at the time of admission and the LUS scores of these women were LUS-1, LUS-2, and LUS-3 respectively. One of the three pregnant women with moderate disease and LUS-1 score was admitted to the ICU 12 days after the admission to the hospital. LUS scores of all hospitalized patients were evaluated according to mortality and it was found statistically significant ($p<0.001$, chi-square=34.810). The maternal mortality rate, according to LUS scores, were 0.37% (1/273), 0.72% (2/277), 2.58% (10/387) and 11.72% (15/128) for LUS 0, LUS 1, LUS 2 and LUS 3, respectively (LUS-3 vs. others; maternal mortality: RR=8.447, 95% CI (4.11-17.34), z=5.814, $p<0.0001$). Demographic, clinical characteristics and laboratory findings of pregnant women with COVID-19 in relation with LUS scores are presented in Table 5. Additionally, clinical severities and LUS scores of all hospitalized patients in detail are presented in Supplementary Table 1.

The laboratory findings of the pregnant women at the time of hospital admission and at the critical stage of the disease are presented in Table 6. At hospital admission 19 (67.9%) patients presented with lymphopenia.

One maternal death occurred during the Omicron wave. She was 39 years old, her body mass index (BMI) was 27.55 and her parity was 3. She had 31+2nd gestational weeks and was admitted to the hospital with shortness of breath. She had hypothyroidism as co-morbidity. On admission to hospital C-reactive protein level was 18 mg/L and the lymphocyte count was 0.44 /10^3 uL. She rejected chest-CT, her chest X-ray revealed pneumonia and her LUS score was 3. After the 5th day of hospital admission, because of rapid deterioration in maternal clinical status cesarean section was performed and she was admitted to the ICU. She passed away due to COVID-19 pneumonia and accompanying acute respiratory distress syndrome (ARDS) after 4 days of follow-up in the ICU.

There were no vaccinated patients in the cohort of this study. Primarily used medications for treating patients are summarized in Supplementary Table 2.

Discussion

The total mortality rate of pregnant women due to COVID-19 was 2.63% among hospitalized patients, and the maternal deaths occurred in one-fifth of the patients who had a severe or critical illness. Maternal deaths were found to have increased approximately 5 times among hospitalized patients during the Delta wave. Almost all maternal deaths occur postpartum and co-morbidities may not be the determining factor for maternal death. The Delta wave and delayed vaccination may be the main reasons for increased maternal mortality. LUS may play a significant role in identifying critical patients. To the best of our knowledge, this study reports the highest number of maternal deaths from a single center.

The maternal mortality rate has been reported differently in each country, Centers for Disease Control in the United States reported a 0.2% maternal mortality rate from COVID-19[2], on the other hand, in Brazil 12% mortality rate was reported by Takemoto et al.[28]. The maternal mortality ratio (2.63%) was relatively high in our study from the commonly reported mortality rates in the literature[29-31]. However, this rate was among hospitalized patients who belonged to a pandemic referral hospital. In a multinational cohort study, the overall maternal mortality was found to be 1.6% that was 22 times higher than that in COVID-19 diagnosed non-pregnant women[32]. In a multicenter retrospective cohort study in Washington State, mortality due to COVID-19 was found to be 13.6 times higher in pregnant women compared with similarly aged individuals[4]. The study from Brazil emphasized that maternal death in pregnancy or postpartum period is always a tragedy and must be considered preventable[33].

The overall maternal death rate was 2.63%, although it was 1.28% before the Delta wave. The Delta variant had become the predominant variant in many parts of the UK and other countries due to increased transmissibility since February 2021[34]. As the pandemic progressed, the risk of critical illness increased, although this may be associated with the increased number of patients or with the effects of the new variants, the data on the reason for this increase have been scarce and confusing[34]. After the Delta variant spread, the death rate during pregnancy was reported to have increased in Mississippi[35] and in Parkland hospital the morbidity was reported to have increased with the Delta wave[36]. In this study, during the Delta wave, the death rate of pregnant women increased approximately 6 times in our hospital. Another reason for the increase in maternal mortality may be the lack of vaccination in pregnant women. Previously, pregnant women were excluded from COVID-19 vaccine research, and countries did not prioritize vaccinating

Table 3. Clinical characteristics of COVID-19 related maternal deaths[a]

	All (n=28)	Pre-Delta (n=10)	Delta (n=17)	p
Total duration of hospitalization (day)	15.86±8.49	18.6±9.05	14.59±8.19	0.309
Intensive care unit duration (day)	10.57±7.94	12.7±7.83	9.71±8.1	0.243
Symptoms at first admission				
Shortness of breath	15 (53.6%)	4 (40%)	10 (58.8%)	
Cough	7 (25%)	3 (30%)	4 (23.5%)	
Fever	2 (7.4%)	-	2 (11.8%)	
Myalgia	1 (3.6%)	1 (10%)	-	
Malaise	1 (3.6%)	1 (10%)	-	
Sore throat	1 (3.6%)	1 (10%)	-	
Diarrhea-nausea	1 (3.6%)	-	1 (5.9%)	
Contact history				
Positive	17 (60.7%)	8	8	0.093
Negative	11 (39.3%)	2	9	
Co-morbidities[b]	14 (50%)	4	9	0.516
Positive PCR test	28 (100%)	10 (100%)	17 (100%)	

Data presented as mean±standard deviation or n (%)
PCR: Polymerase chain reaction, [a]: Only one mother was died during the Omicron wave. Omicron column was not added due to statistical difficulties, [b]: There were 5 pregnant women ≥35 years of age, 4 pregnant women with ≥30 kg/m^2 body mass index, 3 pregnant women with preeclampsia, 2 pregnant women with asthma, 2 pregnant women with hypothyroidism and 1 pregnant women with scoliosis. Co-morbidities can appear simultaneously in a pregnant woman

Table 4. Imaging studies and interventions of COVID-19 related maternal death cases[a]

	n (%)			
Imaging studies	**All (n=28)**	**Pre-Delta (n=10)**	**Delta (n=17)**	
Chest X-ray				
Pneumonia	19 (67.9%)	6 (60%)	12 (70.6%)	
Rejection	9 (32.1%)	4 (40%)	5 (29.4%)	
CT				
Pneumonia	14 (50%)	8 (80%)	6 (35.3%)	
Rejection	11 (39.3%)	2 (20%)	8 (47.1%)	
Negative	3 (10.7%)	-	3 (17.6%)	
Initial LUS score at hospital admission				
LUS 0	1 (3.6%)	1 (10%)		
LUS 1	2 (7.1%)	1 (10%)	1 (5.9%)	
LUS 2	10 (35.7%)	1 (10%)	9 (52.9%)	
LUS 3	15 (53.6%)	7 (70%)	7 (41.2%)	
Interventions				**p**
Supplemental oxygen need with nasal cannula	26 (92.9%)	10 (100%)	15 (88.2%)	0.260
Non-invasive mechanical ventilation	28 (100%)	10 (100%)	17 (100%)	
Invasive mechanical ventilation	23 (82.1%)	9 (%)	13 (76.5%)	0.382
ECMO	10 (35.7%)	2 (%)	8 (47.1%)	0.230

LUS: Lung ultrasound, CT: Chest computed tomography, ECMO: Extracorporeal membrane oxygenation, [a]: Only one mother was died during the Omicron wave. Omicron column was not added due to statistical difficulties

Table 5. Demographic, clinical characteristics and laboratory findings of pregnant women with COVID-19 in relation with lung ultrasound scores

n=1.065	Values			
Demographic characteristics	LUS score 0/1 n=550	LUS score 2 n=387	LUS score 3 n=128	p
Age (year)	28.65±5.35	29.25±5.54	30.69±5.47	0.001
BMI (kg/m^2)	26.24±3.81	26.4±3.78	26.87±3.61	0.242
Gestational age (week)	27.3±10.48	28.06±9.08	29.02±7.72	0.155
Parity	1.25±1.28	1.26±1.2	1.5±1.28	0.117
Laboratory findings				
Leucocyte count (/10^3 uL)	9.1±5.54	8.58±4.19	11.54±6.93	<0.0001
Lymphocyte count (/10^3 uL)	1.5±0.9	1.35±0.99	1.1±0.82	<0.0001
C-reactive protein (mg/L)	3.39±4.94	5.87±14.7	8.04±7.6	<0.0001
Lactate dehydrogenase (IU/L)	210.0 (92)	238.5 (116.5)	296.5 (232.5)	<0.0001
Clinical characteristics				
Duration of hospitalization (day)	3.78±3.86	7.51±4.42	11.59±11.45	<0.0001
Patients admitted to ICU	4 (0.73%)	22 (5.68%)	36 (28.13%)	<0.0001
Patients with severe-critical disease	10 (1.82%)	61 (15.76%)	76 (59.38%)	<0.0001
Maternal death	3 (0.55%)	10 (2.58%)	15 (11.72%)	<0.0001
Supplemental oxygen need with nasal cannula	24 (4.36%)	86 (22.22%)	77 (60.16%)	<0.0001
Non-invasive mechanical ventilation need	5 (0.91%)	34 (8.79%)	47 (36.72%)	<0.0001
Invasive mechanical ventilation need	3 (0.55%)	9 (2.33%)	23 (17.97%)	<0.0001
Extracorporeal membrane oxygenation	1 (0.18%)	4 (1.03%)	10 (7.81%)	<0.0001

Variables are reported as mean ± standard deviation or median (interquartile range) depending on distribution characteristics and n (%), LUS: Lung ultrasound, ICU: Intensive care unit

Table 6. Laboratory parameters of the maternal deaths at two occasions (hospital admission and critical stage of disease)

n=28	At hospital admission Values	During critical stage Values	p
Leucocyte count (/10^3 uL)	8.14±3.4	19.54±8.17	**<0.0001**
Neutrophil count (/10^3 uL)	7.1±3.29	16.26±6.96	**<0.0001**
Lymphocyte count (/10^3 uL)	0.90±0.33	2.07±1.61	**0.002**
Hemoglobin level (g/dL)	10.78±1.35	8.93±1.94	**<0.0001**
Platelet count (10^3/mm^3)	220±84.39	268.04±177.89	**0.191**
Alanine aminotransferase (IU/L)	17.2 (32.8)	25.5 (74.3)	**0.052**
Aspartate aminotransferase (IU/L)	30.5 (34.83)	51.5 (98.77)	**0.009**
C-reactive protein (mg/L)	7.77±5.02	16.9±14.26	**0.001**
Lactate dehydrogenase (IU/L)	295.5 (173.5)	678.5 (440.75)	**<0.0001**
Ferritine (ng/mL) (n=14)	159.2 (239.2)	735.6 (5130.78)	**0.003**
D-dimer (mcg/mL) (n=26)	1.5 (2.55)	5.05 (7.43)	**0.004**
Fibrinogen (mg/dL) (n=26)	562.28±139.87	544.08±290.21	**0.476**
Procalcitonin (ng/mL) (n=16)	0.35 (1.84)	0.88 (3.87)	**0.776**

Variables are reported as mean ± standard deviation or median (interquartile range) depending on distribution characteristics

pregnant women[31]. Later, our government, along with a few other countries, promoted COVID-19 vaccination of pregnant women[32]. Despite this, unfortunately, there were no vaccinated patients in the cohort of this study. Vaccine acceptance is low due to the concerns about vaccine safety in our country[37]. Hospitalization and mortality were not considerably increased during the Delta wave in the vaccinated population, according to an Israeli study[38] and Aslam et al.[7] reported recently that at the 4th wave maternal deaths were all in unvaccinated pregnant women. Due to the relatively higher severity of the Delta variant, the vaccine hesitancy and delayed vaccination might have had an outsized influence on the increased mortality rate during the Delta wave in pregnant women. The mortality rate with the onset of the Omicron wave was 2.6% in the study cohort, this rate was lower than the Delta wave but, higher than the pre-Delta period. However there is no statistical significance between pre-Delta period and Omicron wave maternal mortality rates, and the number of patients in the Omicron wave may be insufficient to determine the maternal mortality rate.

All maternal deaths in this study occurred after delivery, except one patient. Maternal deaths were primarily reported postpartum in Iran, Brazil and Mexico, similar to our cohort of maternal deaths[39,40]. In this study, COVID-19 pneumonia and accompanying ARDS were the primary causes of mortality, except for one maternal death, which was caused by pulmonary embolism. All of the hospitalized patients received either 40 mg or 60 mg enoxaparin according to their weight for thrombophylaxis since COVID-19 increases the risk of thrombosis[41]. Similar to the first maternal death reported in the United caused by basilar artery thrombosis[42], one of our maternal deaths occurred due to a hypercoagulative state of the postpartum period that was pulmonary embolism as a complication of deep vein thrombosis. She had been treated with enoxaparin 60 mg twice daily before her death.

In the current study, if we consider age over 35 and BMI over 30 as risk factors, 14 (50%) pregnant women had co-morbidity. There were 3 women with preeclampsia, 2 women with asthma, 2 women with hypothyroidism and one woman with scoliosis. Half of the pregnant women had no co-morbidity, therefore, co-morbidities may not predict maternal death. However, when compared to similar age non-pregnant women, pregnant women have a higher risk of complications-such as severe pneumonia, hospitalizations, admission to an ICU, and invasive mechanical ventilation-and mortality from COVID-19[43]. Although there has been a report that severe infection can be seen in the early stages of pregnancy[19], late stages of pregnancy are often associated with more severe infection and major unfavorable outcomes[44]. Similar to the case series of maternal deaths from Iran[45] maternal deaths in our hospital occurred in the second and third trimesters.

It would be beneficial to identify COVID-19 patients who can worsen to reduce or even eliminate maternal mortality caused by COVID-19. The leading indicators are common symptoms and nasopharyngeal PCR results, although they might be deceptive and delayed in making decisions, particularly when it comes to hospitalization. When we investigated the relation between LUS scores and maternal death rates, the highest maternal mortality rate was observed among the patients an LUS score of 3 (14/118, 11.9%). Besides, a significant correlation was found between clinical severity and LUS scores. ICU admission, mechanical ventilation and mortality were linked to higher LUS scores, according to a recently published review[18].

Study Limitations

The retrospective nature of this investigation made it difficult to determine the precise number of variant cases. Therefore, the declared period of the Delta and Omicron waves in our country was the basis of our estimation of the maternal mortality rate.

Conclusion

Maternal death is always a tragedy, and it is important to investigate the risk of mortality and take precautions. The maternal mortality rate was relatively high in our referral center of COVID-19. Maternal mortality occurred in the second and third trimesters of pregnancy by a majority in the postpartum period due to COVID-19 pneumonia and related ARDS except for one case in this study. The use of LUS appears to be valuable in identifying critical patients. The Delta wave, delayed vaccination and vaccine hesitancy of pregnant women may have all played important roles in maternal mortality in our hospital. Developing and improving vaccination strategies for pregnant women and prioritizing them, could be crucial in preventing pandemic related deaths in pregnant women.

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Ethics

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Authorship Contributions

Interpretation: M.Y., O.S.G., B.D.T., N.T., Supervision: C.U., N.T., Concept: A.B.T., M.Y., P.B.İ., B.Ö., D.B., O.S.G., Design: A.B.T., E.Y., C.U., G.Ç., B.D.T., N.T., Data Collection or Processing: M.Y., P.B.İ., E.Y., B.Ö., C.U., D.B., O.S.G., G.Ç., B.D.T., N.T., Analysis or Interpretation: A.B.T., M.Y., Literature Search: Writing: A.B.T., P.B.İ., E.Y., C.U.

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Supplementary Table 1. LUS scores and the clinical severity of hospitalized patients

Lung ultrasound scores of hospitalized patients	Clinical severity of patients		
	Asymptomatic- mild disease	Moderate disease	Severe- critical disease
0-1	502 (72.3%)	38 (17%)	10 (6.8%)
2	175 (25.2%)	151 (67.4%)	61 (41.5%)
3	17 (3.1%)	35 (15.6%)	76 (51.7%)
Total	694 (100%)	224 (100%)	147 (100%)

LUS: Lung ultrasound, p<0.0001 (chi-square value=499.039)

Supplementary Table 2. Medications used in the treatment of patients

Medications		n (%)
Anticoagulant	Enoxaparin 40-60 mg subcutaneously as a prophlaxis	28 (100%)
Antiviral	50 mg ritonavir and 200 mg lopinavir (two tablets twice daily in pregnancy)	16 (57.1%)
	Favipiravir 200 mg (eight tablets twice daily postpartum)	2 (7.1%)
Antibiotic	Choice, dose and duration changes with clinical judgement	27 (96.4%)
Corticosteroids	120 mg methylprednisolone daily	28 (100%)
Convalescent plasma therapy		7 (25%)

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Elevated serum YKL-40 levels as a diagnostic and prognostic marker in the placenta accreta spectrum

Plasenta invazyon anomalilerinin tanısı ve prognozunda artmış serum YKL-40 seviyeleri

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Abstract

Objective: Placenta accreta spectrum (PAS) is an important problem with increasing cesarean section (CS) rates recently. There is still no serum marker for the diagnosis. We determined whether serum YKL-40 levels can be used in the diagnosis and prognosis of PAS.

Materials and Methods: The study was conducted with 50 patients with a PAS diagnosis, 27 individuals without PAS, and 33 normal pregnant women. The operations (CS + placental bed suture, CS + excision of the lower segment, CS-hysterectomy) and for individuals who had the excision of the lower segment /CS-hysterectomy, the histopathological diagnoses (accreta, increta, percreta) were recorded. Serum YKL-40 levels were analyzed.

Results: The individuals with PAS possessed significantly greater serum YKL-40 grades (p=0.001). The surgical interventions included 4 CS + excision of the lower segment, 9 CS + placental bed sutures, and 37 CS-hysterectomy. The histopathological outcomes of the individuals who had the excision of the lower segment, CS-hysterectomy and diagnosed 6, 9, and 26 patients with accreta, increta, and percreta, respectively. The accreta, increta, and percreta groups showed statistically significant different serum YKL-40 grades (p=0.001). The receiver operating characteristic analysis was performed to discriminate the cut-off serum YKL-40 level as 32.81 ng/mL with a sensitivity of 66% and specificity of 70.37%. The positive and negative predictive values of YKL-40 in the indicator of PAS were 80.5% and 52.8%, respectively.

Conclusion: Elevated serum YKL-40 grades were correlated with the diagnosis and severity of PAS. If our findings are corroborated and elaborated by larger patient series, the YKL-40 levels should be used along with ultrasonography to construct a model identical to that used in aneuploidy screening.

Keywords: Abnormal placental invasion, cesarean section, placenta accreta spectrum, ultrasonography, YKL-40

Öz

Amaç: Plasenta akreata spektrumu (PAS) artan sezaryen oranlarıyla son yıllarda önemli bir sorun haline gelmiştir. Ancak hastalığın tanısında kullanılabilecek bir serum markeri hala yoktur. Çalışmada serum YKL-40 seviyelerinin, hastalığın tanısı ve prognozunda kullanılabilecek bir marker olup olmadığını ortaya koymak amaçlanmıştır.

Gereç ve Yöntemler: Çalışmaya transabdominal renkli Doppler ultrasonografide (TA-RDS) PAS öngörüsü olan 50 hasta ile PAS olmayıp eski sezaryen öyküsü bulunan 27 kontrol hastası ve 33 normal gebe dahil edildi. Hastaların operasyonlarında ne yapıldığı (sezeryan + servikal yatak suturu, sezeryan + alt segment eksizyon, sezeryan-histerektomi) ve alt segment eksizyon/sezeryan-histerektomi yapılan hastaların histopatolojik bulguları (accreta, increta, percreta) kayıt altına alındı. YKL-40 seviyeleri enzim linked immünosorbent assay yöntemi ile analiz edildi. PAS'nin öngörüsünde serum YKL-40 seviyelerinin optimal cut-off değerini belirlemek için alıcı işletim karakteristiği (ROC) testi kullanıldı.

Bulgular: Hasta grubunun serum YKL-40 seviyesi, diğer gruplara göre anlamlı derecede yüksekti (p=0,001). Elli hastanın operasyonlarında, 4'üne (%8) sezaryen + alt segment eksizyon, 9'una (%18) sezaryen + servikal yatak süturu, 37'sine (%74) sezaryen-histerektomi uygulandı. Alt segment eksizyon ve sezaryen histerektomi uygulanan hastaların histopatolojik sonuçları değerlendirildiğinde 6 (%14,6) hasta accreta, 9 (%22) hasta increta ve 26 (%63,4) hasta percreta tanısı aldı. Accreta, increta ve percreta grupları arasında serum YKL-40 seviyeleri bakımından anlamlı farklılık izlendi (p=0,001). ROC analizinde,

PRECIS: The importance of serum YKL-40 levels on placenta accreta cases.

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%66 duyarlılık ve %70,37 özgüllük ile serum YKL-40 seviyeleri için cut-off değer 32,81 ng/mL olarak bulundu. YKL-40'ın PAS tahmininde pozitif ve negatif prediktif değerleri sırasıyla %80,5 ve %52,8 idi.

Sonuç: Artmış serum YKL-40 seviyeleri PAS'nin varlığı ve derecesi ile ilişkilidir. Bulgularımız daha geniş hasta serileri tarafından desteklenir ve detaylandırılırsa, anöploidi taramasında kullanılana benzer bir model oluşturmak için, YKL-40 seviyeleri ultrasonografi ile birlikte kullanılabilecek bir marker olabilir.

Anahtar Kelimeler: Anormal plasental invazyon, sezaryen section, plasenta acreata spektrum, ultrasonografi, YKL-40

Introduction

Placenta accreta spectrum (PAS), which is described as an unusual trophoblast violation of the myometrium by the placenta, is closely linked to massive obstetric hemorrhage and postpartum hysterectomy[1]. The incidence of PAS has increased due to the rise in cesarean section (CS) rates in recent decades[2]. Histologically, the PAS is allocated according to the harshness of myometrial invasion by extravillous trophoblasts (EVT). In the placenta accreta, there is the partial attachment of the myometrium without invading it by placental villi. In the placenta increta, chorionic villi completely infect the myometrium. While in the placenta percreta, the villi have advanced beyond the myometrium into the uterus serosa and surrounding tissues[3]. The abnormal adherence of EVT in PAS can be caused by an absence of decidua basalis, excessive invasion by EVT, or a combination of these two factors[4]. This assumption is supported by the fact that 80% of PAS patients report a history of CS, myomectomy, and/or curettage operations[5].

Studies have reported various autocrine and paracrine regulators of EVT invasion that include growth factors, matrix metalloproteases, chemokines, cytokines, and adhesion molecules[6]. The YKL-40 (Human Chitinase-3-like protein 1), which is also recognized as the human cartilage glycoprotein 39, acts as a chemoattractant. It is an inflammatory glycoprotein that supports vascular endothelial cells and tubulogenesis and migration[7]. YKL-40 is also linked to the extracellular tissue remodeling, proliferation and differentiation of malignant cells, neovascularization, stimulation of cancer-associated fibroblasts, and inhibition of cancer cell apoptosis[8]. Elevated serum levels are linked to problems in the extracellular matrix and angiogenesis[9].

YKL-40 is secreted predominantly by neutrophils, macrophages, endothelial, stem, and cancer cells[4]. Its levels increase in the presence of various diseases, including cancer, osteoarthritis, cardiovascular diseases, neurological diseases, infections, chronic obstructive pulmonary disease, asthma and preeclampsia, gestational diabetes, and cholestasis of pregnancy[8]. However, the correlation between YKL-40 and PAS is not known.

The contribution of YKL-40 to trophoblast invasion has been investigated by inspecting hysterectomy specimens of PAS patients[7]. However, according to our literature review, there isn't any study that has examined the serum levels of YKL-40 in PAS and attempted to correlate these parameters with the histopathological findings.

Based on the assumption that YKL-40 contributes to excessive EVT invasion, this study determines the serum levels of YKL-40 in the individuals suspected of having PAS and to investigate whether the YKL-40 can be used as a prognostic and diagnostic marker of PAS by correlating these levels with histopathological findings (accreta, increta, and percreta).

Materials and Methods

The patients with a diagnosis of PAS in the Southeast region of our country were referred to our hospital because our hospital is the tertiary referral center. Our study included 50 patients who applied to Gaziantep University, Medical Faculty Obstetrics and Gynecology Polyclinic between the dates of January 2019 and September 2019 and had the PAS in ultrasonography.

Between the dates of January and September 2019, 128 total placenta previa cases were detected in our clinic. In 53 cases, PAS was detected on trans-abdominal-ultrasonography (TA-USG), whereas PAS was not observed in 75 patients. Three of the patients with PAS refused to participate in the study. So, the study included 50 total placenta previa patients in the 3rd trimester of pregnancy with a previous history of CS and who were detected to have PAS on TA-USG in the current pregnancy, 27 total placenta previa patients who had a previous history of CS but did not have PAS in the current pregnancy, and as a control group, 33 normal pregnant women with a history of previous CS. All groups were matched in terms of age, body mass index (BMI), and gestational week.

Pregnant patients who had gestational diabetes, systemic diseases, gestational HT, multiple pregnancies, preeclampsia, low-lying placenta previa, chromosomal or structural fetal anomalies, and pregnant patients who smoked were excluded from the study. This study was conducted in line with the guidelines stated in the Helsinki Declaration, and the approval was obtained from the Clinical Research Ethics Committee of the Faculty of Medicine (approval number: 2019/317).

TA-USG and Doppler USG examinations of the experimental and control groups' patients were performed in the department of Gynecology and Obstetrics by a single experienced physician using a Voluson E8® (GE Healthcare) device. Placenta previa totalis is diagnosed when the placenta stretches directly over the internal cervical os[10]. Using gray-scale/color Doppler TA-USG, we investigated the following items for PAS; loss/unregularity of the echolucent space between the placenta and uterus (clear zone), disruption of the hyperechoic area between the uterine serosa and bladder, and the presence of turbulent placental lacunae[11].

All patients who participated in the study signed a consent form. After fasting for 12 h, venous blood samples were collected from all participants. After coagulation, the samples were centrifuged to obtain serum and kept at -80 °C until analysis. Serum YKL-40 levels were calculated using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Rel Assay DC, Gaziantep, Turkey). The Serum YKL-40 levels were determined as ng/mL. The number of pregnancies and births, age, and previous CSs, gestational week, BMI, the presence of CS-hysterectomy in the current pregnancy; if present, histopathological diagnosis (accreta, increta, and percreta), and the serum YKL-40 levels were recorded for all pregnant participants.

Statistical Analysis

In terms of YKL-40 levels between the groups, a large effect size level (Cohen d=1), the required minimum number of participants in each group was determined as 27 for the expected difference to be statistically significant (α=0.05, 1-β=0.80). Power analysis was done in G-power 3.1.9.2 package program.

The quantitative variables were checked for normal distribution using the Shapiro-Wilk test. ANOVA and LSD tests were used to compare variables with a normal distribution in the three groups, while Dunn and Kruskal-Wallis tests were used for the variables presenting non-normal distributions. The analysis of the receiver operating characteristic (ROC) curve was performed to calculate a cut-off rate for the YKL-40 levels. Statistical analysis were carried out with using the SPSS 22.0 for Windows and Medcalc 17.5.5, and a p-value<0.05 was determined statistically significant.

Results

The groups were not different in terms of gravida, age, parity, the number of previous CSs, gestational week, and BMI (Table 1).

The Mean YKL-40 was determined as 34.07±9.68 ng/mL for the PAS group, 28.4±6.93 ng/mL for those without PAS, and 15.76±2.57 ng/mL for the normal pregnancy group. The mean YKL-40 of the PAS group was significantly greater than that of the other two groups, (p=0.001).

Among the surgical interventions performed on the 50 patients who were detected to have PAS on TA-USG included, 4 of them had CS+excision of the lower segment operations (8%), 9 of them had CS+placental bed suture operations (18%), and 37 of them had CS-hysterectomy operations (74%). Since the placenta was removed in 9 patients and invasion was detected only in the cervical canal, the operation was terminated with CS+placental bed suture, and a Bakri balloon was placed when necessary. The placental pathologies of these patients were "normal." The mean YKL-40 value of 9 patients who were detected to have PAS on TA-USG and only CS+placental bed suture was performed in the operation was 18.75±3.86 ng/mL.

The histopathological outcomes of the participants who underwent excision of the lower segment and CS-hysterectomy were as 6 individuals (14.6%) with accreta, 9 individuals (22%) with increta, and 26 (64.3%) with percreta. The accreta, increta, and percreta groups presented statistically significant different serum levels of the YKL-40 (p=0.001).

The YKL-40 levels were greater in the percreta group than in the increta and accreta groups, and it was greater in the increta group than in the accreta group, significantly (Table 2). YKL-40 values of the patients who had CS + placental bed suture were significantly lower than the accreta, increta and percreta groups

Table 1. Main clinical features of the groups

Variables	Patients with API group (n=50)	Patients without API group (n=27)	Control group (n=33)	p
Age	33.14±4.41	33.56±5.85	33.03±2.05	0.885
Gravida	4.88±1.53	4.41±1.25	4.7±0.64	0.306
Parity	3.38±1.14	3.11±1.12	3.61±0.66	0.066
Number of previous CS	3.12±0.92	3.11±0.93	3.03±0.77	0.988
Gestational week	33.94±1.95	33.89±2.65	34.18±1.21	0.741
BMI (kg/m²)	27.45±2.35	27.86±2.56	27.52±1.12	0.744

API: Abnormal placental invasion, BMI: Body mass index

Table 2. The comparison of YKL-40 rates between the accreta, increta, and percreta subgroups

	Accreta (n=6)	Increta (n=9)	Percreta (n=26)	p
YKL-40 (ng/mL)	27.23±3.32	35.09±4.29	40.6±5.5	0.005[a] 0.001[b] 0.007[c]

[a]: Increta versus accrete, [b]: Percreta versus accrete, [c]: Percreta versus increta

(CS versus accreta, CS versus increta, and CS versus percreta; p=0.002, p=0.001, and p=0.001, respectively). This group was considered the group that TA-USG was a false positive.

The analysis of the ROC curve was performed to calculate a cut-off serum YKL-40 level for indicating PAS. The place under the ROC curve was calculated as 0.68 (95% confidence interval, 0.57-0.98; p=0.002). The cut-off value for YKL-40 was calculated as 32.81 ng/mL with a sensitivity of 66% and specificity of 70.37% (Figure 1). The positive and negative predictive values of YKL-40 in the indication of PAS were 80.5% and 52.8%, respectively.

Discussion

To our knowledge, this study is the first that has analyzed the serum YKL-40 levels in PAS. In this study, we found elevated YKL-40 levels in PAS, which presented a significant increase from accreta to increta, and increta to percreta. Another significant outcome of our study is that the YKL-40 could be a useful prognostic and diagnostic marker for PAS cases with a good level of sensitivity (66%) and specificity (70.37%).

PAS is a condition that can cause both fetal and maternal mortality and morbidity. Maternal complications are generally linked to the difficulty of the operation and bleeding, while neonatal and fatal complications are connected to prematurity that emerges from the operations performed due to bleeding[12]. Therefore, identifying a preoperative diagnostic and prognostic method that could confirm the risk of PAS is quite important. In the antepartum period, the TA-USG is the primary method for the confirmation of placental abnormalities with a high level of sensitivity (85.7%) and a high level of specificity (88.6%)[13]. Some serum markers were also investigated in PAS, and the reliability of the variable was determined depending on the gestational conditions[14]. At present, a sensitive serum marker for the invasive placement is still unclear.

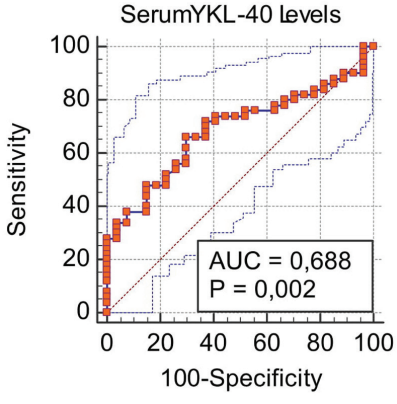

Figure 1. ROC curve of the indicator value of serum YKL-40 rates in API. ROC curve to evaluate the diagnostic value of YKL-40 in PAS. The YKL-40 rates of participants with and without PAS were utilized to create a ROC curve and affiliated values of specificity, sensitivity, and AUC were calculagted.

ROC: Receiver-operating characteristics, AUC: Area under the curve

As our hospital is a tertiary center, it provides care for the PAS cases not only in our city but also in surrounding cities. When treating patients with suspected invasion, preparing for transfusion requirements, conducting the operation under elective conditions, and arranging the surgical team (comprised of a neonatologist, perinatologist, and if needed, a gyneco-oncologist and urologist) are essential to help the family psychologically prepare themselves for a difficult operation. Although ultrasonography is an effective method for showing abnormal placental invasion, it does not provide sufficient information about the depth of invasion. Therefore, it is difficult to determine the abnormal invasion of the accreta, increta, and percreta groups by USG[15].

A serum biomarker that would support the diagnosis of PAS can facilitate us without the need for more expensive magnetic resonance imaging (MRI) method in the preoperative period. In this regard, a marker that would complement ultrasonography in the preoperative period may increase diagnostic accuracy and save the lives of both the fetus and mother. If such a marker is used and the pre-diagnosis of PAS based on TA-USG is confirmed by a serum biomarker, individuals can be referred to tertiary centers for management, and they can be followed up at more frequent intervals.

In our study, there were 9 patients who were detected to have PAS on ultrasonography in the preoperative period, but who did not reveal PAS during the operation. These patients were evaluated as those in which ultrasonography was "false positive." The mean YKL-40 level of these patients was lower than those of both the accreta, increta, and percreta groups (18.75±3.86 ng/mL vs 27.23±3.32; 35.09±4.29; 40.6±5, 5 ng/mL) and the total placenta previa group without PAS (18.75±3.86 ng/mL vs 28.4±6.93 ng/mL), but the mean YKL-40 level of these patients was greater than the normal pregnancy group (18.75±3.86 ng/mL vs 15.76±2.57 ng/mL). This suggests that the YKL-40, which can be used along with ultrasonography in the preoperative period, can help us with the depth of invasion.

YKL-40 plays a crucial part in the restructuring of the extracellular matrix, the activation of the natural immune system, angiogenesis, the growth, differentiation, and anti-apoptosis of tumor cells[8].

Typically, the serum YKL-40 levels increase between the 12th and 20th gestational week, maintain a steady level between the 20th and 25th week, and decrease after the 32nd week. It is not acknowledged whether this increase and the subsequent decrease present the implantation and placental development processes- both processes require angiogenesis and tissue remodeling[16]. Certain studies have implicated the YKL-40 as a potential tumor marker. To this date, *in vitro* studies have determined the YKL-40 overexpression in various tumors, including osteosarcoma, prostate cancer, glioma, colon cancer, and endometrial cancer[8].

We designed this study based on the idea that the YKL-40 levels could be increased in PAS as the abnormal EVT invasion can be

considered a tumorous formation. In a study conducted by Guo et al.[8], the serum YKL-40 rates were determined to be greater in patients with leiomyoma than in the control group, and a positive correlation was determined between the YKL-40 grade and the myoma weight. YKL-40 was revealed to be a useful indicator for detecting leiomyoma with a sensitivity of 82% and specificity of 91%. Similarly, this study found higher YKL-40 grades in the individuals who were detected to have PAS on TA-USG compared with those who were not. The YKL-40 grades were determined to be elevated from the accreta to the percreta. YKL-40 rates exceeding 32.8 ng/mL were associated with an increased PAS risk, with 66% sensitivity and 70.37% specificity.

Although the role of YKL-40 is not obvious, its overexpression has been linked to the signaling pathways of mitogenic activity[7]. Many studies have explored the effects of YKL-40 on extracellular matrix remodeling and the invasion of cancer cells[7]. Accordingly, it is plausible that the YKL-40 affects PAS due to the inhibition of excessive EVT invasion and EVT apoptosis through the extracellular matrix remodeling.

The YKL-40 has also been shown to be related to endothelial dysfunction[17]. Seol et al.[18], conducted a study that determined higher YKL-40 rates in the individuals with preeclampsia and identified a correlation with the severity of the disease. In the cases of placenta accreta and placenta previa without PAS, increased YKL-40 grades may be expected as the scar site undergoes defective maternal vascular remodeling[19]. This hypothesis is corroborated by the findings of our study that indicated increased YKL-40 levels in the PAS group.

A study by Gozukara et al.[7] examined the tissue expression levels of YKL-40 in hysterectomy specimens of the PAS patients and determined the strongest expression in the percreta cases, reported that the YKL-40 grades were correlated with the EVT invasion. Differently from the cited study, we studied the serum rates of YKL-40 in the individuals with PAS and determined that the patients with invasion had elevated levels, which were correlated with the degree of invasion. Correlated with the results of the study by Gozukara et al.[7], which revealed the greatest YKL-40 possession in the percreta group, our study determined the highest YKL-40 rates in the percreta group.

The study showed for the first time that the PAS is affiliated with increased YKL-40 rates and that across the accreta, increta, percreta groups, these levels were greater in the percreta group than in the increta and accreta groups, and greater in the increta group than in the accreta group, significantly. The lower YKL-40 levels of patients who were detected to have PAS on ultrasonography but without PAS during the operation and had CS + placental bed suture, compared to the accreta, increta, and percreta groups supported the increase of YKL-40 rates with the possession of invasion. In these patients, the prediction of PAS on ultrasonography is the false positivity of USG.

Study Limitations

The limitation of this study is that the number of patients who could have been included in each group (accreta, increta, and percreta) was small and the YKL-40 rates were measured only in the third trimester. Our study is a reference study that is the first to divide patients into accreta, increta, and percreta groups according to the serum marker levels and to correlate these findings with the pathology findings.

In this study, it was aimed to present whether the YKL-40 levels would provide a cut-off value between the accreta, increta, and percreata groups that support our ultrasonographic findings. Thus, we planned to determine which disease group (accreta, increta, percreta) the patient would fall in the preoperative period in patients with PAS detected by the ultrasonography and serum YKL-40 level, without the need for an expensive diagnostic tool such as MRI. The fact that the YKL-40 rates in the PAS group were significantly greater than the other 2 groups (normal pregnant and placenta previa without PAS) will guide new studies in terms of using this serum marker as an additional diagnostic tool to ultrasonography in the diagnosis of PAS.

Conclusion

If the results of our study are corroborated by the studies that possess a larger number of participants for each subgroup (accreta, increta, and percreta), it would be possible to confirm both the pathophysiological effects and the prognostic and diagnostic utility of YKL-40 levels in PAS. By confirming the YKL-40 and ultrasonography, a model that is similar to that used for screening the aneuploidy [Alpha-Fetoprotein+USG] may be considered for the PAS. Nonetheless, the assistance of that stands as an unknown feature until greater prospective data from the larger populations are available.

In the cases of recurrent CS, a study with larger samples can be organized on the correlation between the YKL-40 grades and the development of PAS in the early second trimester or the late first trimester. Thus, the YKL-40 grades should become a marker that could be used in the prediction of PAS before the ultrasonographic findings occur.

Ethics

Ethics Committee Approval: This study was conducted in line with the guidelines stated in the Helsinki Declaration, and the approval was obtained from the Clinical Research Ethics Committee of the Faculty of Medicine (approval number: 2019/317).

Informed Consent: All patients who participated in the study signed a consent form.

Peer-review: Externally peer-reviewed.

Authorship Contributions

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

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Relationship between serum cadherin 6 and 11 levels and severe and early-onset preeclampsia: A pilot study

Erken başlangıçlı ve ağır preeklampsi ile serum kaderin 6 ve 11 seviyeleri arasındaki ilişki: Bir pilot çalışma

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Abstract

Objective: Preeclampsia is a highly morbid disease of placental origin, life-threatening condition for both a pregnant woman and her fetus. Cadherin 6 and 11 are adhesion molecules that play an important role in trophoblastic development and placentation. In our study, we investigated the change in serum cadherin 6 and 11 levels in pregnant women with preeclampsia.

Materials and Methods: Pregnant women with preeclampsia were selected and compared with healthy women (as a control group) for a one-year study. The serum alanine aminotransferase, aspartate aminotransferase, and cadherin levels 6 and 11 of participants were analyzed and compared.

Results: A total of 189 pregnant women were subdivided into 2 groups as preeclamptic (n=94) and women with healthy pregnancy (n=95). The cadherin 6 and cadherin 11 levels of the preeclamptic patients were significantly higher than those in the control group (p=0.001), and they were found to be significantly higher mainly in patients with early-onset and severe preeclampsia group (p=0.001). The cut-off cadherin 6 and 11 values for severe preeclampsia were found as 98.174 ng/mL and 1.92 ng/mL; with sensitivity of 88.3% and 84% respectively (p=0.001).

Conclusion: The data analysis showed elevated serum cadherin 6 and 11 levels associated with the severity and early onset of pre-eclampsia. Serum cadherin 6 and 11 levels can be a candidate marker for the prediction of preeclampsia.

Keywords: Cadherin 6, cadherin 11, preeclampsia, severe preeclampsia, early-onset preeclampsia

Öz

Amaç: Preeklampsi, plasenta kaynaklı oldukça morbid bir hastalık olup, hem gebe hem de fetüs için hayatı tehdit edici bir durumdur. Kaderin 6 ve 11, plasenta oluşumu ve trofoblastik gelişimde önemli bir rol oynayan adezyon molekülleridir. Çalışmamızda, preeklampsi olan gebe kadınlarda serum kaderin 6 ve 11 seviyelerindeki değişimleri incelemeyi amaçladık.

Gereç ve Yöntemler: Bir yıllık bir çalışma çerçevesinde preeklampsi olan gebeler seçildi ve kontrol grubu olarak sağlıklı gebelerle karşılaştırıldı. Katılımcıların serum alaninaminotransferaz, aspartate aminotransferaz, kaderin 6 ve 11 seviyeleri analiz edildi ve karşılaştırıldı.

PRECIS: In this study, we evaluated and found higher levels of serum cadherin levels 6 and 11 levels of pregnant women with preeclampsia than in healthy pregnant women.

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Bulgular: Toplam 189 gebe, preeklamptik (n=94) ve sağlıklı gebeliği olan (n=95) olmak üzere iki gruba bölünmüştür. Preeklamptik hastaların kaderin 6 ve kaderin 11 düzeyleri kontrol grubuna göre anlamlı olarak yüksekti (p=0,001) ve özellikle erken başlangıçlı ve şiddetli preeklampsili hastalarda anlamlı olarak yüksek bulundu (p=0,001). Şiddetli preeklampsi için cut-off kaderin 6 ve 11 değerleri sırasıyla %88,3 ve %84 duyarlılıkla, 174 ng/mL ve 1,92 ng/mL olarak bulundu (p=0,001).

Sonuç: Verilerin analizi, yüksek serum kaderin 6 ve 11 seviyelerinin preeklampsinin şiddeti ve erken başlangıcı ile ilişkili olduğunu gösterdi. Serum kaderin 6 ve 11 seviyeleri, preeklampsinin öngörülmesi için aday birer belirteç olabilir.

Anahtar Kelimeler: Kaderin 6, kaderin 11, preeklampsi, ağır preeklampsi, erken-başlangıçlı preeklampsi

Introduction

Preeclampsia is a disease characterized by hypertension (systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg), proteinuria, or end-organ damage, occurring generally after 20 weeks of gestation[1]. According to the gestational week, it is classified as early-onset if diagnosed under 34 weeks, and late-onset preeclampsia if diagnosed at 34 weeks and above[2]. Worldwide, it complicates 4-5% of all pregnancies[3], causing maternal deaths in more than 50.000 mothers per year[4]. Apart from maternal mortality, cerebrovascular events can lead to serious maternal complications, such as liver rupture, pulmonary edema, acute renal failure, or fetal complications, such as preterm labor, intrauterine growth restriction, and fetal death[5].

Placental abnormalities are emphasized in the pathophysiology of preeclampsia. In the normal placental development process, cytotrophoblasts invade maternal spiral arteries at the myometrium level and form vascular spaces[6]. However, inadequate remodeling in the spiral arterioles leads to narrowing, ischemia, and, consequently, the development of preeclampsia[7]. During placental development, trophoblasts develop in two directions: villous trophoblasts that turn into syncytiotrophoblasts, responsible for the secretion of placental hormones, and extravillous trophoblasts (EVTs), responsible for the invasion of blood vessels in the uterus. EVTs destroy the media of the maternal spiral arterioles, displace them the endothelium and turn into endovascular trophoblasts[8]. Cytotrophoblasts must undergo several changes to transform from the epithelial form to the mesenchymal structure, forming the endothelium. Presenting epithelial structure, they are tightly linked to each other by desmosomes, tight junctions, and cadherins that receive support from cytoskeleton structures, such as the actin and catenin basement membrane by integrins[9]. However, in mesenchymal cells, either the inter-cell or inter-basal membrane adhesion molecule expression decreases. These cells are prone to migration and potentially invade the myometrium[10,11].

Cadherins are a transmembrane protein family, provide cell communication with the microenvironment and regulate the structural microarchitecture of the cytoskeleton and cell[12]. Acting as biophysical and chemical sensors in the cell's microenvironment, cadherins also regulate cell growth and behavior[13]. Cadherin 6 is a protein in the class II cadherin group and is involved in the embryological development of the kidney and central nervous system by triggering epithelial-mesenchymal transformation[14,15]. Despite this function in the embryonal period, cadherin 6 is also detected in renal carcinoma in adults and is considered a sign of a poor prognosis[16]. It has been shown that cadherin 6 is also expressed in the endometrial glandular epithelium and stroma in the follicular phase of the menstrual cycle and EVTs[17]. Furthermore, cadherin 11 belongs to the family class II cadherin, responsible for bone, cartilage, and neuronal development in embryonal life[18,19]. It is responsible for the terminal transformation of cytotrophoblasts into syncytiotrophoblasts in the human placenta[20], regulates the relationship between maternal decidua and trophoblasts, and decreases trophoblast proliferation[17].

In this study, considering the potential role of cadherin 6 and 11 in the development of pre-eclampsia condition due to their impact on placental development, we investigated the change in serum cadherin 6 and 11 in pregnant women with preeclampsia and the relationship with disease severity.

Materials and Methods

Study Design

A cohort of pregnant women was enrolled in a case-control study between February 2018 and February 2019. The study was conducted within the guidelines of Helsinki. The patients were all informed about the investigation and conditions for participation at the beginning of enrollment, after the local institutional review board (Committee on Human Research) had approved the study, by grant number KAEK2020/4/2. Written, informed consent was obtained from the participants. All potential enrolled subjects underwent a preliminary screening during pregnancy, a routine obstetric evaluation, and a biochemical routine test for complete blood count and urinalysis. The gestational week was calculated according to the first day of the last menstrual period or the first-trimester ultrasound.

The study setup included a study cohort of patients with preeclampsia, aged between 17 and 44 years and between 26 and 38 gestational weeks of pregnancy, compared with a healthy cohort of pregnant women, matched for the same maternal and gestational age and from the same geographical area (as the control group). Patients with preeclampsia were further categorized as mild and severe, early and late onset preeclampsia according to the following criteria.

Mild preeclampsia was determined[1] when new onset of hypertension was measured twice, at least four hours apart: systolic blood pressure >140 mmHg or diastolic pressure >90 mmHg in a woman who was normotensive at <20 weeks of gestation. Urinary excretion of 300 mg in a 24-hour period or protein to creatinine ratio of 0.3 mg/dL was considered for diagnosis, and a dipstick reading of 1+ was used only if other quantitative methods were not immediately available at screening.

Severe preeclampsia was defined[1] based on additional signs and symptoms: 1) systolic blood pressure >160 mmHg or diastolic pressure >110 mmHg on two occasions at least four hours apart while the patient was on bed rest; 2) proteinuria of >5 g in a 24-hour period or 3+ on a urine dipstick; 3) hemolysis (peripheral blood smear or lactate dehydrogenase >480 U/L), elevated liver function (serum aspartate amino transferase >64 U/L or serum alanine amino transferase >80 U/L), thrombocytopenia (platelets <100.000), oliguria (<500 mL in 24 hours); 4) creatinine >1.1 mg/dL or a doubling of the serum creatinine concentration, with no known renal dysfunction or disease, cerebral or visual disturbances, or convulsions, with no history of seizure disorders. While early-onset pre-eclampsia was defined as pre-eclampsia occurring before 34 weeks, late-onset preeclampsia was defined as pre-eclampsia causing 34 weeks or latter.

Exclusion criteria for both enrolled groups were preexisting medical conditions, such as thyroid disorders, chronic hypertension, diabetes mellitus, multiple pregnancies, infection signs, and taking any medication.

Pregnant women whose gestational week was below 37 at delivery were administered two doses of betamethasone.

The following demographic, obstetric, and biochemical parameters were collected and compared for the pregnant women: age, gravida, parity, delivery type, alanine aminotransferase, aspartate aminotransferase, cadherin 6 and 11 levels, body mass index (BMI), birth weight of newborns, Apgar score at the fifth minute (min), and the need for a newborn intensive care unit (NICU).

Biochemical Analysis

Five milliliters of blood was obtained from the antecubital vein of the pregnant women at the time of application and centrifuged (Shimadzu UV160A, S. No: 28006648, Japan) at 3,000 x g for 10 min, and the sera were stored at -80 °C. On the evaluation day, the samples were melted at room temperature. All assays were conducted according to the manufacturer's instructions. The samples, which had a higher concentration, were diluted and measured in duplicate.

The concentrations of cadherin 6 serum were measured using a commercially available Human Cadherin 6 Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Bioassay Technology Laboratory, Cat No. E6937Hu, Shanghai, China). The concentrations of cadherin 11 in serum were measured, using a commercially available Human Cadherin 11 Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Bioassay Technology Laboratory, Cat No. E3272Hu, Shanghai, China). The enzymatic reactions were quantified in an automatic microplate photometer. The concentrations of cadherin 6 and 11 were determined by comparing the samples' optic density with the standard curve. All assays were conducted according to the instructions of the manufacturer. The mean inter-assay and intra-assay coefficients of variation percentage for cadherin 6 were <10% and <8%, respectively. The assay ranges of the kit are 10-2.000 ng/L. The sensitivity of the test is 4.93 ng/L for cadherin 6. The assay ranges of the kit are 0.05-20 ng/mL. The sensitivity of the test is 0.025 ng/mL for cadherin 11. The rest of the blood analyses were carried out within 2 h of blood sampling, using a hematology analyser (GEN-S; Beckman-Coulter Inc., USA).

Statistical Analysis

The sample size was calculated according to a medium effect size, 80% power, and a significance level of 0.05. IBM SPSS 21 software (SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) was used for statistical analysis. Descriptive statistical methods (median, frequency, percentage, minimum, maximum) were used to evaluate the study data. The suitability of quantitative data for normal distribution was tested by the Kolmogorov-Smirnov and Shapiro-Wilk tests and graphical evaluations. The Kruskal-Wallis test was used to compare three groups of non-normally distributed data Pearson chi-square and Yates chi-square tests were used to examine the relationship between cadherin 6, 11 and preeclampsia and control group. Diagnostic screening tests (sensitivity, specificity, positive predictive value, negative predictive value) and the receiver operating characteristic (ROC) curve analysis assessed the cadherin 6 and 11 cutoff. Significance was evaluated at the level of $p<0.05$.

Results

At the beginning of the study, 108 pregnant women with preeclampsia and 103 healthy pregnant women agreed to participate, but 14 pregnant women were excluded from the preeclampsia group (two women refused to participate, six had chronic hypertension, four had thyroid disorders, and two had signs of infection). Furthermore, eight pregnant women were excluded from the control group (three refused to participate, two were expecting twins, and three had gestational diabetes). Finally, 94 pregnant women with preeclampsia and 95 healthy pregnant women were included in the study (Figure 1).

The control, mild, and severe preeclampsia groups were similar in median maternal age (p=0.867), gravidity (p=0.150), and parity (p=0.173). The BMI values of the women with severe preeclampsia (p=0.003) and mild preeclampsia (p=0.004) were significantly higher than those of the controls, and the BMI values of women with severe and mild pre-eclampsia were similar (p=0.857).

The birth weight and Apgar score at the fifth min of the women with severe (p=0.001) and mild preeclampsia (p=0.001) were significantly lower than the controls. Also, the birth weight (p=0.001) and Apgar score at the fifth min (p=0.02) of the subjects with severe preeclampsia was significantly lower than those with mild pre-eclampsia.

The NICU admission (p=0.001) and cesarean section rates (p=0.001) of the women with severe preeclampsia were higher than those of the women with mild preeclampsia and the controls. While the CS rates of the women with mild preeclampsia were higher than those of the controls (p=0.001), the NICU admission rates were similar (p=0.165).

All maternal and neonatal characteristics according to the severity of preeclampsia are shown in Table 1.

The median serum cadherins 6 and 11 levels of the participants with severe preeclampsia were higher than those of the pregnant women with mild preeclampsia and the controls (p=0.001). The median serum cadherins 6 and 11 of pregnant women with mild preeclampsia were higher than those of controls (p=0.001). The median serum cadherins 6 and 11 levels of the study groups concerning the severity of preeclampsia are shown in Table 2.

As presented in Table 3, the median serum cadherin 6 and 11 levels of the women with early-onset pre-eclampsia (n=32) were higher than those of the controls and women with late-onset pre-eclampsia (n=62) (p=0.001). The median serum cadherins 6 and 11 of pregnant women with late-onset preeclampsia were also higher than those of the control subjects (p=0.001).

The ROC curve analysis of serum cadherin 6 and 11 data revealed that for preeclampsia prediction, with a cut-off value of more than 98.174 ng/mL, cadherin 6 had a sensitivity and specificity of 92% and 91.4%, respectively (p=0.001). It also showed that above 98.174 ng/mL, the risk of severe preeclampsia increased 121.71 fold. Moreover, for predicting preeclampsia, with a cut-off value of more than 1.92 ng/mL, cadherin 11 had a sensitivity and specificity of 86% and 88.5%, respectively (p=0.001), and above 1.92 ng/mL, severe pre-eclampsia risk increased 47.22 fold (Figure 2 and Figure 3).

Discussion

In this study, it was shown that serum cadherin 6 and 11 levels increased with the severity and onset of pre-eclampsia.

Both severe and early-onset preeclampsia caused a meaningful increase in the level of these biomarkers, and clinical and obstetric variables were in accordance with the clinical presentation of preeclampsia.

In placental development, cadherins play an important role[21], and it is therefore vital that experimental studies be conducted to examine the relationship between cadherin 6 and 11 and preeclampsia, as none currently exist. Cadherin 6 affects the prognosis of pregnancies, starting from the preimplantation period. In the endometrium, expression of cadherin 6 predominates in the follicular phase of the menstrual cycle, whereas expression of cadherin 11 predominates in the luteal phase and prepares the endometrium for implantation. However, if the cadherin 6 to 11 change cannot be achieved

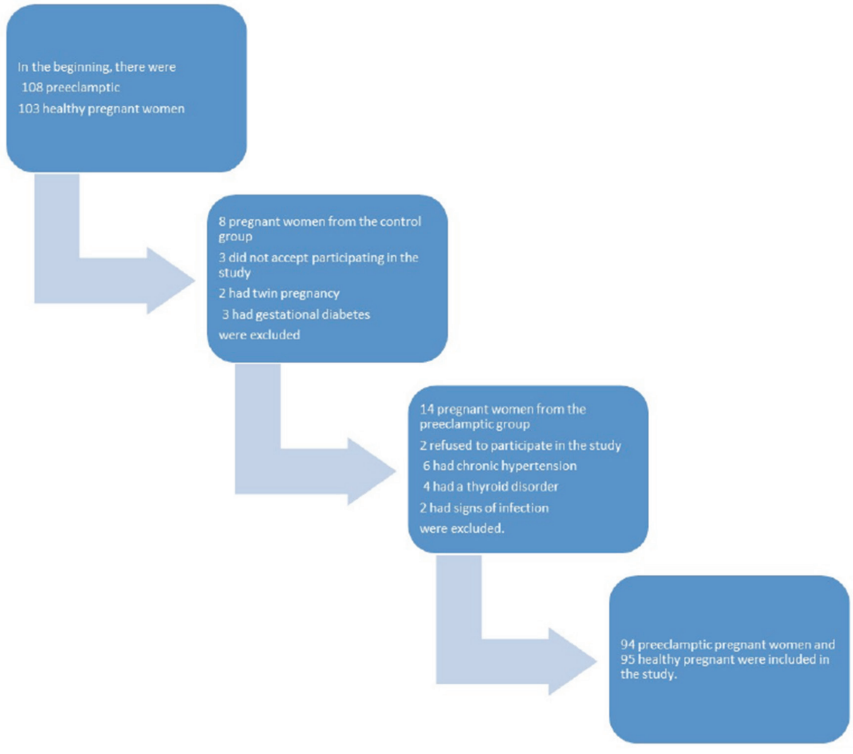

Figure 1. Study flowchart

and cadherin 6 continues to be expressed in high amounts, an accurate implantation cannot succeed and adverse pregnancy outcomes, such as miscarriage, preeclampsia, and intrauterine growth retardation, may occur[22].

In our study, we found that cadherin 6 levels were higher in pregnant women severe and early pre-eclampsia. Cadherin 6 is the dominant marker in invasive EVTs and regulates the communication between maternal blood vessels, myometrium, and EVTs. Furthermore, Dunne et al.[23] showed that cadherin 6 is expressed on the platelet surface and supports platelet adhesion, aggregation, and thrombus formation. Additionally, when the integrin αIIbβ3 receptor, which cadherin 6 is related, is blocked, platelet aggregation is inhibited[23]. Bouck et al.[24] showed in mice that cadherin 6 expression in

Table 1. Maternal and neonatal characteristics according to the severity of preeclampsia status

		Preeclampsia status			p
		Controls	Mild	Severe	
		(n=95)	(n=44)	(n=50)	
Age (year)	Min-Max (Median)	17 - 40 (27)	19 - 43 (26.5)	19 - 40 (28)	[a]**0.867**
BMI (kg/m²)	Min-Max (Median)	18 - 34.77 (23.12)	20 - 36.77 (23.885)	18.12 - 37 (25.165)	[a]**0.009***
Gravidity	Min-Max (Median)	1 - 7 (2)	1 - 5 (2)	1 - 6 (2.5)	[a]**0.150**
Parity	Min-Max (Median)	0 - 6 (1)	0 - 3 (1)	0 - 5 (1)	[a]**0.173**
Birth weight (gr)	Min-Max (Median)	1500 - 5200 (3200)	2040 - 4100 (2900)	950 - 4500 (1800)	[a]**=0.001***
Apgar score at 5th minute	Min-Max (Median)	7 - 9 (9)	0 - 9 (9)	4 - 9 (8)	[a]**=0.001***
NICU admission	**No**	88 (58.2%)	38 (25.16%)	25 (16.55%)	[b]**=0.001***
	Yes	7 (18.42%)	6 (15.78%)	25 (65.78%)	
Delivery type	**Vaginal**	94 (71.21%)	23 (17.42%)	15 (11.36%)	[b]**=0.001***
	Cesarean section	1 (1.75%)	21 (36.84%)	35 (61.40%)	

[a]Kruskal-Wallis H test, [b]Pearson ki-kare test, *p<0.05
NICU: Newborn Intensive Care Unit, BMI: Body mass index, Min: Minimum, Max: Maximum, Data are expressed as range (median) and number (line percentage) as appropriate

Table 2. Laboratory findings according to the severity of preeclampsia status

		Preeclampsia status			p
		Controls	Mild	Severe	
		(n=95)	(n=44)	(n=50)	
Cadherin 6	Min-Max (Median)	67.15 - 2553.4 (81.26)	73.44 - 146.7 (92.83)	81.24 - 2090.49 (202.75)	[a]**=0,001***
Cadherin 11	Min-Max (Median)	0.35 - 14.91 (0.99)	0.57 - 12.32 (1.45)	0.88 - 21.78 (3.51)	[a]**=0,001***

[a]Kruskal-Wallis H test, *p<0.05, Min: Minimum, Max: Maximum

Table 3. Laboratory findings according to onset of preeclampsia status

		Preeclampsia status			p
		Controls	Early onset preeclampsia	Late onset preeclampsia	
		(n=95)	(n=32)	(n=62)	
Cadherin 6	Min-Max (Median)	67.15 - 2553.4 (81.26)	81.24 - 2090.49 (265.63)	73.44 - 996.19 (94.03)	[a]=0,001*
Cadherin 11	Min-Max (Median)	0.35 - 14.91 (0.99)	0.88 - 21.78 (4.62)	0.57 - 19.46 (1.82)	[a]=0,001*

[a]Kruskal-Wallis H test, *p<0.05, Min: Minimum, Max: Maximum

platelets contributes to thrombus formation. Considering that preeclampsia causes widespread endothelial damage in many organs and atherosis development in the placental vessels[25], the prothrombotic state, due to high levels of cadherin 6, could contribute to the aggravation of the disease and the course of eclampsia. Also, MacCalman et al.[17] showed that the lack of cadherin 6 expression in invasive EVTs may result in excessive trophoblast invasion into the maternal tissue. Considering the opposite of this physiological situation, that is, when cadherin 6 is at a high level, an inadequate invasion may develop and pre-eclampsia may occur.

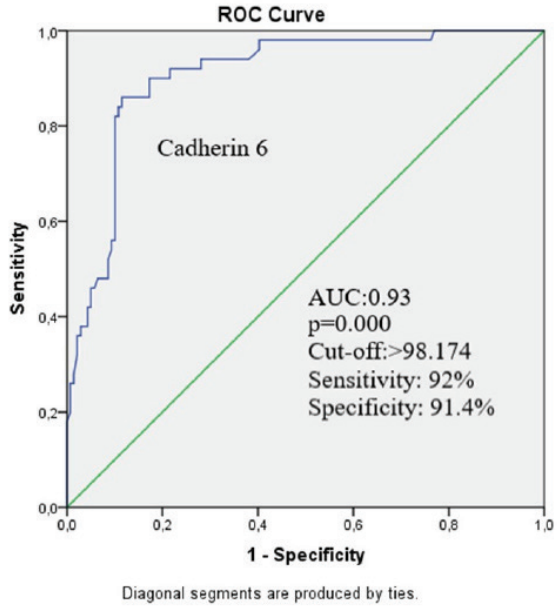

Figure 2. Receiver operating characteristic curve of serum cadherin 6 in women with severe preeclampsia

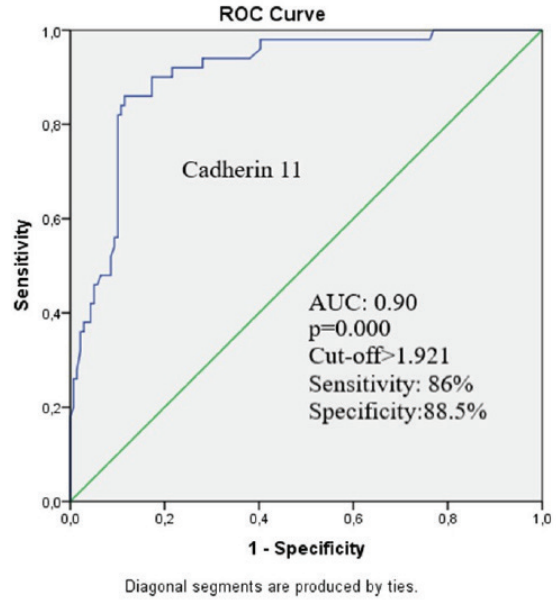

Figure 3. Receiver operating characteristic curve of serum cadherin 11 in women with severe preeclampsia

Cadherin 11 is responsible for the terminal transformation of cytotrophoblasts into syncytiotrophoblasts into the placenta, reducing cell proliferation[20]. The trophoblast assumes critical tasks in decidual development and early implantation by arranging the decidua and trophoblast communication through the growth factor ß-1 (TGFß-1). Finally, when placental development is completed, cadherin 11 expression decreases[17]. Garrido-Gomez et al.[26] evaluated the microenvironment during pregnancy in the culture medium and the decidualisation of the cells derived from placental tissue of pregnant women with severe preeclampsia and endometrial stromal cells of women with a history of severe preeclampsia. In the Gomez study, although the transcription profile was the same as the healthy control group, the cells of the patients with severe pre-eclampsia and a history of severe pre-eclampsia had undergone defective decidualization and cytotrophoblast invasion. While defective decidualization is the basis of preeclampsia, it can be prevented with preventative therapies.

Our results showed that the cadherin 11 level was higher in pregnant women with severe and early onset preeclampsia. There is some evidence in the literature that supports our outcome. Cadherin 11 induces the formation of large cellular aggregates and multinucleated cells from EVTs by inducting through TGFß-1 in cell culture medium[27]. Thus, when the cadherin 11 level is higher than it should be, uncontrolled cellular aggregates may affect placental perfusion. Also, it was shown that cadherin 11 is predominantly expressed in complete hydatidiform mole[20,28] and preeclampsia can be seen in the first trimester in hydatidiform mole. Similarly, the risk of preeclampsia in the second trimester in triploidy pregnancies is 35%[29]. It could be speculated that the relationship between preeclampsia risk and hydatidiform mole might be due to the cadherin 11 protein.

Study Limitations

The limitations of the authors' investigation are that the study was conducted in a single centre and the samples were taken from confirmed cases of pre-eclampsia, not randomly during pregnancy; and not just in the suspected cases of pre-eclampsia to confirm the progressive increase in biomarkers, depending on the severity of the disease. In future studies, to clarify the cause-effect relationship between preeclampsia and cadherin 6 and 11 level, changes of these markers at the tissue levels will be examined.

Conclusion

The authors detected a significant relationship between the increases in cadherins 6 and 11 with pre-eclampsia development, promoting both biomarkers as tools for the diagnosis and severity of the disease. These findings could contribute to the elucidation of the pathogenesis, screening, and diagnosis of preeclampsia, even if larger studies must determine the roles

of these cadherins during placentation and the course of pre-eclampsia.

Ethics

Ethics Committee Approval: The study was approved by the the local institutional review board by grant number KAEK2020/4/2 (Samsun Training and Research Hospital).
Informed Consent: Written informed consent was obtained from the participants.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Supervision: Ş.H., A.T., Concept: S.Ç., Ş.H., Design: H.G., C.S.Ç., Data Collection or Processing: C.S.Ç., S.Ç., Analysis or Interpretation: H.G., N.Y., B.A., Literature Search: N.Y., B.A., Writing: H.G., A.T.
Conflict of Interest: No conflict of interest was declared by the authors.
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Outcome in women undergoing uterine artery embolization for arterio-venous malformation diagnosed post-pregnancy-A retrospective study

Gebelik sonrası tanı konulan arteriyovenöz malformasyon nedeniyle uterin arter embolizasyonu uygulanan kadınlarda sonlanım-Retrospektif bir çalışma

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Abstract

Objective: To analyse the outcome of patients with symptomatic arterio-venous malformation (AVM), formed following pregnancy and managed by uterine artery embolization (UAE).

Materials and Methods: This retrospective study was conducted after ethical approval and included 15 patients presenting with abnormal uterine bleeding following pregnancy, who were suspected to have an AVM which later was confirmed by angiography and managed with UAE. Presenting symptoms, post-UAE complications and subsequent fertility outcomes were noted. Follow-up period ranged from 6 months to 2.5 years.

Results: The mean age was 28.4±3.82 years and mean parity was 1.3. Out of 15 cases, 9 (60%) presented after abortion, 4 (26.6%) after normal vaginal delivery and 2 (13.3%) after cesarean delivery; of these 10/15 (66.7%) patients had a history of curettage. The most common presenting symptom was continuous bleeding per-vaginum since the antecedent pregnancy in 9/15 (60%) patients and 6/15 (40%) patients had irregular bleeding. The mean duration of symptoms was 91±85.7 (30-360) days. For UAE, embolic agents used were polyvinyl alcohol (PVA) particles (300-500 µm) in 2 (13.3%), 30% glue injection in 3 (20%), the combination of PVA with glue injection in 4 (26.6%) and PVA with gelfoam in 6 (40%) patients. After UAE, bleeding responded within 3.6±0.97 (3-6) days in all but one patient who required repeat UAE one month later. All women resumed their normal menstrual cycle in 31.3±5.2 (24-42) days. Ten patients desired conception, of whom 5 (50%) conceived within 13.2±5.1 (6-19) months after UAE. Two women carried pregnancy to term, one underwent preterm cesarean for growth restriction with oligohydramnios. One patient had postpartum hemorrhage, which was managed medically. One had spontaneous abortion at 6 weeks gestation and the other is 13 weeks pregnant at present.

Conclusion: UAE is an effective treatment modality for the management of symptomatic post-pregnancy AVMs.

Keywords: Uterine artery embolization, arteriovenous malformation, post-pregnancy, outcomes

Öz

Amaç: Gebelikten sonra oluşan ve uterin arter embolizasyonu (UAE) ile tedavi edilen semptomatik arterio-venöz malformasyonlu (AVM) hastaların sonuçlarını analiz etmek.

Gereç ve Yöntemler: Bu retrospektif çalışma, etik onay alındıktan sonra yapıldı ve gebelik sonrası anormal uterin kanama ile başvuran, AVM'si olduğundan şüphelenilen ve daha sonra anjiyografi ile doğrulanan ve UAE ile tedavi edilen 15 hastayı içeriyordu. Başvuru semptomları, UAE sonrası komplikasyonlar ve sonraki doğurganlık sonuçları not edildi. Takip süresi 6 ay ile 2,5 yıl arasında değişmekteydi.

PRECIS: UAE for management of symptomatic AVMs following pregnancy is an effective treatment modality.

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Bulgular: Ortalama yaş 28,4±3,82 yıl ve ortalama parite 1,3 idi. On beş olgunun 9'u (%60) kürtaj, 4'ü (%26,6) normal vajinal doğum ve 2'si (%13,3) sezaryen sonrası başvurdu. On (%66,7) hastanın küretaj öyküsü vardı. En sık başvuru semptomu, 9/15 (%60) hastada önceki gebelikten beri sürekli vajinal kanama idi ve 6/15 (%40) hastada düzensiz kanama mevcuttu. Ortalama semptom süresi 91±85,7 (30-360) gündü. UAE için kullanılan embolik ajanlar, 2 hastada (%13,3) polivinil alkol (PVA) partikülleri (300-500 µm), 3 hastada (%20) %30 tutkal enjeksiyonu, 4 hastada (%26,6) PVA ile tutkal enjeksiyonu kombinasyonu ve 6 hastada (%40) PVA ile jel köpüğü kombinasyonu kullanıldı. BAE'den sonra, bir ay sonra UAE'nin tekrarlanması gereken bir hasta dışında tüm hastalarda kanama 3,6±0,97 (3-6) gün içinde durduruldu. Tüm kadınlar 31,3±5,2 (24-42) günde normal adet döngüsüne döndüler. UAE'den sonra 10 hasta gebe kalmak istedi ve 5 hasta (%50) 13,2±5,1 (6-19) ay içinde gebe kaldı. İki kadında terme kadar gebelik devam ederken, bir kadına oligohidramnios ve gelişme geriliği nedeniyle erken sezaryen uygulandı. Bir hastada medikal olarak tedavi edilen doğum sonu kanama vardı. Biri 6 haftalık gebeyken spontan düşük yaptı ve diğeri şu anda 13 haftalık hamiledir.

Sonuç: UAE gebelik sonrası semptomatik AVM'lerin yönetiminde etkili bir tedavi yöntemidir.

Anahtar Kelimeler: Uterin arter embolizasyonu, arteriyovenöz malformasyon, gebelik sonrası, sonlanım

Introduction

Uterine arterio-venous malformations (AVM) are rare, representing 1-2% of all genital hemorrhages[1]. AVMs are characterized by abnormal communications between arteries and veins in the same vicinity, which can be of varying sizes. Uterine AVMs may be congenital or acquired. Congenital AVMs are the result of defect in embryonic vascular differentiation[2] and usually present as pubertal heavy menstrual bleeding. Acquired uterine AVMs are usually traumatic, resulting from prior curettage, uterine surgery, or direct uterine trauma. Less commonly, these may also be associated with neoplasm, infection, endometriosis, diethylstilbestrol and intrauterine devices[3].

AVMs usually occur in women of reproductive age but have been reported up 72 years and are rare in nulliparous women. Patients mostly present with abnormal uterine bleeding (AUB) due to the disruption of thin walled abnormal vessels. Bleeding may be insidious or acute[3]; heavy, irregular, or scanty but prolonged. Patients with AVM formed post-pregnancy may present as postpartum hemorrhage or bleeding may ensue post-instrumentation of the gravid uterus following abortions[4,5]. Patients may also present with pain in the lower abdomen, anemia, and rarely congestive heart failure due to a large AV shunt. Approximately 30% AVM cases require blood transfusion[6].

Treatment options for uterine AVMs include expectant management in women with mild symptoms, medical management for symptomatic relief and surgical management in severe cases. Though hysterectomy is the definitive cure, uterine sparing unilateral or bilateral uterine artery ligation is also an option. In today's era, uterine artery embolization (UAE) is emerging as a promising conservative treatment due to its potency and safety[7,8]. However, the literature-regarding post-pregnancy AVM is limited. This retrospective study was conducted to evaluate the efficacy and safety of UAE for symptomatic uterine AVMs developing the following pregnancy.

Materials and Methods

The study was conducted in the Department of Obstetrics and Gynecology, All India Institute of Medical Sciences New Delhi India, a tertiary care hospital, from July 2017 to December 2019. The inclusion criteria were patients with angiography confirmed AVM who presented with AUB following a pregnancy and who were managed with UAE. Exclusion criteria were patients with no history of antecedent pregnancy before AVM diagnosis; patients in whom angiography report was not available and patients who were not managed with UAE.

After obtaining ethical approval from the Institute Ethics Committee (IEC-660/03.07.2020), data for these cases were retrieved from the hospital records. The patients were followed up telephonically after obtaining verbal consent. Patient's socio-demographic profile was noted. Detailed history, including presenting symptoms with duration, details of antecedent pregnancy and its outcome, obstetric history, previous menstrual history, investigations, ultrasonography and Doppler findings were extracted from case records.

All patients underwent UAE after discussion regarding their need and feasibility based on the severity of symptoms, failed medical management and ultrasound findings. Pelvic digital subtraction angiography (DSA) was performed in all patients at the time of UAE, which confirmed AVM. UAE was performed under local anesthesia with asepsis. The bilateral common femoral artery was punctured using an 18 gauge needle and a 6F sheath was placed inside the internal iliac artery under fluoroscopic guidance. Embolization was performed using polyvinyl alcohol (PVA) particles, 30% glue injection (Endocryl, Samarth Life Sciences Pvt. Ltd., India) or gelfoam (Spongostan, Ferrosan Medical Devices A/S, Søborg, Denmark), as per availability.

Procedural details such as the type of embolic agent used and intra-procedural difficulty or complications were noted. Post procedure imaging for vascularity and resolution of the lesion was performed after 24 hr. Time taken for the relief of symptoms was noted. The menstrual pattern and any subsequent pregnancy in women keen on conception were determined on telephonic interview. Follow-up period ranged from 6 months to 2.5 years.

Statistical Analysis

Data was analyzed using the statistical package STATA version 12.0 (Texas, USA). Descriptive statistics such as mean, standard deviation and range values were computed for all continuous variables.

Results

Total 15 women underwent UAE for AVM, which developed post-pregnancy, over a span of two years. The mean age and parity were 28.4±3.82 years and 1.3 respectively. Nine (60%) patients presented after abortion, including two 2nd trimester abortions, 4 (26.6%) patients presented after normal vaginal delivery and 2 (13.4%) after cesarean section. Symptoms started immediately the following pregnancy in 5/15 (33%) patients and there was no history of curettage. Rest 10 (67%) patients had symptoms following curettage performed for managing antecedent pregnancy complications. Details of patients included in the study are shown in Table 1 and Figure 1.

The mean hemoglobin at hospital admission was 8.9±1.97gm/dL. The most common presenting symptom was continuous bleeding per vaginum (BPV) since the antecedent pregnancy in 9/15 (60%) patients, 6/15 (40%) patients had irregular but heavy BPV and four of these required blood transfusions. The mean time interval since symptom onset and UAE was 91±85.7 (30-360) days (Table 1).

The embolic agents used for UAE were PVA particles (300-500 µm diameter) in 2 (13.3%) patients, 30% glue injection in 3 (20%) patients, the combination of PVA with glue injection in 4 (26.6%) and PVA with gelfoam in 6 (40%) patients, as per availability (Table 1). Bilateral UAE was successful in 14/15 (93.3%) patients; with complete symptomatic relief achieved in 3.6±0.97 (3-6) days (mean ± standard deviation, range). One patient required a repeat embolization due to persistent vascularity on Doppler and had symptomatic relief five days after the 2nd UAE. Hence, failure rate in the present study was 6.7%. Figure 2 and Figure 3 show DSA spot images and pre- and post-embolization ultrasound Doppler images, respectively.

Procedure related complications were seen in 3 (20%) patients. One patient developed a 2 cm hematoma at the femoral puncture site, which was managed conservatively by pressure bandage and resolved in three days. Two patients developed mild fever with lower abdominal pain and were managed with antipyretics and analgesics. None of the patients had any severe adverse event.

All patients resumed the menstrual cycle in 31.3±5.2 (24-42) days post-procedure. Five (33.3%) patients complained of hypomenorrhoea with mean 1.9±0.7 bleeding days. Three (20%) patients had an increased frequency of cycles (mean-20.6±2.5 days) compared to their previous cycles.

Ten out of 15 patients (66.7%) desired conception, of whom 5 (50%) conceived within 13.2±5.1 (6-19) months after UAE. Two patients carried pregnancy to term with no complications, one delivered vaginally and the other underwent cesarean for failed induction. The third patient had preterm cesarean at 34 weeks' gestation done for severe oligohydramnios with fetal growth restriction and had PPH, which was managed

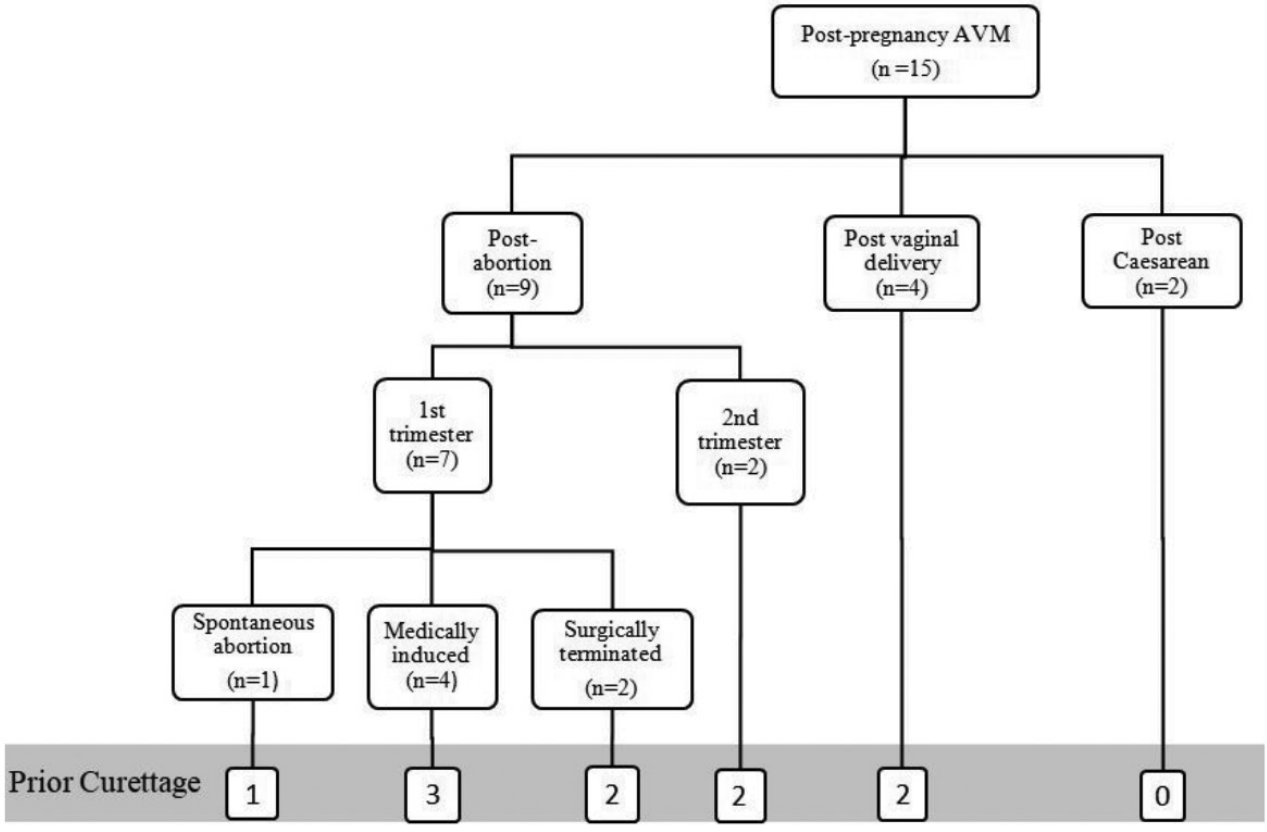

Figure 1. Showing antecedent pregnancies and history of curettage in women included in study

medically. One patient had spontaneous abortion at 6 weeks gestation and pregnancy is ongoing in another patient who is 13 weeks pregnant (Table 2). However, 3 patients who developed hypomenorrhoea tried for conception but none of them conceived with in follow-up duration.

Discussion

Surgical manipulations such as curettage lead to an increased immune response and angiogenesis, disturbing uterine physiology[9,10]. Peitsidis et al.[9] in a systematic review of 91 studies, reported acquired AVM after curettage in 95 of 103 patients with AVM. Obstetric association of AVM even without prior curettage is also reported; proposed mechanism being aberrant regression of the placental bed or abnormal vascular communication after chorionic villi necrosis[5]. In a retrospective study by Kim et al.[1], of 19 patients who developed AVM following delivery, approximately a quarter of patients had no history of curettage, which was almost similar to this study, with about 30% patients lacking prior curettage; though in Vilos et al.'s[10] case-series of five patients 60% did not have prior curettage.

In the past, AVMs have been diagnosed incidentally on histopathology after hysterectomy performed for heavy

Table 1. Overview of patients developing AVM following pregnancy and managed with UAE

Case	Age (in years)	Parity	Antecedent pregnancy	Symptoms duration at presentation (In days)[1]	Clinical presentation	Medical treatment prior to UAE	Embolic agent	Pregnancy	Interval between UAE and conception (months)
1	33	P3L4A1	Abortion	30	Continuous BPV	TA	PVA	Not desired	
2	27	A3	Abortion	60	Irregular&HMB	TA	Glue	Conceived[2] (Caesarean)	19
3	22	P1l1A1	FTNVD	180	Irregular & HMB	TA	Glue	Not conceived	
4	27	P1L1A2	Abortion	96	Continuous BPV	TA& OCP	PVA and glue	Conceived (Caesarean)	17
5	32	P2L2A1	Abortion	120	Continuous BPV	TA	PVA and glue	Not desired	
6	30	P3L3	FTLSCS	90	Irregular &HMB	OCP	PVA	Not desired	
7	28	P1L1A1	Abortion	45	Continuous BPV	TA	Glue	Conceived (VD)	13
8	30	PLL1A3	Abortion	360	Irregular &HMB	TA	PVA and glue	Not conceived	
9	24	P1L0A1	Abortion	60	Continuous BPV	TA	PVA and glue	Conceived[3] (abortion at 8 week)	11
10	31	P1L1A1	Abortion	50	Continuous BPV	TA	PVA and Gelfoam	Not conceived	
11	27	P1L1	FTNVD	54	Continuous BPV	TA& OCP's	PVA and Gelfoam	Conceived (ongoing pregnancy-13 weeks)	6
12	25	P1L1	FTNVD	30	Continuous BPV	TA	PVA and Gelfoam	Not conceived	
13	26	P1L1A2	PTVD	30	irregular & HMB	OCP's	PVA and Gelfoam	Not conceived	
14	28	P1L2	PTLSCS	40	Continuous BPV	TA	PVA and Gelfoam	Not desired	
15	37	P2L2A1	Abortion	120	irregular & HMB	TA	PVA and Gelfoam	Not desired	

[1]: Onset of symptoms was immediately following pregnancy in 5/15 (33%) patients without history of curettage. Rest 10 (67%) patients had onset of symptoms following curettage done for the management of antecedent pregnancy complications.
[2]: This patient underwent repeat UAE 48 hours after first UAE due to persistent bleeding and persistent vascularity on Doppler.
[3]: This patient developed a 2 cm hematoma.
TA: Tranexamic acid, VD: Vaginal delivery, HMB: Heavy menstrual bleeding, PVA: Polyvinyl alcohol, UAE: Uterine artery embolization, AVM: Arterio-venous malformation

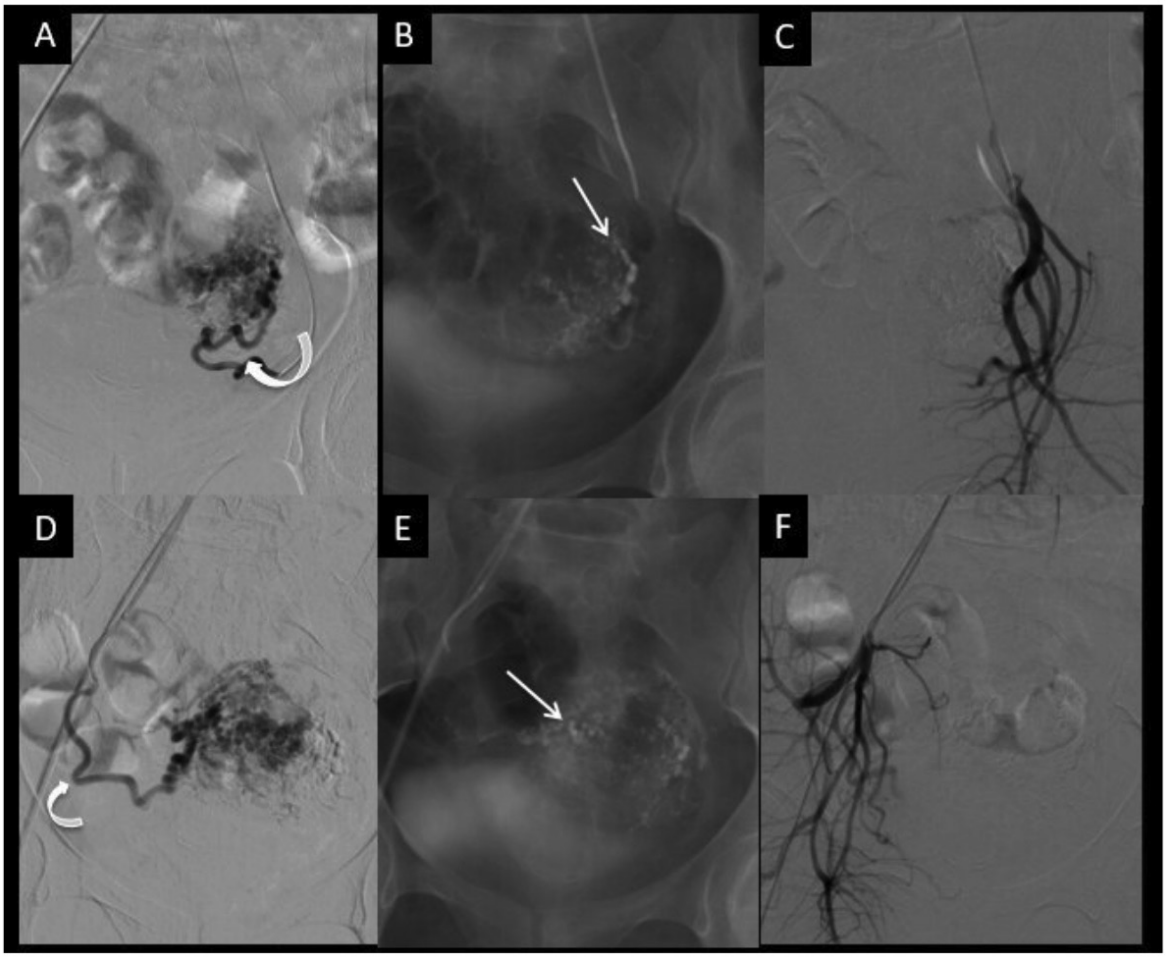

Figure 2. Diagnostic subtraction angiography (DSA) spot images of UAE: A & D showing bilateral hypertrophied uterine arteries supplying the nidus of uterine AVM (curved arrow); B & E showing vessels being embolized sequentially using glue mixed with lipiodol (arrows); C &F showing post embolization angiograms showing non filling of the uterine AVM suggestive of successful embolization

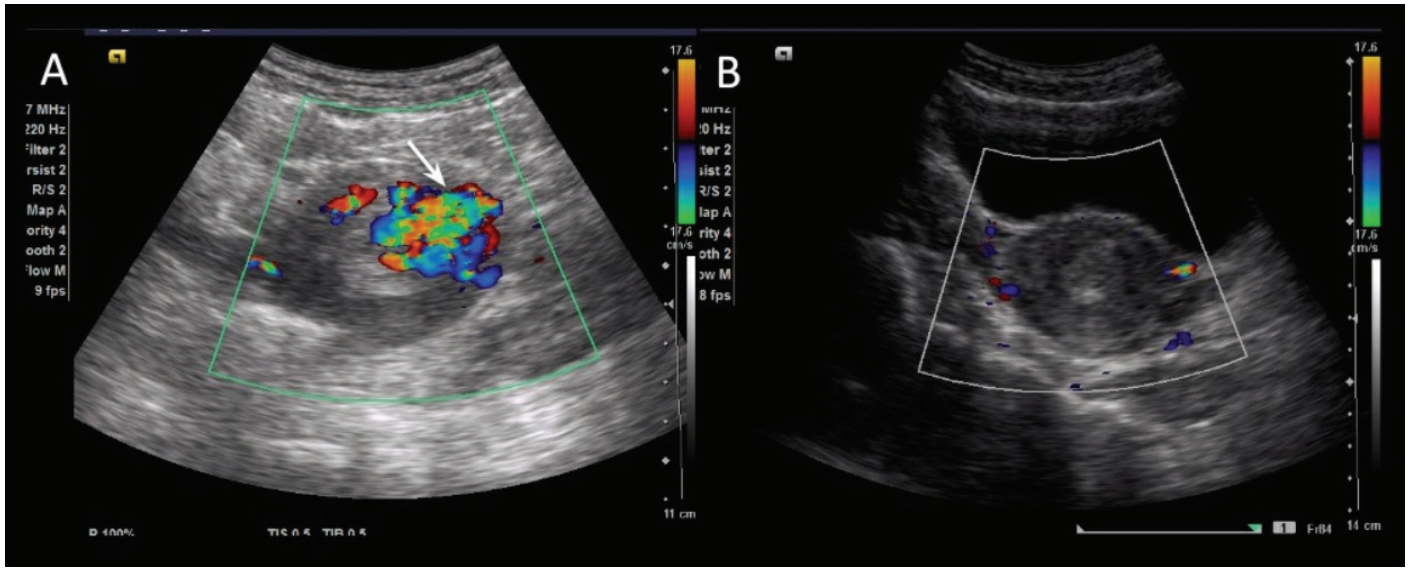

Figure 3. (A) Pre-embolization ultrasound Doppler image **(A)** showing bunch of vascular channels within the uterine wall (arrow) suggestive of uterine AVM. **(B)** Post embolization Doppler image **(B)** showing complete obliteration of nidus of AVM

Table 2. Pregnancy outcomes in patients of AVM who conceived after UAE

Pregnancy	Total n=5	Complication	Particle used for UAE	Interval between UAE & conception(months)
Full term delivery **Total** Caesarean Vaginal	 2 1 1		 PVA and glue Glue injection	 17 13
Prterm Caesarean	1 1	Severe oligohydramnios, FGR, PPH	 Glue injection	 19
Abortion	1	Spontaneous abortion at 6 weeks	PVA and glue	11
Ongoing	1 (at 13weeks gestation)		PVA and gel foam	6

UAE: Uterine artery embolization, FGR: Fetal growth restriction, PPH: Postpartum haemorrhage, AVM: Arterio-venous malformation

bleeding[9]. The advent of imaging modalities such as colour-Doppler ultrasonography, magnetic resonance imaging, computed tomography and pelvic angiography has made diagnosis easy and early. Pelvic angiography remains the gold standard for diagnosing AVM[11], though not used routinely as ultrasonography with color Doppler has good detection[6]. In the present study, all cases could be diagnosed with Doppler ultrasound, and confirmed with angiography at the time of UAE.

Treatment options for post-pregnancy AVM remain the same as AVM of other etiologies and include medical treatment, uterine sparing UAE or hysterectomy, which is the definitive treatment. Forssman et al.[12] in 1982 reported the first conservative treatment of uterine arteriovenous aneurysm which was occluded at laparotomy by introducing gelfoam into the uterine artery. In a review including a hundred women with iatrogenic AVM after diagnostic curettage and presenting with acute abnormal uterine bleeding, 59% patients underwent UAE, 29% had hysterectomy, 6% responded to methylergometrine, and 6% had a spontaneous resolution[9]. All patients in our study were managed conservatively with UAE and only one patient required the second session of embolization, which is comparable to 5.3% (1/19) reported by Kim et al.[1], though 60% (3/5) patients required repeated embolization in the study by Vilos et al.[10].

The embolic agent most commonly used in patients desiring fertility is gelfoam because of its temporary nature[10,13], but no difference is reported in clinical outcome with other embolic agents[14,9].

Reported complications of the procedure are puncture site superficial hematoma in 0.6-14.8%, uterine artery rupture during manipulation, contrast allergy, adult respiratory distress syndrome and femoral artery hematoma, pseudoaneurysm and arterio-venous fistula[1,13,15]. In present study, one patient had puncture site hematoma which was managed with compression.

There is concern of diminished ovarian function and consequent amenorrhea and subfertility post-UAE. However, in Peitsidis' review, most patients resumed normal menstruation within two months and none developed amenorrhea[9]. As in our series, evidence also suggests that the ovarian function might not be affected or, if affected, recovers in young patients undergoing UAE[16]. Fifty percent (5/10) of our patients desiring pregnancy conceived spontaneously within two years of UAE and only one of them had placental insufficiency requiring pre-term delivery. Pregnancy in other patients were uneventful. Two patients were delivered by cesarean due to obstetric indications and other delivered vaginally. Peitsidis et al.[9] reported a pregnancy rate of up to 29% following UAE for AVM with 15 months as mean time to conceive, whereas up to 50% pregnancy rate was reported by Delplanque et al.[8] within mean 38 months from UAE who also studied peak systolic velocity with the success of UAE in AVMs. Post-UAE pregnancy outcomes in a meta-analysis of 227 pregnancies conceived after UAE performed for leiomyoma showed 35.2% risk of abortion, which was three fold higher than controls; 66% caesarean rate; with no increase in preterm delivery rate[17].

In this study, UAE was successful in the symptomatic management of all fifteen patients presenting with post-pregnancy AVM with no significant complications, and half of the woman desiring pregnancy conceived. Hence, UAE can be offered to young patients with post-pregnancy AVM, though the retrospective nature and small sample size are the limitations of this study.

Conclusion

UAE is an effective and safe option for managing symptomatic AVMs developing post-pregnancy in women of reproductive age.

Ethics

Ethics Committee Approval: After obtaining ethical approval from the Institute Ethics Committee (IEC-660/03.07.2020), data for these cases were retrieved from the hospital records.
Informed Consent: Retrospective study.
Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: V.K., Sw.S., J.M., S.G., S.S., N.S., S.K., V.D., Design: V.K., Sw.S., J.M., S.G., S.S., N.S., S.K., V.D., Data Collection or Processing: V.K., Sw.S., J.M., S.G., S.S., N.S., S.K., V.D.,

Analysis or Interpretation: V.K., Sw.S., J.M., S.G., S.S., N.S., S.K., V.D., Literature Search: V.K., Sw.S., J.M., S.G., S.S., N.S., S.K., V.D., Writing: V.K., Sw.S., J.M., S.G., S.S., N.S., S.K., V.D.

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Obstetric hemorrhage and surgical emergencies training workshops on fresh cadavers and simulators result in high application in daily practices and decreased patient referral

Taze kadavralar ve simülatörler üzerinde obstetrik kanama ve cerrahi acil durumlar eğitim atölyeleri, günlük pratikte yüksek uygulama ile sonuçlanır ve hasta sevkini azaltır

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Abstract

Objective: In this study, we aimed to evaluate the effects of obstetric emergencies workshops combining theoretical lectures, simulation trainers and fresh cadaver models on daily practices and referrals of obstetrics and gynecology specialists.

Materials and Methods: This is a prospective observational study involving obstetrics and gynecology specialists attending the Turkish Society of Obstetrics and Gynecology endorsed obstetrics hemorrhage management courses held in 2018 and 2019. The training course is an annually organized two-day course, one day allotted to theoretical lectures and the other day to practical training on both simulators and fresh cadavers. Trainees participating in the course was surveyed with an anonymous questionnaire on their motivation to attend the course and their juridical history of obstetric patient management. Attendees were asked to disclose whether they had ever independently performed the procedures stated in the questionnaire or not, before commencing the course and 1 year after attending the course.

Results: Among the attendees 22% (n=32) had at least one obstetrical malpractice lawsuit and 36.1% (n=52) were accused of Health Care Complaints Comission. The main motivation of the attendees for taking this course was Professional development followed by reducing maternal mortality and morbidity and avoiding malpractice. One year after the course, attendees applying uterine devascularization surgery increased by 36.1% (52/144), uterine compression sutures increased by 52.7% (76/144), and ability to apply uterine balloon tamponade increased by 59.7% (86/144). Besides improvement in other obstetric surgical skills an indirect effect seen that the attandees operated on high-risk cases increased in placenta previa (15.3%), placenta acreata spectrum (30.5%), operative deliveries (27.7%), peripartum hysterectomy (24.9%) and relaparotomy for postpartum hemorrhage (34.7%).

Conclusion: Opportunities of attending well organized fresh cadaveric workshops on managing postpartum hemorrhage and other obstetric surgeries, can quickly adapt to daily practice, restore the professional confidence of obstetric and gynecology specialists, and eventually decrease patient referral.

Keywords: Obstetric emergencies, cadaver course, obstetric emergency training

PRECIS: Obstetric hemorrhage and surgical emergencies training workshops.

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Öz

Amaç: Bu çalışmada teorik dersler, simülasyon eğitmenleri ve taze kadavra modellerini bir araya getiren obstetrik acil durum çalıştaylarının kadın hastalıkları ve doğum uzmanlarının günlük uygulamalarına ve sevklerine etkisini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Türk Kadın Hastalıkları ve Doğum Derneği onaylı obstetrik kanama yönetimi kurslarına devam eden kadın doğum ve kadın hastalıkları uzmanlarının 2018 ve 2019 yıllarında katıldığı prospektif gözlemsel bir çalışmadır. Eğitim kursu, yılda bir kez düzenlenen iki günlük bir kurs olup, bir gün teorik derslere, diğer gün ise hem simülatörler hem de taze kadavralar üzerinde uygulamalı eğitime ayrıldı. Kursa katılan kursiyerlere kursa katılma motivasyonları ve obstetrik hasta yönetimi ile ilgili hukuki geçmişleri hakkında isimsiz bir anket uygulandı. Katılımcılardan kursa başlamadan önce ve kursa katıldıktan 1 yıl sonra ankette belirtilen işlemleri bağımsız olarak yapıp yapmadıkları soruldu.

Bulgular: Katılımcıların %22'si (n=32) en az bir obstetrik malpraktis davasına sahip ve %36,1'i (n=52) Sağlık Şikayet Komisyonu'na şikayet edilmişti. Katılımcıların bu eğitimi almalarının ana motivasyonu, mesleki gelişim, ardından anne ölüm ve hastalık oranlarını azaltmak ve malpraktisten kaçınmaktı. Kurstan bir yıl sonra uterus devaskülarizasyon cerrahisi uygulayanların oranı %36,1 (52/144), uterus kompresyon sütürleri %52,7 (76/144) ve uterus balon tamponadı uygulama yeteneği %59,7 (86/144) arttı. Diğer obstetrik cerrahi becerilerindeki gelişmelerin yanı sıra yüksek riskli olgularda ameliyata girenlerde dolaylı bir etki görüldü; plasenta previa (%15,3), plasenta akreata spektrumu (%30,5), operatif doğumlar (%27,7), peripartum histerektomi (%24,9), doğum sonu kanama için relaparotomi (%34,7).

Sonuç: Doğum sonu kanama ve diğer obstetrik ameliyatları yönetme konusunda iyi organize edilmiş taze kadavra atölyelerine katılma fırsatları, günlük uygulamaya hızla adapte edilir, doğum ve jinekoloji uzmanının profesyonel güvenini geri kazandırır ve sonunda hasta sevkini azaltır.

Anahtar Kelimeler: Obstetrik aciller, kadavra kursu, obstetrik aciller eğitimi

Introduction

Obstetrical emergencies are a paramount concern in obstetricians' daily practice. The majority of the severe obstetric emergencies occur in otherwise healthy low-risk term pregnancies and complicate approximately 15% of deliveries[1]. Peripartum hemorrhage, one of the most prominent of these obstetric emergencies, is the leading cause of maternal mortality in developing countries and is responsible for 27% of maternal deaths worldwide[2]. The management of obstetric emergencies requires an organized team work that competency of the obstetrician plays the role of a keystone.

The ultimate goal of surgical training is to render physician capable of performing safe independent practice. Nevertheless, the lack of uniformity in obstetrics and gynecology training has the potential to induce undesirable consequences[3]. Standardization and quality perfection in obstetrics and gynecology training is a widespread concern and efforts to achieve these goals are ongoing[4]. However, substantial variations in the complexity and educational value of operating room cases as well as the experience of individual mentors, place additional difficulties in standardization of training. Therefore, despite all attempts at standardizing and improving the quality of residency training, surgical training could still be seen as a raffle of obtaining adequate tuition from competent instructors in good surgical cases[5].

Obstetrics is one of the most risky fields in medicine regarding the severity of patient outcomes and related ramifications[6]. Unsatisfactory obstetrics and gynecology training, displays its effects on the daily practicesof postgraduate surgeons, curbs professional confidence, engenders circumstances that would end up with litigations against obstetricians and most importantly worsens patient outcomes. Previously, cadaver simulation models have been proposed as a solutions to overcome these problems in various fields of surgery[7-9]. Nevertheless data on the efficiency of cadaver simulation training in obstetrical emergencies are lacking.

In this study, we aimed to evaluate the effects of obstetric hemorrhage and surgical emergencies workshops combining theoretical lectures, simulation trainers and fresh cadaver models on the daily practices of obstetrics and gynecology specialists.

Materials and Methods

This is a prospective observational study involving obstetrics and gynecology specialists attending the Turkish Society of Obstetrics and Gynecology endorsed obstetrics hemorrhage management courses held in 2018 and 2019. The training course is an annually organized two-day course, one day is allotted to theoretical lectures and video presentations, the other day to practical training on both simulators and fresh cadavers. One trainee and six attendees were assigned to each cadaver and simulator along with one instructor for each procedure. list of the theoretical and video training procedures and topics were as follows: Surgical and medical management of postpartum hemorrhage, uterine devascularization techniques such as uterine artery ligation, utero-ovarian artery ligation, hypogastric artery ligation, uterine compression sutures, episiotomy, 3rd and 4th degree perineal lacerations, upper 1/3 vaginal tear repair, vaginal hematoma management, postpartum hysterectomy, management of uterine rupture, postpartum re-laparotomy and abdominal packing, and management of bladder injuries.

Practical courses on day two included pelvic anatomy, uterine devascularization surgery such as uterine artery ligation, utero-ovarian artery ligation, hypogastric artery ligation, abdominal packing, uterine compression sutures, perineal laceration repair, upper vaginal anatomy and tear repair, uterine balloon tamponade application on simulators. Trainees were surveyed with an anonymous questionnaire before the course. Attendees

were asked to disclose whether they had ever independently performed the procedures stated in the questionnaire or not. Attendees' age, years in practice, obstetric examinations performed per day, deliveries performed per month, medico-legal histories and motivation to enroll the course were also surveyed in the questionnaire. Motivation of attendance of participants was evaluated with a visual analog scale (VAS). Participants were asked to mark the number extending from 0 to 10 for each statement in the VAS scale that fits themselves most as 10 represents a statement is fully compliant with attendees' motivation and 0 represents no compliance. Participants were asked to complete the questionnaire before commencing the course and 1 year after attending the course. In this study, we included data obtained from two - year courses, held on 7-8 July 2018 and 5-6 July 2019 in Istanbul.

Statistical Analysis

Statistical analyzes were performed using IBM SPSS v20. The distribution of data was assessed by Shapiro-Wilks test. Mean ± standard deviations were calculated for normally distributed data and median (minimum-maximum) were calculated for non-normally distributed data. Differences between pre-course and post-course answers in the questionnaires were evaluated with McNemar's test. P-value <0.05 is considered significant.

Results

A total of 144 obstetrics and gynecology specialists, with an independent working experience of at least 1 year, attended the course. Mean age of the attendees was 38.6±6.5 years. The number of mean daily obstetric examinations performed by attendees was 36.8±24.6 and mean monthly deliveries were found as 51.2±59.2. Judicial history of attendees revealed 22.2% (32/144) of them were trialed for obstetrical malpractice at least once in their independent practices. Whilst 18 of them (56.2% of trialed attendees) were acquitted, 14 (43.2% of trialed and 9.7% of all attendees) of them were convicted and imposed judicial punishments, within convicted attendees, 12 of they imposed a fine (8.3% of all attendees) and 2 of them (1.3% of all attendees) were administered a suspended sentence. Demographic characteristics and judicial histories of attendees are given in Table 1. VAS scale assessment revealed that the leading motivation of surgeons in attending the course was professional development, followed by reducing maternal mortality and morbidity and avoiding malpractice (Mean VAS scores: 9.3±1.8; 8.4±3.1; 7.3±3.7 respectively). Evaluation of the motivations of participants in attending the workshop is shown in Table 2.

Table 1. Demographic characteristics and judicial history regarding medical practice of attendees at the commencement of workshop

Total number of attendees	144
Meanage (years)	38.6±6.5 (28-54)
Mean working experience as obs/gyn specialist (years)	6.4±6.4 (1-21)
Mean number of daily obstetric examinations	36.8±24.6 (3-120)
Mean number of monthly deliveries	51.2±59.2 (5-300)
Institution of past residency training	
University Hospital n (%)	74 (51.4)
Health Ministry Training and Research Hospital n (%)	70 (48.6)
Attendees with a history of obstetrical malpractice lawsuit (n, %)	32 (22.2)
Acquitted n (%)	18 (56.2)
Judicial punishments n (%)	14 (43.7)
Judicial fine sentences	12
Suspended sentences	2
Accusations to Health Care Complaints Comissionn (%)	52 (36.1)
Acquitted	32 (61.5)
Condemned for accusations n (%)	20 (38.5)

Table 2. Evaluation of motivation of surgeons to attend to workshop with VAS scale

Motivation of attending the course; professional development (VAS score 0-10)	9.3±1.8
Motivation of attending the course; avoiding malpractice (VAS score 0-10)	7.3±3.7
Motivation of attending the course; reducing maternal mortality and morbidity (VAS score 0-10)	8.4±3.1

VAS: Visual analog scale

The rate of attendees independently performing questioned procedures before and one year after the workshop are summarized in Table 3. As most of the questioned procedures were lifesaving in nature and performed for emergent situations that are nearly impossible to manage without conducting them, attendees who never performed procedures were asked their ways of coping with these emergent occurrences in their daily practice. These attendees described their ways of dealing with emergent circumstances as refraining from risky patients, transferring them to tertiary centers and calling the assistance of a more experienced colleague when surgical measures are in escapably required as their limited surgical inventories usually fall short. One year after the course, number of attendees that independently conducted procedures was found to be significantly higher for every surgical intervention introduced in the workshop compared to the commencement of the course. One year after the course, attendees applying uterine devascularization surgery increased by 36.1% (52/144), uterine compression sutures increased by 52.7% (76/144), and the ability to apply uterine balloon tamponade increased by 59.7% (86/144). The third and fourth degree perineal tear repairing specialist increased by 23.6 and 33.3% respectively. Abdominal packing increased by 55.5%, upper vaginal laceration repair by 12%, Schirodkar cerclage by 45.8%, Mc Donald cerclage by 20.8% and urinary bladder injury repair by 26.4%.

Besides improvement in other obstetric surgical skills an indirect effect seen that the on high-risk cases increased in placenta previa (15.3%), placenta acreata spectrum (30.5%), operative deliveries (27.7%), peripartum hysterectomy (24.9%) and relaparotomy for postpartum hemorrhage (34.7%). A comparison of the management of other obstetric emergency interventions not included in the practical course one year before and after the course are presented in Table 4. The number of trainees that performed uterine rupture repair increased by 38.9%, vaginal hematom adrian age by 16.6%.

Discussion

Emerging studies have demonstrated that training courses of obstetric emergencies are improving health outcomes[10]. Simulation is recommended as a method of training to provide competency to professionals, to increase the quality ofcare and to reduce mortality and morbidity associated with adverse obstetric and gynecological circumstances[11]. Simulation practice with trainers is shown to improve patient outcomes in obstetric emergencies like postpartum

Table 3. The main procedures applied on cadaveres and simulators. Comparison of rate of attendees that never independently performed procedures before and one year after cadaveric courses

	Trainees that never performed the procedure before the course n (%)	Trainees that never performed the procedure 1 year after the course n (%)	Delta % change	p-value
Uterine artery ligation	68 (47.2)	16 (11.1)	-36.1	<0.001
Utero-ovarian artery ligation	80 (55.6)	22 (15.3)	-40.3	<0.001
Hypogastric artery ligation	86 (59.7)	50 (34.7)	-25	<0.001
Any devascularisation surgery	68 (47.2)	16 (11.1)	-36.1	<0.001
B-lynch suture	88 (61)	24 (16.7)	-44.3	<0.001
Other compression sutures	106 (73.6)	46 (32)	-41.6	<0.001
Any uterine compression suture	88 (61)	12 (8.3)	-52.7	<0.001
Uterine tamponade with Bakri balloon	86 (59.7)	30 (20.8)	-38.9	<0.001
Uterine tamponade with sengstaken-blakemore tube	140 (92.7)	50 (34.7)	-58	<0.001
Uterine tamponade with foley catheter	86 (59.7)	24 (16.7)	-43	<0.001
Any uterine tamponade	86 (59.7)	0	-59.7	<0.001
Abdominal packing	122 (84.7)	42 (29.2)	-55.5	<0.001
3rd degree tear repair	40 (27.8)	6 (4.2)	-23.6	<0.001
4th degree tear repair	86 (59.7)	38 (26.4)	-33.3	<0.001
Repair of lacerations in 1/3 upper vagina	24 (16.7)	6 (4.2)	-12.5	<0.001
Mc Donald cerclage	52 (36.1)	22 (15.3)	-20.8	<0.001
Schirodkar cerclage	118 (81.9)	52 (36.1)	-45.8	<0.001
Repair of bladder injury	54 (37.5)	16 (11.1)	-26.4	<0.001

Table 4. Comparison of other obstetric emergency interventions not included in the practial course. Comparison of rate of attendees that never independently performed procedures before and one year after cadaveric courses

	Trainees that never performed the procedure before the course n (%)	Trainees that never performed the procedure 1 year after the course n (%)	Delta % change	p-value
Placenta previa C/S	36 (25)	14 (9.7)	-15.3	<0.001
Placenta invasion anomaly C/S	72 (50)	28 (19.5)	-30.5	<0.001
Vacuum extraction	46 (31.9)	26 (4.2)	-27.7	=0.001
Forceps delivery	112 (77.8)	74 (51.4)	-26.4	<0.001
Re-laparotomy for postpartum hemorrhage	68 (47.2)	18 (12.5)	-34.7	<0.001
Peripartum hysterectomy	46 (31.9)	10 (7)	-24.9	<0.001
Repair of uterine rupture	70 (48.6)	14 (9.7)	-38.9	<0.001
Vaginal hematoma drainage	28 (19.4)	4 (2.8)	-16.6	<0.001

hemorrhage, perimortem cesarean section, shoulder dystocia and umbilical cord prolapse[11]. Cadaver simulation models have demonstrated to increase the competency of trainees in the fields of general surgery, urology, gynecology and vascular surgery in previous studies[12-14]. A recent study by Soler-Silva et al.[8] described the thiel cadaver simulation model as the most realistic surgical simulation and found thiel cadavers superior to any other simulation model in terms of précising surgical skills and enhancing trainees' confidence. Although obstetric emergencies, particularly the peripartum hemorrhages, are the most problematic scenarios of daily obstetric practice, information regarding the efficacy of courses including cadaver simulation models for obstetrics emergencies is lacking.

In our study the rate of surgeons independently performing critical interventions is significantly increased for all the procedures introduced in the workshop. Courses including cadaver simulation models are shown to be effective in improving daily practices of obstetricians regarding obstetric emergencies, particularly in the management of peripartum hemorrhages.

Hypogastric artery ligation is a life-saving and fertility preserving procedure used in controlling peripartum hemorrhages and it is advised to be taught to all pelvic surgeons[15]. Owing to close anatomical relationships of the internal iliac artery with some important structures, serious complications may arise in the hands of incompetent surgeons. Despite its value, ligating the internal iliac artery is a skill that are not usually acquired in residency training mostly due to the infrequency of cases that require hypogastric artery ligation. Recently, Mahale et al.[16] demonstrated the effectiveness of cadaver simulations on practicing hypogastric artery ligation and gaining the necessary skills to perform the procedure. Supporting their findings, we found that a significantly higher number of attendees were performing the procedure 1 year after the workshop along with other measures against postpartum hemorrhage. Therefore, fresh cadaver models combined with theoretical lectures and simulators appear effective in excelling on procedures where only assisting a mentor is inadequate due to the low number of cases and hands on training is necessary, such as hypogastric artery ligation.

Studies indicated declining rates of vaginal deliveries and forceps deliveries and vacuum extractions in the last two decades[17]. Availability of better techniques increased legal liabilities, or lack of technical proficiency of obstetricians may all contribution to this trend. In spite of the advances in obstetric techniques, there are still cases in which operative delivery is preferable over cesarean section. However expertise of forceps delivery is diminishing in educational institutions. A low volume of patients requiring a forceps delivery is described as one of the major obstacles to transferring these skills from mentors to trainees[18]. Although contemporarily most obstetricians prefer vacuum extraction over forceps in operative deliveries due to ease of use, the application of vacuum extraction also requires competent training. In our study, we found that a significantly higher number of attendees started to apply forceps deliveries and vacuum extractions one year after the course although operative deliveries were not part of the course. This increase in the willingness to perform operative deliveries can be explained by the surgeons increased ability to manage hemorrhage, vaginal tears and perineal tears after the course, which are common complications of obstetric deliveries and are included in the course program.

As mentioned in the introduction section, the incompetency of the surgical skills of obstetricians could be caused by cumulative effects of multiple factors extending through their residency training periods. As recommended in a collaborative publication named "Quality Patient Care on Laborand Delivery: A Call to Action," simulation models could be incorporated into obstetrics and gynecology residency training to ameliorate the educational deficiencies of residents[19].

Conclusion

Opportunities of attending well organized fresh cadaveric workshops on managing postpartum hemorrhage and other obstetric surgeries, can quickly adapt to daily practice, restore the professional confidence of obstetric and gynecology specialists, and eventually decrease patient referral.

Ethics

Ethics Committee Approval: The study was approved by the Alanya Alaaddin Keykubat University Ethics Committee (approval number: 12, date: 25.05.2022).
Informed Consent: Written consent from all participants were obtained.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: E.Ç., Design: B.A., Y.C., Data Collection or Processing: A.K., E.K., Analysis or Interpretation: A.K., E.K., Literature Search: B.A., Writing: B.A., Y.C., E.Ç.
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Correlation of serum leptin and ghrelin levels with endocrine and reproductive parameters in women with clomiphene citrate resistant polycystic ovary syndrome

Klomifen sitrat rezistant polikistik over sendromlu hastalarda ghrelin ve leptin düzeylerinin endokrin ve reprodüktif parametrelerle korelasyonu

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Abstract

Objective: To investigate the changes in serum ghrelin and leptin levels in patients with clomiphene-resistant polycystic ovary syndrome (PCOS).

Materials and Methods: Thirty-five patients who could not achieve ovulation or pregnancy despite using 150 mg/day of clomiphene citrate (CC) participated in the study. Thirty-five patients who were compatible with the study group in terms of age and body mass index (BMI) but did not have clinical and laboratory findings of PCOS constituted the control group. On the third day of the cycle, in addition to the basal hormone profile, ghrelin and leptin levels were also measured. Patients in both groups went to IVF/ICSI. Basal hormone values, leptin, ghrelin, metabolic, demographic parameters, and clinical pregnancy rates were correlated.

Results: Patients in both groups were recorded to be similar in terms of age (29.4±0.11 vs 28.5±7.30), BMI (24.3±3.07 vs 23.8±1.55), and infertility time (6.14±4.30 vs 6.03±1.28). Serum ghrelin levels of the PCOS group were significantly lower than the control group (0.48±2.21 vs 1.19±4.02) Serum leptin levels of the PCOS group were significantly higher than the control patients (45.6±304 vs 16.5±0.32). Serum leptin levels and BMI (r=0.65, p<0.01) A positive correlation was found between luteinizing hormone (LH) (r=0.53, p<0.02), and insulin resistance (r=0.74, p<0.03). There was a negative (r=-0.76, p<0.03) correlation between serum ghrelin and LH. A positive and significant correlation was found between serum ghrelin, testosterone, mature oocyte, and implantation rates.

Conclusion: Serum ghrelin correlates with fertility outcomes in women with CC-resistant PCOS undergoing IVF/ICSI.

Keywords: Clomiphene resistance, polycystic ovary syndrome, ghrelin, leptin, HOMA-IR, LH, fertility outcome

Öz

Amaç: Bu çalışmanın amacı klomifen sitrata dirençli polikistik over sendromlu (PKOS) hastalarda serum leptin ve ghrelin düzeylerinin endokrin parametreler ve implantasyon oranları üzerine etkisini göstermektir.

Gereç ve Yöntemler: 150 mg klomifen sitratla yapılan ovulasyon indüksiyonuna cevap vermeyen otuz beş PKOS hastası çalışmaya dahil edilmiştir. Kontrol grubuna da benzer yaş ve vücut kitle indeksine (VKİ) sahip PKOS olmayan otuz beş infertil kadın alınmıştır. Serum ghrelin, leptin ve diğer hormon değerleri siklusun üçüncü gününde ölçülmüştür. Her iki grupta da IVF/ICSI uygulanmıştır.

PRECIS: This study was planned to determine the effect of serum ghrelin and leptin levels on endocrine parameters and implantation rates in patients with clomiphene-resistant polycystic ovary syndrome (PCOS).

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Bulgular: Yaş (29,4±0,11 vs 28,5±7,30), VKİ (24,3±3,07 vs 23,8±1,55), infertilite süresi (6,14±4,30 vs 6,03±1,28) açısından iki grup arasında anlamlı farklılık yoktur. Serum ghrelin düzeyleri PKOS grubunda kontrol grubuna kıyasla anlamlı olarak düşük bulunmuştur (0,48±2,21 vs 1,19±4,02). Serum leptin düzeyleri ise PKOS grubunda kontrol grubuna göre anlamlı yüksek bulunmuştur (45,6±304 vs 16,5±0,32). Serum luteinleştirici hormon (LH), testesteron insülin düzeyleri ve insülin rezistansı PKOS grubunda kontrol grubuna göre anlamlı olarak yüksek bulunmuştur. Serum leptin, VKİ (r=0,65, p<0,01), LH (r=0,53, p<0,02), insülin ve insülin rezistansı (r=0,74, p<0,03) arasında pozitif ve anlamlı korelasyon mevcuttur. Serum ghrelin ve LH düzeyleri (r=-0,76, p<0,03) arasında negatif ve anlamlı korelasyon bulunmuştur. Serum ghrelin, testosterone düzeyleri, toplanan matür oosit sayısı (r=0,70, p<0,02), ve implantasyon oranları (r=0,79, p<0,03) arasında pozitif ve anlamlı korelasyon bulunmuştur.

Sonuç: PKOS hastalarında serum ghrelin düzeyleri ile implantasyon oranları arasında korelasyon bulunmaktadır.

Anahtar Kelimeler: Klomifen sitrat rezistan PKOS, ghrelin, leptin, HOMA-IR, LH, gebelik sonuçları

Introduction

Because of its properties similar to the metabolic syndrome, polycystic ovary syndrome (PCOS) negatively affects the reproductive outcome by causing both endocrine and adipose tissue dysfunction[1,2]. Despite recent advances in assisted reproduction techniques, difficulties in conceiving patients with PCOS persist. We can list the possible causes of subfertility we encounter in patients PCOS as follows. (i) anovulation, (ii) hyperandrogenism, (iii) endocrine dysfunction, (iv) impairment in endometrial responsiveness. In addition to these factors, it is thought that dysfunction detected in the adipose tissues of PCOS cases in the last decade may cause subfertility by affecting the release of peripheral peptides[1-3]. Peptides either cause subfertility by directly affecting the ovary and endometrium or indirectly affect hypothalami-pituitary neurons[1,2,4].

Ghrelin and leptin are peptides that contribute to subfertility in cases of PCOS[1,2,4]. These two peripheral peptides disrupt both gonadotropin secretion and follicle development by having negative or positive energy balance as well as their effects on the release of insulin and other hormones and contribute to the subfertility seen in PCOS[1]. Leptin and ghrelin show their central effects through the GnRH pulse generator[1,2]. The main synthesis site of ghrelin is the gastrointestinal system and shows its effect in the arcuate nucleus[4,5]. Ghrelin increases the feeling of eating but decreases follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion[6,7]. The effect of ghrelin on GnRH release is mediated by the GH secretagogue receptor (GHS-R)[8]. Ghrelin inhibits FSH, LH release, and estrogen and progesterone secretion by binding its receptor[4]. Ghrelin also contributes to follicling maturation by preventing apoptosis via GHS-R1a found in follicles[9]. Although the primary synthesis site of leptin is adipose tissue, it is also synthesized in granulosa cells[10]. Since GnRH neurons do not contain a leptin receptor, leptin stimulates GnRH release by acting through kisspeptin neurons[11]. While physiological leptin levels stimulate follicle development and steroidogenesis, cystic development may occur in the ovaries in the presence of high leptin[12]. Relationships between serum leptin and ghrelin levels and other parameters and reproductive outcome in infertile patients with PCOS have been discussed in previous studies[1,2,4,10]. However, the effect of serum levels of these two peptides on implantation, pregnancy rates, and other parameters in clomiphene-resistant PCOS cases were not investigated.

The aim of the study was to determine the relationship between serum ghrelin and leptin levels and reproductive outcome and endocrine parameters in women with CC-resistant PCOS.

Materials and Methods

A total of 70 patients who decided to on IVF/ICSI were included in the study. While 35 of these patients were clomiphene-resistant patients with PCOS, the remaining 35 were non-PCOS control patients. Both groups were matched for age and body mass index (BMI). In this way, adiposity affecting ghrelin and leptin synthesis was disabled. Failure to achieve ovulation or pregnancy despite 150 mg clomiphene citrate (CC) Daily for six months was accepted as CC resistance. The control group was selected from unexplained infertile women. They did not carry the clinical, laboratory, or dermatological findings of PCOS. In the control group, 13 of 35 (37.1%) patients complied with the metabolic syndrome criteria. Letrozole treatment was administered to CC-resistant cases before IVF/ICSI. PCOS cases unresponsive to letrozole were referred to IVF/ICSI. Serum samples were taken on the third day of the cycle of the patients in the PCOS and control groups. Serum LH, FSH, testosterone, insulin, ghrelin, and leptin levels were measured in fasting blood samples. Serum Leptin levels were measured by ELISA kit following the manufacturer's protocol. The sensitivity of the kit was 2 pg/mL and ranged 2-400 pg/mL. Ghrelin levels were measured with an enzyme immunoassay kit following the manufacturer's protocol. The sensitivity of the kit was 0.13 ng/mL and the range was 0.13-1.34 ng/mL. The homeostatic model assessment (HOMA-IR) Formula was used for insulin resistance. The cases who became pregnant with letrozole were excluded. Women with a history of antiandrogens, antidiabetics, and lipid-lowering drugs were excluded. Females with known causes of infertility like uterine fibroids, male factors, and other metabolic disorders were excluded from the study. Local Ethics Committee approval was obtained from Memorial Kayseri Hospital. Both PCOS and non-PCOS participants underwent conventional antagonist protocol. Relationship between serum ghrelin and leptin levels and implantation rates (IR), clinical pregnancy rate (CPR), and other metabolic and demographic parameters.

Statistical Analysis

The Statistical Package for Social Sciences software 21.0 for Windows software software (SPSS, Inc., Chicago, IL, USA)

was used for the analysis of collected data. The normality of disturbance was analyzed with the Kolmogorov-Smirnov test. A t-test was used for data comparisons. Pearson correlation analyses were used to detect possible correlations among the data. A p-value of <0.05 was set as significant. Data were expressed as mean ± SD.

Results

Table 1 shows all endocrine, metabolic, and demographic parameters. The age (29.4±0.11 vs 28.5±7.30), BMI (24.3±3.07 vs 23.8±1.55), and duration of infertility (6.14±4.30 vs 6.03±1.28) were similar. Serum total Ghrelin levels of PCOS cases were found to be significantlylower whencomparedwithnon-PCOS cases in the control group (0.48±2.21 vs 1.19±4.02). Serum levels of leptin in women with PCOS were significantly higher than in the patients in the control group (45.6±304 vs 16.5±0.32). The cases in the PCOS grouparenon-obese and their BMI values and ages are similar to the control cases. Therefore, fluctuations in leptin and ghrelin levels depending on age and BMI values were eliminated. The PCO group had significantly higher LH, testosterone, and insulin levels compared with the control group. Both groups had similar serum FSH and glucose levels. PCOS group had higher HOMA-IR than those in the control group. Ghrelin and leptin levels were not correlated with any group. Serum leptin showed positive correlation with BMI (r=0.65, p<0.01) LH (r=0.53, p<0.02), insulin and HOMA-IR (r=0.74, p<0.03). Serum leptin levels were not correlated with the number of mature oocytes (MII). Any correlation was not detected between CRP and leptin. Serum ghrelin and LH levels were negatively correlated (r=-0.76, p<0.03). Serum ghrelin showed a positive correlation with testosterone, mature oocytes (r=0.70, p<0.02), and implantation rates (r=0.79, p<0.03). No correlation was not detected between ghrelin, insulin, HOMA-IR, age, and BMI. Ghrelin did not show any correlation with CPR (Table 2).

Discussion

The impact of serum ghrelin and leptin on metabolic and demographic findings of infertile women with PCOS has been investigated in previous studies[1,13]. However, the effect of serum ghrelin or leptin on the metabolic parameters of women with clomiphene-resistant PCOS has not been studied to date. This study is the first clinical study investigating the relationship between serum ghrelin and leptin levels and endocrine and reproductive parameters in CC-resistant PCOS cases and is important in this respect. Our study clearly showed that, while a significant increase in serum leptin levels, a significant decrease in ghrelin levels in CC-resistant PCOS. While these changes in leptin and ghrelin levels are consistent with the results of some previous PCOS studies, they are different from the results of some[1,2].

Table 1. Demographic, hormonal and reproductive characteristics of CC resistant PCOS and control groups

	CC resistant PCOS (n=35)	Non-PCOS (n=35)	p
Age (y)	29.4±0.11	28.5±7.30	0.60
BMI (kg/m^2)	24.3±3.07	23.8±1.55	0.33
Infertility duration (y)	6.14±4.30	6.03±1.28	0.07
Testosterone (ng/mL)	0.87±5.03*	0.39±7.60	0.001
Day 3 Estradiol (pg/mL)	39.5±2.11	38.8±3.04	0.65
Day 3 Progesterone (ng/mL)	0.24±0.10	0.25±0.012	0.32
LH (mIU/mL)	11.33±0.11*	5.44±1.43	0.002
FSH (mIU/mL)	6.22±3.44	5.88±1.70	0.65
Insulin (mU/L)	12.2±3.43*	7.01±8.33	0.002
HOMA-IR	3.66±5.12*	1.74±3.02	0.001
Glucose (mg/dL)	91.4±4.05	88.5±644	0.21
MII oocyte	13.4±4.69*	7.40±6.10	0.002
Implantation rate (%)	40.0%	37.1%	0.057
Clinical pregnancy (%)	51.4%	48.7%	0.06
Ghrelin (ng/mL)	0.48±2.21*	1.19±4.02	0.02
Leptin (pg/mL)	45.6±304*	16.5±0.32	0.001

Data presented as means ± SD. BMI: Body mass index, FSH: Follicle-stimulating hormone, HOMA-IR: Homeosta-sis model assessment of insulin resistance, LH: Luteinizing hormone, PCOS: Polycystic ovary syndrome, MII: Mature oocyte, *p<0.05

Table 2. The resulst of correlation analysis between leptin, ghrelin levels and other measured parameters

	Age	BMI	HOMA-IR	LH	MII oocyte	Testosterone	IR	CPR
Leptin	r=0.34 p=0.43	r=0.65 p<0.01*	r=0.74 p<0.03*	r=0.53 p<0.02*	r=0.32 p=0.22	r=0.50 p=0.65	r=-0.60 p=0.52	r=0.42 p=0.12
Ghrelin	r=0.43 p=0.33	r=0.30 p=0.54	r=0.31 p=0.66	r=-0.76 p<0.03*	r=0.70 p<0.02*	r=0.876 p<0.01*	r=0.79 p<0.03*	r=0.51 p=0.32

BMI: Body mass index, HOMA-IR: homeostasis model assessment of insulin resistance, LH: Luteinizing hormone, IR: Implantation rate, CPR: Clinical pregnancy rate, MII: Mature oocyte, *p<0.05

When we evaluated both peptides separately, the high leptin levels we found were consistent with those in other studies[14-16]. However, there are studies reporting normal serum leptin levels in infertile cases of PCOS[17,18]. However, serum leptin levels showed a positive correlation with BMI, LH, insülin levels, and HOMA-IR in CC-resistant PCOS cases. The correlation findings we obtained are compatible with the literature except for LH[15,16,19]. The relationship between high leptin levels and insülin resistance in infertile patients with PCOS is known fact[14], and we found the same finding in CC-resistant PCOS cases. The positive relationship between BMI and leptin may be evidence that adipose tissue content and function change in CC-resistant PCOS cases. As for the positive relationship between LH and leptin, increased peripheral leptin levels may increase LH by stimulating GnRH release via kisspeptin receptors in arcuate neurons[1,11].

We detected a positive but insignificant correlation between leptin and the number of mature oocytes. Similarly, there was no significant correlation between leptin levels and implantation rates, and clinical pregnancy rates. These data do not mean that there is no relationship between leptin and reproductive outcome. While physiological amounts of leptin induce sex steroid synthesis and oocyte development in the ovaries, supraphysiological concentrations of leptin may lead to an ovarian cyst formation[12]. However, in patients with hypothalamic amenorrhea, leptin treatment regulates the LH pulse frequency[1,20]. However, in the presence of high leptin, there is a decrease in the response of the ovaries to gonadotropins[21,22]. High HOMA-IR levels may also limit the physiological effects of leptin on the ovary. Insulin ensures successful ovulation by regulating both gonadotropic hormone receptors and GnRH pulse frequency in the ovaries[23]. Since all physiological pathways will be dysregulated in the presence of high leptin, it will not be possible for leptin to positively affect reproductive parameters in CC-resistant PCOS cases. However, the presence of central leptin resistance due to chronic inflammation in PCOS cases may also prevent leptin from fully performing its physiological functions in the hypothalamic-pituitary-ovarian axis[24].

The second parameter that we evaluated in CC-resistant PCOS cases and found a significant decrease in their levels is ghrelin. In most of the studies in the literature, decreased serum ghrelin levels have been reported in infertile PCOS cases[15,17]. Our study is the first clinical study to report a decrease in ghrelin levels in CC-resistant PCOS cases. We found a negative correlation between a decrease in ghrelin levels and an increase in LH levels. Under normal conditions, physiological levels of ghrelin block LH release[4]. Ghrelin exerts this inhibitory effect on LH in both animals and humans through GnRH neurons[25,26]. Because of the decreased ghrelin levels, found in our study, the suppressive effect of ghrelin on LH may have been neutralized and increased LH. However, the positive correlation we found between Ghrelin levels and serum testosterone levels may also be a physiological consequence of the LH increase. This is how we can explain the positive correlation between ghrelin and testosterone synthesis, as increasing LH levels stimulate androgen synthesis in the ovary.

One of the most important results of the current study is the presence of a positive correlation between ghrelin levels and the number of mature oocytes collected and implantation rates. In CC-resistant PCOS cases, we can explain the increase in the number of mature oocytes and implantation rates due to the decrease in ghrelin level in two ways. Due to decreased ghrelin levels, the inhibitory effect of Ghrelin on GnRH neurons is removed and FSH and LH release are activated[1,4]. As a result, the number of mature eggs collected in IVF/ICSI casesduetoincreased FSH and LH release also increases, which increases the implantation rates. The most interesting finding of our study was that there was no correlation between clinical pregnancy rates and ghrelin levels. The physiological amount of ghrelin increases follicle maturation and quality due to its anti-apoptotic effect on ovarian follicles[4,9]. The decreased ghrelin levels found in CC-resistant cases may in oocytes and reduce the follicle quality. This may explain the lack of a correlation between clinical pregnancy rates and serum ghrelin.

Conclusion

In this observational study where we compared serum levels of ghrelin or leptin with endocrine, demographic, and reproductive parameters of CC-resistant PCOS and non-PCOS cases. We found a significant decrease in ghrelin levels despite an increase in leptin levels in the PCOS group. Despite the significant correlation between leptin levels and BMI, LH, insülin levels, and HOMA-IR, we did not detect a relationship between implantation and pregnancy rates and leptin levels.

However, in addition to the correlation between ghrelin levels and serum testosterone and LH levels, ghrelin levels correlated significantly with the total number of mature oocytes and implantation rates. Thanks to more comprehensive studies evaluating ghrelin and leptin levels as well as other peptides in CC-resistant PCOS cases, we can reach more definite conclusions about the reproductive outcome in cases with metabolic syndrome.

Ethics

Ethics Committee Approval: Local Ethics Committee approval was obtained from Memorial Kayseri Hospital (approved number: 16481, date: 21.01.2021).
Informed Consent: Written consent from all participants were obtained.
Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

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Comparison of the impact of laser-assisted hatching on fresh cleavage and blastocyst embryo transfer and association with pregnancy outcomes

Lazer destekli yuvalamanın taze klivaj ve blastokist embriyo transferleri üzerindeki etkisinin karşılaştırılması ve gebelik sonuçları ile ilişkisi

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Abstract

Objective: Assisted hatching (AH) techniques can improve live birth (LB) and clinical pregnancy (CP) rates. Since there are limited data regarding this subject, we investigated the impact of laser-assisted hatching (LAH) on fresh embryo transfer (ET) and association with pregnancy outcomes in unselected patient population.

Materials and Methods: This retrospective study included the fresh ETs performed at our center between April 2010 and April 2019. Among 3.782 fresh ETs, 3.286 underwent LAH (n=1.583 at cleavage stage and n=1.703 at blastocyst stage) while 496 underwent non-assisted hatching (NAH) (n=213 at cleavage stage and n=283 at blastocyst stage). The ETs were performed at the blastocyst or cleavage stages, and single or double embryos were transferred. LB rate was the primary outcome, while secondary outcomes were the pregnancy test, monozygotic twinning (MZT), and CP rates.

Results: The LAH and NAH groups showed similar LB, pregnancy test, CP, and MZT rates at cleavage and blastocyst stages. On the other hand, LAH significantly affected LB rates at the blastocyst stage (20.6% at blastocyst stage vs. 16% at the cleavage stage, p=0.001).

Conclusion: In conclusion, LAH does not improve reproductive outcomes of fresh blastocyst-stage and cleavage-stage ETs. However, LAH significant impacts LB rates in the blastocyst stage than the cleavage stage.

Keywords: In vitro fertilization, laser-assisted hatching, fresh embryo transfer, cleavage stage, blastocyst stage

Öz

Amaç: Destekli yuvalama teknikleri canlı doğum ve klinik gebelik oranlarını iyileştirebilir. Bu konuyla ilgili sınırlı veri olduğundan, seçilmiş olmayan hasta popülasyonunda lazer destekli yuvalamanın taze embriyo transferi üzerindeki etkisini ve gebelik sonuçları ile ilişkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Bu retrospektif çalışmaya merkezimizde Nisan 2010 ile Nisan 2019 tarihleri arasında gerçekleştirilen 3,782 taze embriyo transferleri dahil edildi. Klivaj aşamasındaki 3,286 embriyoya lazer destekli yuvalama işlemi uygulanırken, 496 embriyo (klivaj aşaması n=213, blastokist aşaması n=283) kontrol grubu olarak değerlendirildi. Embriyo transferleri klivaj (n=1,583) ve blastokist (n=1,703) aşamalarında gerçekleştirildi ve tek veya iki embriyo transfer edildi. Birincil sonuç olarak canlı doğum oranı, ikincil olarak ise gebelik testi, klinik gebelik ve monozigotik ikizlik oranları gruplar arasında karşılaştırıldı.

PRECIS: Impact of laser-assisted hatching on outcomes of fresh cleavage vs. blastocyst embryo transfer.

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Bulgular: Lazer destekli yuvalama ve kontrol grubu klivaj ve blastosist aşamalarında benzer canlı doğum, gebelik testi, klinik gebelik ve monozigotik ikizlik oranları gösterdi. Öte yandan, lazer destekli yuvalama, blastokist aşamasında canlı doğum oranlarını önemli ölçüde etkiledi (blastokist aşamasında %20,6 ve klivaj aşamasında %16, p=0,001).

Sonuç: Sonuç olarak, lazer destekli yuvalamanın taze klivaj ve blastokist evresi embriyo transferlerinin üreme sonuçlarını iyileştirmediği gösterildi. Bununla birlikte, lazer destekli yuvalamanın, blastokist aşamasındaki canlı doğum oranlarını klivaj aşamasına kıyasla anlamlı düzeyde artırmış olduğu saptandı.

Anahtar Kelimeler: Tüp bebek, lazer destekli yuvalama, taze embriyo transferi, klivaj aşaması, blastokist aşaması

Introduction

Assisted hatching (AH) methods involve the handling of zona pellucida (ZP) and are implemented as part of assisted reproductive technologies (ART)[1]. The ZP is a coat enveloping the oocyte; it prevents polyspermy and protects the embryo before implantation[2]. After fertilization, hatching of ZP is crucial for implantation in the receptive endometrium. Failure to hatch is one of the primary reasons for failure to implantation[3].

To date, different AH techniques have been developed to increase implantation ratios in women going through intracytoplasmic sperm injection (ICSI) or in vitro fertilization (IVF)[4]. The AH techniques can be performed chemically, mechanically, or by laser[5,6]. All AH methods are implemented to create a gap in the ZP or to thin the ZP for supporting the embryo during hatching when the blastocyst is ready for expansion and implantation[7].

Among the AH methods, laser-assisted hatching (LAH) has become popular since it is relatively simple and less time-consuming[8]. Also, in this method, the target can be precisely controlled, allowing the creation of gaps in the ZP less risk of injury to the embryo[9]. As per the Society for Assisted Reproductive Technology report[10], AH was applied in 56.3% of cleavage stage and 22.8% of blastocyst stage fresh embryo transfer (ET) patients in 2010. However, the retrospective and prospective studies assessing the impact of AH on reproductive outcomes[11,12] gave conflicting results. While some studies reported that AH might improve the clinical pregnancy (CP) and the multiple pregnancy rates, others reported either no improvement or a decrease in implantation and live birth (LB) rates[13,14]. On the other hand, it was recommended by the American Society for Reproductive Medicine[15] that AH might be clinically beneficial for women aged 38 or older, who went through at least two IVF/ICSI cycles, or those who have poor-quality or cryopreserved embryos. Although several studies[16-18] investigated the impact of AH on patients who underwent frozen-thawed cycles, patients who experienced repeated implantation failure (RIF), and those with advanced maternal age, little data obtained in the literature respecting the impact of AH in an unselected patient population undergoing IVF/ICSI.

We examined the impact of LAH on the clinical outcomes, including pregnancy test, CP, LB, and monozygotic twinning (MZT) ratios in a general patient group undergoing IVF/ICSI.

Materials and Methods

Patients

This study was performed at the infertility clinic of the University of Health Sciences Turkey, Etlik Zubeyde Hanim Women's Health Training and Research Hospital and approved by the Ethical Review Committee (17.01.2020-01/20). Patients who went through fresh IVF/ICSI cycles at this center between April 2010 and April 2019 constituted the target population of this study. In total, 3.782 fresh ETs were performed. Among these, 3.286 underwent LAH (n=1.583 at cleavage stage and n=1.703 at blastocyst stage), 496 underwent NAH (n=213 at cleavage stage, and n=283 at blastocyst stage). While one of the main inclusion criteria was single or double fresh ETs on day 3 or day 5, patients who underwent frozen-thawed embryo cycles were excluded.

Controlled Ovarian Stimulation, ICSI, and Embryo Culture

Women partners were stimulated using gonadotropin-releasing hormone (GnRH) antagonist or agonists after evaluating the ovarian reserve. The medication doses were determined based on the patients' body mass index (BMI), age, antral follicle counts, and basal serum follicle-stimulating hormone (FSH) levels. Oocyte maturation was induced by injecting 10.000 IU of human chorionic gonadotropin (hCG) (Pregnyl, Schering-Plough, Turkey) subcutaneously. When at least three follicles ≥16-18 millimeter in size, transvaginal ultrasound-guided oocyte pick-up was performed 36 hours after the hCG injection. Retrieved oocytes were amassed in G-IVF plus medium (Vitrolife, Gothenburg, Sweden) covered by 3 mL Ovoil (Vitrolife, Gothenburg, Sweden) in an atmosphere of 5% O_2, 6% CO_2, and 95% humidity at 37 °C for 2-4 hours.

The mature oocytes were inseminated by ICSI. Fertilization was confirmed as the presences of the two distinct pronuclei and second polar body 16-18 h after insemination. Zygotes were cultured in 30 µL drops of G-TL medium (Vitrolife, Gothenburg, Sweden) covered by 3 mL Ovoil (Vitrolife, Gothenburg, Sweden) in an atmosphere of 5% O_2, 6% CO_2, and 95% humidity at 37 °C.

Luteal support was initiated on the oocyte retrieval day by administering 100 mg progesterone in oil (Progestan, Kocak, Istanbul) daily or vaginal progesterone (Crinone® 8% progesterone vaginal gel, Merck, Germany). Luteal support lasted until 10-12 weeks of gestation. Pregnancy was accepted

positively when serum level of hCG was ≥10 IU/L 2 weeks after oocyte retrieval.

The LAH Procedure, Embryo Morphology, and Embryo Transfer

The LAH was applied to each embryo by creating a hole using an infrared diode laser (1.480 nm/400 mW, 40x objectives, Saturn 3 Laser System, Research Instruments Ltd., Cornwall, UK) and a non-contact 630-650 nm pilot laser on day 3 of embryo development. Cleavage embryos were graded based on their quality[19]. They were graded as grade 1 if they had 6-8 blastomeres on day 3 with <10% fragmentation without morphological abnormalities. The cleavage embryos were accepted as grade 2 if they had uneven blastomeres with mild variation in refractility and <10% fragmentation. Embryos with 3-6 blastomeres and 20-50% fragmentation were graded as grade 3, while those with less than three blastomeres and >50% fragmentation were graded as grade 4. Thus, the grade 1 and 2 embryos were categorized as good quality ones, while grade 3 and 4 were accepted as poor quality for the cleavage stage. Therefore, the grade 4 embryos were not transferred.

Blastocysts were graded as per the Gardner classification[20] and scored based on expansion status, inner cell mass (ICM), and trophectoderm (TE) development. The expansion status was graded as follows: Early blastocysts were graded as grade 1, blastocysts as grade 2, full blastocysts as grade 3; expanded blastocysts as grade 4; hatching blastocysts as grade 5 and hatched blastocysts as grade 6. The ICM was graded based on the presence of tightly packed cells (A), loosely packed cells (B), and very few cells (C). The TE was graded as A if several cells formed a cohesive epithelium, B if few cells formed a loose epithelium, and C in the presence of only very few large cells. Blastocysts having a grade of at least 3BB were classified as good-quality embryos. The ET was performed by transabdominal ultrasonography guidance on day 3 or day 5 of embryo development. When we have at least three good-quality embryos on day 3, the embryos were cultured to the blastocyst stage in the G-TL medium (Vitrolife, Gothenburg, Sweden) and transfers were performed on day 5. The other ETs were performed on day 3.

Clinical Outcomes

Serum hCG levels were measured two weeks after ETs. CP was described as the presence of gestational sac via vaginal ultrasonography by six weeks of pregnancy.

Statistical Analysis

The normal distribution of the continuous parameters was tested by the Shapiro-Wilk test. If the variables were not normally distributed, the Mann-Whitney U test was used to compare the LAH and NAH groups. The Fisher's Exact and chi-square tests

Table 1. Demographic data and controlled ovarian stimulation parameters of cleavage stage embryo transfer cycles

Parameters	LAH (n=1.583)	NAH (n=213)	p*
	Mean ± SD (min-max)	Mean ± SD (min-max)	
Maternal age	32.04±5.09 (18-48)	33.25±3.74 (25-44)	**<0.001**
BMI	29.43±9.17 (16.4-316)	28.32±4.14 (19.1-43.7)	**0.014**
Antral follicle count	11.59±7.6 (0-45)	11.24±8.29 (1-30)	0.121
Endometrial thickness on hCG day (mm)	7.86±1.78 (5-15.6)	7.76±1.35 (5-11.6)	0.739
Endometrial thickness on OPU day (mm)	7.94±1.81 (5-15.4)	7.66±1.29 (5-11.5)	0.251
Total oocyte count	10.63±7.41 (1-52)	10.47±7.82 (1-44)	0.466
Mature oocyte count	7.21±5.63 (1-36)	7.67±5.87 (1-30)	0.232
2PN	3.78±3.31 (0-23)	4.03±3.92 (1-28)	0.620

*p-value calculated by Mann-Whitney U test, LAH: Laser-assisted hatching, NAH: Non-assisted hatching, SD: Standard deviation, BMI: Body mass index, hCG: Human chorionic gonadotropin, mm: Millimeter, OPU: Oocyte pick-up, PN: Pronuclei

were implemented to analyze the categorical data. Descriptive statistics of the continuous variables were expressed as medians, means, standard deviations, interquartile ranges, and minimum and maximum values. The categorical parameters were given as frequencies (n) and percentages (%). The IBM SPSS statistics software was used for all statistical analyses.

Results

During the study period, 3,782 IVF/ICSI cycles were performed. In total, 1,796 patients underwent ET at the cleavage stage. Among these patients, 1,583 underwent LAH while 213 underwent NAH. The demographic data and controlled ovarian stimulation parameters of the patients are displayed in Table 1. The maternal age was significantly higher, and BMI was significantly lower in the NAH group than in the LAH group (p<0.001, p=0.014). Two groups were similar regarding other parameters. The embryo grades, numbers of transferred embryos, and reproductive outcomes in two groups are shown in Table 2.

While the number of grade 2 embryos was significantly higher in the LAH group, the number of grade 3 embryos was significantly higher in the NAH group (p<0.001 and p<0.001). However, the two groups were similar regarding reproductive outcomes, including the rates of pregnancy tests, clinical pregnancy, and live birth (p=0.311, p=0.368, p=0.23). The MZT rates were also similar between the LAH and NAH groups.

Our review revealed that 1986 patients underwent ET at the blastocyst stage. Among these patients, 1703 underwent LAH while 283 underwent NAH. Data of these patients are presented in Table 3.

There was a significant difference between the two groups regarding BMI, endometrial thickness on hCG and oocyte retrieval days, and the number of embryos with 2 pronuclei (2PN) embryos (p=0.005, p<0.001, p<0.001, p=0.007).

The blastocyst scores and reproductive outcomes are displayed in Table 4. The two groups were significantly different concerning blastocoel expansion, ICM, and TE scores, and numbers of transferred embryos (p<0.001, p<0.001, p<0.001, p=0.012). However, the two groups were similar regarding the pregnancy test, clinical pregnancy, live birth, and MZT rates (p=0.498, p=0.231, p=0.208, p=1).

Comparison of the pregnancy test, CP, LB, and MZT rates for investigating the effects of LAH on reproductive outcomes following fresh cleavage-stage or blastocyst-stage ETs revealed no significant difference regarding pregnancy test, clinical pregnancy, and MZT rates between cleavage and blastocyst stage ETs (p=0.249, p=0.698, p=0.735). In contrast, there was a statistically significant difference concerning live birth rates. The live birth rates were significantly higher in the blastocyst-stage ETs than cleavage-stage ETs (p=0.001) (Table 5).

Discussion

In our retrospective review, we analyzed the impact of LAH on fresh ETs in a general IVF population. Our findings indicate that LAH does not advance pregnancy outcomes of fresh cleavage and blastocyst-stage ETs. However, it increased a remarkable enhancement in the LB rate at the blastocyst stage ETs. The LAH procedure did not significantly reproductive outcomes, including CP, pregnancy test, LB, and MZT rates, irrespective of embryo morphology.

Several studies, which analyzed the impact of LAH on reproductive outcomes, but the results are conflicting[16,17,21,22]. Most of these studies[16-18] focused on the effects of LAH on specific patient populations, including those who underwent

Table 2. Embryo grades and reproductive outcomes of cleavage-stage embryo transfer cycles

Parameters		LAH n (%)	NAH n (%)	Total n (%)	p
Embryo grade	1	632 (40.5)	78 (36.6)	710 (40.0)	**<0.001**
	2	773 (49.5*)	83 (39.0)	856 (48.2)	
	3	157 (10.1)	52 (24.4*)	209 (11.8)	
Embryo transfer	1	1024 (65.6)	167 (78.4*)	1191 (67.1)	**<0.001**
	2	538 (34.4*)	46 (21.6)	584 (32.9)	
Pregnancy test	Positive	582 (37.3)	87 (40.8)	669 (37.7)	0.311
	Negative	980 (62.7)	126 (59.2)	1106 (62.3)	
Clinical pregnancy	Positive	459 (29.4)	69 (32.4)	528 (29.7)	0.368
	Negative	1103 (70.6)	144 (67.6)	1247 (70.3)	
Live birth	Positive	250 (16.0)	41 (19.2)	291 (16.4)	0.230
	Negative	1312 (84.0)	172 (80.8)	1484 (83.6)	
Monozygotic twinning	Positive	30 (1.9)	4 (1.9)	34 (1.9)	1.00**
	Negative	1532 (98.1)	209 (98.1)	1741 (98.1)	

p-values were calculated by chi-square test, *p-values lower than 0.05, **p-values calculated by Fisher's Exact test, LAH: Laser-assisted hatching, NAH: Non-assisted hatching

frozen-thawed IVF/ICSI-ET cycles, those with advanced maternal age, and patients previously diagnosed with RIF. Zeng et al.[21] conducted a systematic review analyzing twelve randomized controlled trials and concluded that LAH was affiliated with higher CP and implantation rates and a higher risk of multiple pregnancies in women receiving thawed embryos. In a retrospective trial, Hiraoka et al.[16] studied the impact of the ZP openings with different sizes in LAH performed on high-quality blastocysts originated from slow frozen-thawed cleavage stage embryos in women with RIF. They detected a remarkable improvement in pregnancy, implantation, and delivery rates with the opening of 50% of the ZP (74%, 52%, 65%) while the improvements in the control (17%, 10%, 13%; p<0.01) and 40 µm ZP opening (43%, 27%, 38%, p<0.04) groups were less significant[18]. These authors also reported significantly lower delivery rates in the control group than the 50% ZP opening and the 40 µm ZP opening groups[16]. Another randomized trial, Wan et al.[17] performed a quarter ZP opening by LAH and investigated its impact on the clinical parameters after transferring vitrified-warmed blastocysts originating from low-grade cleavage stage embryos. These researchers reported a remarkable increase in the CP and implantation rates while the LB rates did not change significantly (p=0.034, p=0.021, p>0.05). Ng et al.[4] showed in a retrospective trial conducted on vitrified-warmed blastocyst transfers that LAH did not impact the rates of implantation (26.2% vs. 27.3%), conception (38.7% vs. 42.1%), clinical pregnancy loss, LB, CP and MZT. They also reported that five pairs of dichorionic/diamniotic twins developed from single ETs.

Only a few studies have analyzed the effects of LAH on fresh ETs[18,23,24]. In a study by Sagoskin et al.[23] conducted ZP drilling by LAH on the day of fresh ET in women going through the transfer of cleavage embryos (day 3) in a selected patient population with good prognostic factors, including normal serum FSH and E2 levels, maternal age ≤39, have good-quality embryo on day 3 and history of no more than one failed IVF/ICSI cycle. Patients with unfavorable prognostic factors were excluded from this study. The presence of spontaneous pregnancy loss (13% vs. 16%), fetal cardiac activity (53% vs. 54%), and LB (47% vs. 46%) rates were similar between the groups who underwent LAH and those who did not undergo AH. These authors concluded that LAH did not benefit this selected patient population. In a prospective randomized study, Razi et al.[24] performed LAH to open a hole in ZP on day 2 of embryo development in patients undergoing ICSI due to male factor infertility during their initial IVF/ICSI cycle. Comparison of LB and CP rates between LAH and NAH groups revealed insignificant differences (11.11%

Table 3. Demographic and controlled ovarian stimulation parameters of the blastocyst-stage embryo transfer cycles

	LAH (n=1.703)	NAH (n=283)	
Parameters	**Mean ± SD (min-max)**	**Mean ± SD (min-max)**	**p**
Maternal age	30.65±4.77 (19-50)	31.3±5.68 (19-47)	0.135
BMI	28.74±13.42 (16.4-316)	29.33±5.33 (19-43.5)	**0.005**
Antral follicle count	13.01±8.49 (0-31)	13.14±9.46 (0-30)	0.439
Endometrial thickness on hCG day (mm)	8.1±1.92 (5-16.5)	7.32±1.45 (5-11.5)	**<0.001**
Endometrial thickness on OPU day (mm)	8.12±2.5 (5-17)	7.3±1.36 (5-12)	**<0.001**
Total oocyte count	10.21±6.63 (1-42)	10.73±7.87 (1-43)	0.856
Mature oocyte count	7.32±5.08 (0-31)	7.71±5.67 (1-32)	0.629
2PN	4.94±3.42 (1-25)	4.75±3.92 (1-20)	**0.007**

p-values were calculated by Mann-Whitney U test, LAH: Laser-assisted hatching, NAH: Non-assisted hatching, SD: Standard deviation, BMI: Body mass index, hCG: Human chorionic gonadotropin, mm: Millimeter, OPU: Oocyte pick-up, PN: Pronuclei, min: Minimum, max: Maximum

vs. 8.6%, p=0.6, and 20% vs. 23.9%, p=0.3). Additionally, these authors reported that there were multiple pregnancies (twin) in both LAH and control groups. One congenital anomaly was present in the LAH group. Tannus et al.[18] worked on patients with advanced maternal age (i.e., mean age 41.1±1.1). This retrospective study showed that LAH was affiliated with reduced LB and CP rates in fresh ETs performed during cleavage but not the blastocyst stage. In a retrospective study by Xu et al.[25] have evaluated the effect of LAH on the low-grade cleavage stage embryos. They reported the total blastocyst rate (50.7% vs. 40.2, p<0.001), usable blastocyst rate (31% vs. 18.6%, p<0.001) were significantly higher in the LAH group. Additionally, CP rates were not different between groups (49.4% vs. 40%, p>0.05).

This study found that clinical outcomes were similar between NAH and LAH when the latter was performed in cleavage-stage ETs. Our study also showed that LAH did not improve the pregnancy test (p=0.311), LB (p=0.230), and CP (p=0368) rates in cleavage-stage ETs. Similarly, we did not detect a remarkable difference in the positive pregnancy test (p=0.498), CP (p=0.231), and LB (p=0.208) rates in blastocyst-stage ETs. On the other hand, LAH significantly improved LB rates in blastocyst-stage ETs than the cleavage-stage ETs (p=0.001). A study by Schwärzler et al.[26] analyzed the pregnancy outcomes of blastocyst-stage and cleavage-stage ETs. Additionally, it was reported that[27] the blastocyst could improve the synchronization between embryo and endometrium and permit the selection of more advanced embryos considered the most appropriate for transfer. Also, it is known that blastocyst transfer leads to relatively higher LB and implantation rates. In line with these findings, we found that LB rates were remarkably higher in blastocyst-stage ETs than in cleavage-stage ETs. The differences in the previously published reports and our study results can be ascribed to the differences in the study population, AH timing, and technique.

The use of micromanipulation techniques in ART is related to a higher risk of MZT. Several researchers have reported that multiple factors, including maternal age, prolonged embryo culture until the blastocyst stage, embryo biopsy for preimplantation genetic testing, fresh or frozen-thawed ET, ovarian stimulation, and ZP manipulation as ICSI and AH might account for this increased risk[28-31]. However, several authors reported that blastocyst transfer was related to an increased risk of MZT[27], the others did not report such an association[30,31]. Our results also revealed an insignificant difference in MZT rates between cleavage and blastocyst stage ETs.

Table 4. Blastocyst scores and reproductive outcomes of blastocyst stage embryo transfer cycles

Parameters		LAH n (%)	NAH n (%)	Total n (%)	p
Blastocoel expansion	3	439 (25.8)	176 (62.2*)	615 (35.7)	**<0.001**
	4	717 (42.1*)	69 (24.4)	786 (45.6)	
	5	260 (15.3*)	29 (10.2)	289 (16.8)	
	6	26 (1.5)	9 (3.2)	35 (2.0)	
Inner cell mass score	A	148 (8.7)	64 (22.6*)	212 (10.7)	**<0.001**
	B	611 (35.9)	107 (37.8)	718 (36.2)	
	C	944 (55.4*)	112 (39.6)	1.056 (53.2)	
Trophoectoderm score	A	22 (1.3)	66 (23.3*)	88 (4.4)	**<0.001**
	B	609 (35.8)	131 (46.3*)	740 (37.3)	
	C	1.072 (62.9*)	86 (30.4)	1.158 (58.3)	
Embryo transfer	1	1239 (72.8)	226 (79.9*)	1.465 73.8)	**0.012**
	2	464 (27.2*)	57 (20.1)	521 (26.2)	
Pregnancy test	Positive	668 (39.2)	105 (37.1)	773 (38.9)	0.498
	Negative	1.035 (60.8)	178 (62.9)	1.213 (61.1)	
Clinical pregnancy	Positive	511 (30.0)	75 (26.5)	586 (29.5)	0.231
	Negative	1.192 (70.0)	208 (73.5)	1.400 (70.5)	
Live birth	Positive	350 (20.6)	49 (17.3)	399 (20.1)	0.208
	Negative	1.353 (79.4)	234 (82.7)	1.587 (79.9)	
Monozygotic twinning	Positive	30 (1.8)	5 (1.8)	35 (1.8)	1.00**
	Negative	1.673 (98.2)	278 (98.2)	1951 (98.2)	

p-values were calculated by chi-square test, *p-values lower than 0.05, **p-values calculated by Fisher's Exact test, LAH: Laser-assisted hatching, NAH: Non-assisted hatching

Table 5. Effect of LAH on cleavage and blastocyst stage ET

		Day 3 AH n (%)	Day 5 AH n (%)	Total n (%)	p
Pregnancy test	Positive	582 (37.3)	668 (39.2)	1250 (38.3)	0.249
	Negative	980 (62.7)	1035 (60.8)	2015 (61.7)	
Clinical pregnancy	Positive	459 (29.4)	511 (30.0)	970 (29.7)	0.698
	Negative	1103 (70.6)	1192 (70.0)	2295 (70.3)	
Live birth	Positive	250 (16.0)	350 (20.6*)	600 (18.4)	**0.001**
	Negative	1312 (84.0*)	1353 (79.4)	2665 (81.6)	
Monozygotic twinning	Positive	30 (1.9)	30 (1.8)	60 (1.8)	0.735
	Negative	1532 (98.1)	1673 (98.2)	3205 (98.2)	

p-values were calculated by chi-square test, *p-values lower than 0.05. LAH: Laser-assisted hatching, ET: Embryo transfer, AH: Assisted hatching

Study Limitations

Our study has several limitations such as its retrospective design, completed at a center and consisted of small patient population. Our some results were not reached statistical significance because of the small patient population. Larger prospective and multi-center studies must enhance our knowledge on the effect of LAH on fresh ETs.

Conclusion

In conclusion, our findings were shown that LAH has insignificant impact on the rates of CP, MZT and pregnancy test between cleavage and blastocyst stage ETs, but a significant effect on LB rate in blastocyst stage ETs.

Ethics

Ethics Committee Approval: This study was performed at the Infertility Clinic of the University of Health Sciences, Etlik Zubeyde Hanim Women's Health Training and Research Hospital and approved by the Ethical Review Committee (17.01.2020-01/20).
Informed Consent: Retrospective study.
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Authorship Contributions

Surgical and Medical Practices: O.A., R.Ö., S.D., Concept: S.H., İs.K., A.A.Ö., O.A., R.Ö., İ.K., S.D., Design: S.H., İs.K., A.A.Ö., O.A., R.Ö., İ.K., S.D., Data Collection or Processing: S.H., İ.K., A.A.Ö., O.A., R.Ö., S.D., Analysis or Interpretation: S.H., A.A.Ö., İ.K., Literature Search: S.H., İs.K., O.A., R.Ö., İ.K., S.D., Writing: S.H., İs.K., A.A.Ö., O.A., R.Ö., İ.K., S.D.
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A clinical scoring system for the diagnosis of adenomyosis

Adenomiyozis tanısı için klinik bir skorlama sistemi

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Abstract

Objective: To develop a scoring system using clinical evaluation methods to predict the presence of adenomyosis.

Materials and Methods: A cohort of 232 patients who underwent hysterectomy for benign gynecologic disorders was prospectively enrolled. A detailed anamnesis was obtained and physical/pelvic examinations with trans-vaginal ultrasound imaging were performed one day before the hysterectomy. The diagnosis of adenomyosis was based on histopathologic examination. Findings were compared between patients with (n=55) and without (n=166) adenomyosis. Factors associated with adenomyosis were assessed with regression analysis and odds ratios (OR) were calculated. The variables found to be significant were chosen for the scoring system. Receiver operating characteristic analysis was carried out to find the cut-off values for these variables.

Results: Number of parity, dyspareunia and dysmenorrhea visual analogue scale (VAS) scores, age of menarche, presence of uterine tenderness and detection of heterogeneous myometrium and myometrial cysts during ultrasonography were found to be the significant parameters. OR for the presence of myometrial heterogeneity, myometrial cysts, uterine tenderness were 27.2, 3.6 and 9.3 respectively. Cut-off values were calculated; 3 for parity (OR=2.8), 13-years for menarche (OR=1.6), 2 for dyspareunia VAS scores (OR=1.9) and 4 for dysmenorrhea VAS scores (OR=1.2). The total sum of maximum OR that a patient can obtain was calculated as 47.6 and this value was assumed to predict the presence of adenomyosis 100%. The multiplication of the sum of the OR in a patient by 2.1 (100/47.2) was found to have a predictive ability for the presence of adenomyosis.

Conclusion: A scoring system is developed to predict adenomyosis non-invasively based on clinical evaluation.

Keywords: Adenomyosis, clinical evaluation, non-invasive, pelvic examination, scoring system

Öz

Amaç: Klinik değerlendirme yöntemleri kullanılarak adenomiyozis varlığını öngörmeye yarayacak bir skorlama sistemi geliştirmek.

Gereç ve Yöntemler: Benign hastalıkları için histerektomi yapılan 232 kişilik bir kohort prospektif olarak değerlendirildi. Tüm hastalara histerektomiden bir gün önce detaylı anamnez alınarak, fizik/pelvik muayeneler ile birlikte transvajinal ultrasonografik inceleme yapıldı. Adenomiyozis tanısı histopatolojik inceleme ile konuldu. Adenomiyozisi olan (n=55) ve olmayan (n=165) hastaların bulguları karşılaştırıldı. Adenomiyozis ile ilişkili olduğu bulunan faktörler regresyon analizi ile değerlendirildi ve olasılık oranları (OO) hesaplandı. Anlamlı bulunan değişkenler skorlama sistemi için kullanıldı. Bu değişkenlerin eşik değerlerinin bulunması için alıcı işlem karakteristikleri analizi kullanıldı.

Bulgular: Parite sayısı, disparoni ve dismenore görsel analog ölçek (VAS) skorları, menarş yaşı, uterin hassasiyet varlığı ve ultrasonografik incelemede heterojen miyometriyum ile miyometriyal kistlerin görülmesi anlamlı parametreler olarak bulundu. Miyometriyal heterojenite, miyometriyal kist ve uterin hassasiyet varlığı için OO sırasıyla 27,2, 3,6 ve 9,3 olarak bulundu. Parite için 3 (OO=2,8), menarş için 13 yaş (OO=1,6), disparoni VAS skoru için 2 (OO=1,9) ve dismenore VAS skoru için 4 (OO=1,2) eşik değerler olarak hesaplandı. Bir kişinin alabileceği maksimum OO değerlerinin toplamı 47,6 olarak hesaplandı ve bu değerin adenomiyozis varlığını yüzde yüz öngöreceği kabul edidi. Bir hastadaki OO'nun toplamının 2,1 ile çarpılmasının (100/47,2) o hastada adenomiyozis varlığı için öngörücü bir yeteneğe sahip olduğu sonucuna ulaşıldı.

Sonuç: Klinik değerlendirmeye dayalı olan, non-invaziv olarak adenomiyozisi tahmin etmek için bir skorlama sistemi geliştirilmiştir.

Anahtar Kelimeler: Adenomiyozis, klinik değerlendirme, non-invaziv, pelvik muayene, skorlama sistemi

PRECIS: Using simple, noninvasive, clinical evaluation methods, a clinical scoring system for the diagnosis of adenomyosis is developed.

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Introduction

Adenomyosis is a relatively common benign disorder in which endometrial gland and stroma are located within the myometrium resulting angiogenesis of the spiral vessel, hypertrophy of the surrounding smooth muscles and enlargement of the uterus. We have recently showed that the disease mimics the malignant process in terms of angiogenesis, apoptosis, hypoxia and energy metabolism; however, the etiology and pathogenesis remain unclear[1]. Although in most cases it is asymptomatic, it may cause abnormal uterine bleeding, especially menorrhagia, dysmenorrhea, dyspareunia, pelvic pain and subinfertility[2]. There is significant overlapping in the presentations with other gynecologic disorders and in many cases there is concomitant endometriosis or leiomyomas[3]. In addition there are no specific laboratory tests and reliable clinical standards for the diagnosis. Therefore, the diagnosis and evaluating the response to treatment are challenging. So far the definitive diagnosis still requires a histologic analysis of the hysterectomy specimens or hysteroscopic or laparoscopic biopsy.

Clinical examination, transvaginal ultrasonography (TVS), magnetic resonance imaging (MRI), hysteroscopy guided biopsies have all been suggested as diagnostic methods with various clinical usefulness[4]. The clinical examination alone cannot detect adenomyosis[4]; however, it provides the exclusion of other gynecologic pathologies and gives detailed information about severity and complexity of the disease in the planning of medical or surgical treatment. TVS has been suggested to be the primary imaging modality for the diagnosis of adenomyosis with a range of 65-81% sensitivity and 65-100% specificity[5]. The detection of asymmetric thickening of the myometrium, myometrial cysts, linear myometrial striations, loss of a clear endomyometrial border and a heterogeneous myometrium which is reported to be the most predictive finding, raise the probability of the presence of adenomyosis[6]. MRI has similar sensitivity and specificity for diagnosing adenomyosis as TVS and it is recommended only for the cases where conservative management is planned and the differentiation between adenomyosis and uterine myomatosis is required[7]. Hysteroscopy guided biopsies improve the specificity of diagnosis from 60 to 89%[8]; however, it is an invasive procedure with high costs and not a common practice that should be reserved for clinical situations in which a malignancy needs to be excluded.

It is estimated that adenomyosis is present in 20 to 35% of women[9]. Although the disease has been deemed the disease of middle-aged, multiparous women, the disease is increasingly diagnosed in young women and in infertility patients[10]. It is surprising that the awareness of the disease is poor as there are relatively few studies for a disease that has a very high prevalence and unfortunately there are still no international guidelines to follow for preoperative diagnosis and management of this disorder[11]. The preoperative diagnosis of adenomyosis, which has still been diagnosed histopathologically, would prevent unnecessary therapies, loss of time and use of resources in vain. Therefore, precise prediction of this disease without surgery gains importance. Developing a scoring system with clinical evaluation for this purpose will be very helpful in solving this problem. From this point, we developed a scoring system that will predict the presence of adenomyosis with high sensitivity using clinical evaluation methods such as history, physical examination, ultrasonography and laboratory tests.

Materials and Methods

A prospective cohort study was conducted in Mersin University Faculty of Medicine, Department of Obstetrics and Gynecology between 10.02.2017 and 10.08.2017 with 232 patients who had undergone hysterectomy for benign disorders. The indications for hysterectomy were leiomyoma, dysfunctional uterine bleeding, which was resistant to medical therapy, adnexal mass, cervical and endometrial pathologies, postmenopozal bleeding, dysmenorrhea, dyspareunia or pelvic pain, pelvic abcess and uterine prolapse. Patients with postoperative diagnosis of gynecologic malignancies and who were pregnant were excluded. Ten patients with postoperative diagnosis of malignancies and 1 patient with coexisting pregnancy were excluded and the remaining 221 patients were enrolled. The minimum number of patients to be included in this prospective study was calculated with power analysis. To calculate the minimum number of patients, the number of hysterectomies performed in the clinic during the first 6 months of the previous year, 2016, was obtained (240 cases). It was calculated to reach at least 40% of the population to predict the population in 2017[12]. To develop a scoring system that can be an alternative to the histopathological evaluation in the diagnosis of adenomyosis, the aim was to develop a scoring system that is 0.9 compatible with the histopathological results and with this purpose the required minimum number of cases was calculated to be 221 with 0.05 type 1 error and 0.2 type 2 error (80% power)[13]. Mersin University Clinical Trials Ethics Committee approved the study (2017/22) and informed consent was obtained from each patient.

The patients who were admitted to the hospital with the hysterectomy indications for benign pathologies were visited before the operation and detailed anamnesis was obtained. Physical and pelvic examinations with transvaginal ultrasound were examined by the same investigator. Demographic characteristics, obstetric and gynecologic histories were noted. The amount of perceived pain was measured using visual analog scale (VAS)[14]. During pelvic examination uterine size with more than 10 weeks gestational age was considered enlarged[15]. Observing myometrial cysts, enlarged uterus, heterogeneous myometrium and or focal nodular areas during TVS was considered to suggest adenomyosis[16]. The uterus was measured in the anteroposterior, longitudinal, and transverse planes. The uterine volume was calculated using the ellipsoid algorithm. The laboratory results were noted.

The hysterectomy specimens were evaluated by the department of pathology. The diagnosis of adenomyosis was based on the presence of glandular extension ≥2.5 mm below the endometrial myometrial interface[17] and routine endometrial sampling was performed from 4 sites if there were no additional pathologies. Pathologic results were accepted as the definitive diagnosis.

Statistical Analysis

Statistical analysis was accomplished with SPSS (version 11.5, Illinois, Chicago, USA). The normality of the data was tested both with visual methods, including histograms and probability plots and Kolmogorov-Smirnov test. Normally distributed data were expressed as mean ± standard deviation and non-normally distributed data were expressed as median interquartile range. Student t-tests, Mann-Whitney U tests were used for comparisons where appropriate. Categorical parameters were expressed as number (%) and compared with chi-square test. To obtain the scores to predict the presence of adenomyosis, the odds ratios (OR) that were calculated from the binary logistic regression analysis in which adenomyosis was assumed to be the dependent variable were used. The variables found to be significant were chosen for the scoring system. Receiver operating characteristic (ROC) analysis was carried out to find the cut-off values for these variables. The variables which were not found to be significant in logistic regression analysis but known to be associated with adenomyosis and were found to be significantly different from the cases without adenomyosis in the univariate analysis, were also included in the binary logistic regression analysis in which adenomyosis was taken as the dependent variable (present/absent). OR were calculated for these variables that were found to be significant. For variables other than menarche, reference group was taken as the first group, the reference score was assigned as 0 and the OR were calculated accordingly. In the menarche variable the reference group was assigned as the last group and the reference score was assigned as 0. The sum of the maximum OR that a person can obtain was assumed to be 100 percent and a coefficient was calculated to convert the sum of OR to percentages to predict the presence of adenomyosis. The sum of the OR that a patient obtains was multiplied with this coefficient to get the adenomyosis risk percentage. The statistical significance was set at $p<0.05$.

Results

Adenomyosis was diagnosed in 24.9% (n=55/221) of the patients. The most common complaints were pelvic pain (27.1%) and menometrorhagia (22.2%), and the most common indications for hysterectomy were leiomyomas (29.4%) and abnormal uterine bleeding (14%).

The comparison of the demographic characteristics of the patients with and without adenomyosis is provided in Table 1. Groups were similar with respect to assessed parameters. The comparison of patterns of menstrual bleeding and perceived pain VAS scores are shown in Table 2. The mean age of menarche of the patients with adenomyosis was significantly lower compared to the patients without adenomyosis (13.2±1.7 vs 13.8±1.5 years, p=0.031). The groups were similar with respect to menstrual cycle length, menstrual flow duration and rate of intermenstrual bleeding; however, the number of sanitary pads per day (5.3±2.5 vs 4.5±2.6, p=0.004) and need for diaper usage (32.5% vs 47.3%, p=0.036) were significantly higher in the patients with adenomyosis (Table 2). Similarly, median dysmenorrhea and dyspareunia VAS scores were significantly higher in the adenomyosis group [3 (6) vs 2 (4), p=0.016 and 2 (4) vs 0 (2.3), p=0.007, respectively].

Table 1. Comparison of the demographic characteristics of patients with and without adenomyosis

	Adenomyosis (n=55)	No adenomyosis (n=166)	p
Age (years)	50.6±7.8	51.1±8.8	0.869
BMI (kg/m^2)	30.6±5.1	29.9±5.1	0.369
Gravidity	4 (2)	3 (3)	0.281
Parity	3 (2)	3 (2)	0.101
Vaginal delivery (n)	3 (3)	2 (3)	0.224
Cesarean section (n)	0 (0)	0 (0)	0.892
Surgical abortion number	1 (1)	1 (2)	0.772
Smoking status	50 (30.1%)	14 (25.5%)	0.316
Previous myomectomy	6 (3.6%)	4 (7.3%)	0.117
COC history	37 (22.3%)	13 (23.6%)	0.485
History of intrauterine device	66 (39.8%)	19 (34.5%)	0.3

BMI: Body mass index, COC: Combined oral contraceptive, Data were expressed as mean ± standard deviation, median (interquartile range), number and percentage. $p<0.05$ was considered significant

In the pelvic examination the incidence of a large uterus (>10 gestational weeks large) and uterine tenderness were significantly higher in the patients with adenomyosis (Table 3). The ultrasonographic findings showed increased uterine volume in the adenomyosis patients [180 (155) vs 122 (164) cm^3, p=0.041]. In 50.9% of the patients with adenomyosis, heterogeneous myometrium was observed with ultrasonography that was only present in 3.6% of the patients without adenomyosis (p<0.0001). Similarly, more patients with adenomyosis had myometrial cysts detected with sonography (20% vs 5.4%, p=0.002) (Table 3). Concomitant leiomyoma was present in 32.7% of the patients with adenomyosis (Table 3).

The groups were similar with respect to laboratory complete blood count results. The hemoglobin, hematocrit values and platelet counts in the patients with and without adenomyosis were 12.4±1.4 g/dL, 38±3.4% and 320.000±99.000 mL and 12.3±1.8 g/dL, 37.8±4.5% and 303.000±70.000 mL respectively (p>0.05 for all). Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios were also similar (data not shown).

In a pathologic examination the median weight of the uteruses was found to be 181 (111) g in the adenomyosis patients, which was 145 (139) g in the patients without adenomyosis (p=0.044). Histopathologically confirmed concomitant endometrioma was significantly more common in patients with adenomyosis compared to the patients without adenomyosis (9.1% vs 2.4%, p=0.045).

To develop a scoring system a regression analysis was carried out to find the parameters that were associated with the presence of adenomyosis. Number of parity, dyspareunia and dysmenorrhea VAS scores, age of menarche and detection of heterogeneous myometriums during ultrasonography were found to be the significant parameters. OR were calculated for these independent factors (Table 4). With ROC analysis cut-off values were calculated; 3 for parity, 13 years for menarche, 2 for dyspareunia VAS scores and 4 for dysmenorrhea VAS scores (Table 4). Variables that were found to be significantly different in the univariate analysis were also analyzed within each other and the presence of uterine tenderness and detection of myometrial cysts with ultrasonography was also found to be independent predictors of adenomyosis (respectively β=2.225, p=0.09;

Table 2. The comparison of patterns of menstrual bleeding and perceived pain VAS scores

	Adenomyosis (n=55)	No adenomyosis (n=166)	p
Menarche (years)	13.2±1.7	13.8±1.5	0.031*
Menstrual cycle (days)	29.3±4.8	28.6±3.1	0.831
Menstrual flow duration (days)	7.3±3	5.7±2	0.831
Intermenstrual bleeding	41 (24.7%)	19 (34.5%)	0.107
Number of sanitary pads used per day	5.3±2.5	4.5±2.6	0.004*
Need for diapers usage	26 (47.3%)	54 (32.5%)	0.036**
Dysmenorrhea VAS	3 (6)	2 (4)	0.016*
Dyspareunia VAS	2 (4)	0 (2.3)	0.007*

VAS: Visual analog scale, Data were expressed as mean ± standard deviation, median (interquartile range), number and percentage. p<0.05 was considered significant
*: statistically significant, t-test
**: statistically significant, chi-square test

Table 3. Comparison of pelvic examination findings and transvaginal ultrasonographic findings in patients with and without adenomyosis

	Adenomyosis (n=55)	No adenomyosis (n=166)	p
Large uterus (>10 gestational weeks)	32 (58.2%)	68 (41%)	0.019**
Uterine tenderness	7 (12.7%)	2 (1.2%)	0.001**
Uterine volume (cm^3)	180 (155)	122 (164)	0.041*
Presence of heterogenous myometrium	28 (50.9%)	6 (3.6%)	<0.0001**
Presence of myometrial cysts	11 (20%)	9 (5.4%)	0.002**
Presence of leiomyoma	18 (32.7%)	66 (39.8%)	0.221

Data were expressed as mean ± standard deviation, median (interquartile range), number and percentage. p<0.05 was considered significant
*: statistically significant, t-test
**: statistically significant, chi-square test

Table 4. Clinical scoring system for prediction of adenomyosis

	Risk factor	Score
Parity	≤3	0
	>3	2.8
Age of menarche	≤13	1.6
	>13	0
Dysmenorrhea VAS score	≤4	0
	>4	1.2
Dyspareunia VAS score	≤2	0
	>2	1.9
Heterogenous myometrium	No	0
	Present	27.2
Myometrial cyst	No	0
	Present	3.6
Uterine tenderness	No	0
	Present	9.3

VAS: Visual analog scale

OR=9.250, 95% confidence interval: 0.75-48.830 and β=1.29, p=0,013, OR: 3.631, 95% confidence interval:1.316-10.020). These two parameters were also included in the scoring system. The total sum of maximum OR that a patient can obtain was calculated as 47.6 and this value was assumed to predict the presence of adenomyosis 100%. To find a coefficient to convert the sum of OR to percentages, 100 was divided by 47.6 and 2.1 was found as the coefficient. Finally, multiplication of the sum of the OR in a patient by 2.1 was found to have a predictive ability for the presence of adenomyosis.

Discussion

This study aimed to develop a simple and useful clinical scoring system to predict the presence of adenomyosis that has remained a histopathological diagnosis. Preoperative prediction of this benign disease would provide initiation of targeted medical therapies and the need for radical surgeries would decrease. In literature the risk factors have been identified; however, there are still no effective preinterventional diagnostic methods. In this prospective study the patients who were to undergo hysterectomy had been assessed preoperatively and based on the histopathological results, the preoperative diagnostic effectiveness of each factor associated with adenomyosis had been revealed. A clinical predictive scoring system was developed using parity, age at menarche, VAS scores of dysmenorrhea and dysparaneu, detection of heterogeneous myometrium and myometrial cysts.

Parity has been suggested to be a risk factor for adenomyosis. The hormonal milieu and the myometrial trophoblastic invasion are the proposed mechanisms[18]. Prior uterine surgeries including cesarean sections and intrauterine interventions have been reported to be associated with adenomyosis due to the disruption of endometrial - myometrial border[19] in some studies; however, other studies did confirm these results[20]. We showed that parity, if more than three, increased the risk of adenomyosis significantly. The incidence of adenomyosis was not different in patients who had undergone prior cesarean section, myomectomy or curettage in this study. Although the invagination of the endometrial tissue into the weakened myometrium resulted from prior surgical trauma is one of the proposed mechanisms[21]; it is not enough to explain all the clinical pictures. Adenomyosis may develop *de novo* from embryological misplaced pluripotent Müllerian remnants, invagination of the basalis proceeds along the intramyometrial lymphatic system may lead to adenomyosis and adenomyosis may originate from bone marrow stem cells that are displaced through the vasculature[22]. Therefore, a history of previous uterine surgery was excluded in the scoring system.

Younger age at menarche is another reported risk factor for adenomyosis. The mechanism is increased estrogen exposure[22]. In adenomyotic tissue higher expression of estrogen receptors has been shown[21]. The adenomyotic tissue also contains aromatase and estrogen sulphatase enzymes that locally produce estrogens[21]. A menarche age at or younger than 13 years increased adenomyosis risk by 1.6 times.

Dysmenorrhea and dyspareunia VAS scores were significantly higher in the patients with adenomyosis and cut-off scores that significantly have a predictive potential were calculated as 4 and 2 for dysmenorrhea and dyspareunia, respectively. Dysmenorrhea is a commonly assessed parameter and is found in 15-30% of the patients with adenomyosis. The proposed mechanisms are the hemorrhage and enlargement of the entrapped endometrium in the myometrium and or increased prostaglandin and eicosanoid synthesis in the adenomyotic tissue compared to the normal myometrial tissue[23]. Dyspareunia has been reported to be present in 7-10% of the patients with adenomyosis[24]. In this study both complaints have been found to be useful and significant predictors of adenomyosis; however, they are with relatively low OR and have emerged as the least influential factors on the scoring.

Today, imaging modalities have been started to be used more commonly in the differential diagnosis of adenomyosis. Especially, ultrasonography and MRI are prominent modalities[22]. Detection of heterogeneous myometrial echogenicity myometrial cysts and globularly enlarged uterus are the most commonly reported ultrasonographic findings[22,25]. The most predictive ultrasound finding is suggested to be the presence of myometrial heterogeneity[26]. Similarly, we found that the presence of myometrial heterogeneity, myometrial cysts and enlarged uterus were all significantly more common in the patients with adenomyosis. Detection of heterogeneous myometrium and myometrial cysts are found to be the predictive factors that were significant to be included in the scoring system. Specifically heterogeneous myometrial appearance alone

increases the risk of adenomyosis 27 times. Heterogeneity and myometrial cysts, which can be easily detected in experienced hands in ultrasonography, have a critical place in the prediction of adenomyosis in clinical evaluation. Studies comparing MRI, which is useful in the detection of adenomyosis, with TVUS, report that both methods yield similar results[25]. For this reason, TVUS, which is a cheaper and faster method, should be the preferred method.

In literature increased body mass index, oral contraceptive usage history and short menstrual periods, and cigarette smoking have been reported to be associated with adenomyosis as they all affect estrogen exposure[22]. However, in this study none of these parameters have been associated with adenomyosis. Tamoxifen treatment is also reported to be a risk factor for adenomyosis[27]. Unfortunately the number of patients under this medication in the assessed population was not enough to make a statistical analysis.

Heavy menstrual bleeding is the most common finding of adenomyosis, seen in approximately 40-60% of patients. This may be secondary to the increased endometrial surface of the enlarged uterus or to increased vascularization of the endometrial layer[24]. Other suggested reasons are inappropriate uterine contractions during menstrual periods and excess prostaglandin and estrogen production[28]. When the bleeding characteristics related to the presence of adenomyosis were examined, it was seen that the number of pads and diaper usage rates were significantly higher in the adenomyosis group, which is similar to the literature[29]. However, hemoglobin and hematocrit values, which reflect the amount of bleeding, did not differ in patients with and without adenomyosis. Again, none of the parameters related to bleeding were found to have significance to be included in the clinical scoring.

Studies have shown that the number of samples taken from pathological specimens affects the rates of adenomyosis diagnosis. The frequency reported in hysterectomy materials may vary depending on the number of sections and histopathological criteria. For example, when three routine sections were taken, adenomyosis was diagnosed in 31% of the hysterectomy samples, while taking six sections increased the rate to 61%[30]. In our pathology clinic, we make four sections in routine examination. Therefore, there is a theoretical possibility that existing adenomyosis cases could have been missed. However, the fact that the pathologist evaluating all specimens is a single person and that she is an experienced person dealing only with gynecopathology for many years eliminates the validity of this limitation. Again, a second issue that may be a limitation is that ultrasonographic evaluation and pelvic examination may differ between researchers due to the potential for variability. To overcome this limitation, all pelvic and ultrasonographic examinations were undertaken by the same person.

The study strengths, on the other hand, are the determination of the number of subjects by performing power analysis and including the determined number of subjects, the clinical evaluations were carried out by the same person, and all specimens were evaluated by the same experienced pathologist. A prediction model is more accurate when the overall probability reaches to ≥80%[31], therefore probability more than 80% may guide the management.

Conclusion

In conclusion adenomyosis is a common disease which has still been diagnosed histopathologically. To predict adenomyosis noninvasively, methods based on clinical evaluation with high sensitivity and specificity are needed. In our study, we have created a clinical scoring system for this purpose. In this scoring system, there are simple parameters that can be easily used by the clinician, have a low cost and are repeatable. The effect of each parameter on predicting adenomyosis is different, and the total effect can be calculated according to the answers to be given to all questions. In this simple scoring system, parity, menarche, VAS scores of dysmenorrhea and dyspareunia, myometrial heterogeneity in ultrasonography and presence of tenderness during pelvic examination was found to be useful parameters in predicting the diagnosis of adenomyosis. This prospective cohort study had an adequate sample size with a-80% power and was carried out by the same investigators and an experienced pathologist which all constituted the study strengths. The main limitation was the potential variability in ultrasonographic and pelvic examinations. This scoring system should be validated in the future, its reliability should be evaluated and the aspects that need to be improved, if any, should be revealed.

Ethics

Ethics Committee Approval: Mersin University Clinical Trials Ethics Committee approved the study (2017/22).

Informed Consent: Informed consent was obtained from each patient.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.Y., H.A., H.D., İ.G., Concept: M.Y., H.A., Design: M.Y., H.A., Data Collection or Processing: M.Y., H.A., H.D., İ.G., Analysis or Interpretation: M.Y., H.A., H.D., İ.G., Literature Search: M.Y., H.A., Writing: M.Y., H.A.

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Does "no-touch" technique hysteroscopy increase the risk of infection?

"No-touch" teknik histeroskopi enfeksiyon riskini artırır mı?

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Abstract

Objective: Today, thanks to its many advantages, hysteroscopy with a vaginoscopic approach (no-touch) is increasingly being used more in outpatient diagnoses and treatments. However, there are concerns that the "no-touch" technique increases ascending genital tract infections since a speculum is not inserted, and disinfection of the cervix cannot achieve.

Materials and Methods: Between 2011 and 2017, 302 patients who underwent office hysteroscopy with the vaginoscopic approach (group 1) and 254 patients who underwent hysteroscopy with the standard method under anesthesia in the operating room (group 2) were compared in terms of early complications (within two weeks postoperatively). The primary outcome was early postoperative infection, and the secondary outcome was other early complications, such as bleeding and rupture.

Results: In this study, the success rate of hysteroscopy with the vaginoscopic approach was 96.4%. According to the visual analog scale scoring system, 88.7% of the patients described mild-to-moderate pain. When group 1 and 2 were compared in terms of postoperative infection (3% and 2.4%, respectively) and other early complication rates (0% and 0.8%, respectively), no statistically significant difference was found (p>0.05).

Conclusion: Hysteroscopy with a vaginoscopic approach continues to be the gold standard method that is safe and well-tolerated by patients.

Keywords: Hysteroscopy, vaginoscopy, office hysteroscopy, complication, infection

Öz

Amaç: Günümüzde pek çok avantajı nedeniyle, vajinoskopik (no-touch) yaklaşımla histeroskopi, ayaktan tanı ve tedavilerde, giderek daha fazla kullanılmaktadır. Ancak spekulum yerleştirilmediği ve serviksin dezenfeksiyonu sağlanamadığı için "no-touch" tekniğinin asendan genital sistem enfeksiyonlarını artırdığına dair endişeler mevcuttur.

Gereç ve Yöntemler: 2011-2017 yılları arasında vajinoskopik yaklaşımla ofis histeroskopisi yapılan 302 hasta (grup 1) ile ameliyathanede, anestezi altında standart yöntemle histeroskopi yapılan 254 hasta (grup 2) erken (postoperatif 2 hafta içindeki) komplikasyonlar açısından karşılaştırıldı. Birincil sonuç erken postoperatif enfeksiyondu, ikincil sonuç ise kanama ve rüptür gibi diğer erken komplikasyonlardı.

Bulgular: Bu çalışmada vajinoskopik yaklaşımla histereskopinin başarı oranı %96,4 olarak bulundu. Görsel anolog skala skorlama sistemine göre hastaların %88,7'si hafif ve orta şiddette ağrı tanımladı. Grup 1 ve grup 2, postoperatif enfeksiyon (sırasıyla %3 ve %2,4) ve diğer erken komplikasyon oranları (sırasıyla %0 ve %0,8) açısından karşılaştırıldığında aralarında istatistiksel olarak anlamlı fark bulunmadı (p>0,05).

Sonuç: Vajinoskopik yaklaşımla histereskopi, güvenli ve hastalar tarafından iyi tolere edilen, altın standart yöntem olmaya devam etmektedir.

Anahtar Kelimeler: Histereskopi, vajinoskopi, ofis histereskopisi, komplikasyon, enfeksiyon

PRECIS: In this study, we evaluated whether the risk of infection increases in no-touch vaginoscopic hysteroscopy compared to the standard method.

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Introduction

Hysteroscopy is accepted as the gold standard and minimally invasive method in the diagnosis and treatment of intrauterine pathologies[1]. Inserting a speculum, cleaning the cervix, applying a tenaculum to the cervix, and initiating cervical dilatation in traditional hysteroscopy are painful procedures and are performed in the operating room, under local or general anesthesia[2]. Since 1990, office hysteroscopy (outpatient hysteroscopy) has been increasingly used by clinicians with the concept of "see and treat" intrauterine pathologies in the same session[3]. Over time, the vaginoscopic "no-touch" technique[4] has proven to be better tolerated than the traditional technique in the office setting, without the use of anesthesia or analgesia[5]. With small-diameter, continuous-flow hysteroscopes manufactured with the latest technological advances, office hysteroscopy has become safe, efficient, inexpensive, less invasive, and less painful without the risk of anesthesia[6]. However, there are also concerns that the vaginoscopic approach is more likely to result in postoperative genital tract infections because the cervix is not cleaned before the hysteroscope being inserted into the uterine cavity[5]. In this study, we evaluated whether office hysteroscopic procedures with the vaginoscopic approach, which are increasingly used, are associated with an increase in infections and any other early complications (such as bleeding, uterine perforation and fluid overload) compared with the traditional method.

Materials and Methods

This study was conducted retrospectively at a tertiary referral university hospital. Ethical approval was obtained from the institutional ethics committee (approval number: 2020.07.2.07.108). By examining patient records, 302 women who underwent office hysteroscopy with the vaginoscopic approach between 2011 and 2017 were considered group 1. In the same data range, 254 women who underwent hysteroscopy with the standard method under local or general anesthesia in the operating room were designated as group 2. Diagnostic or operative hysteroscopy was planned for the patients with various indications, such as abnormal uterine bleeding, submucosal fibroids, increased endometrial thickness, uterine anomalies, and intrauterine devices that cannot be removed. Diagnostic or operative hysteroscopy was also planned for those with recurrent pregnancy loss, unexplained infertility, endometrial and cervical polyps. Office hysteroscopy with the vaginoscopic approach was first offered to patients who were prescribed diagnostic or short-term hysteroscopic procedures. Hysteroscopy under direct general or local anesthesia was planned for women with intracavitary fibroids or polyps larger than 2 cm, for those who preferred the procedure under anesthesia, and for those who had a history of severe cervical stenosis or vaginismus. Informed consent of the patients was obtained for the procedure. Suspected pregnancy, heavy uterine bleeding, ongoing vaginal infections, pelvic inflammatory disease, and a history of cervical or endometrial premalignant lesions were the exclusion criteria. All procedures were performed in the early proliferative phase of the menstrual cycle in the premenopausal patients. There were no restrictions regarding the day of the procedure in the postmenopausal patients. All the women were informed that menstrual-type cramping may occur during or after the procedure. They were assured that the hysteroscopy could be terminated at any time upon their request. All office hysteroscopy procedures were performed using the vaginoscopic approach by three experienced gynecologists following aseptic rules, in the gynecological position, without analgesia or anesthesia, without vaginal disinfection, without the use of a vaginal speculum and tenaculum[4]. A 2.9 mm diameter, 30° lens system, 5 mm outer diameter continuous flow Bettocchi® hysteroscope was used for outpatient procedures (Karl Storz SE&Co. KG, Germany). For the diagnostic and surgical procedures performed under anesthesia in the operating room, a 4 mm diameter, 12° lens system continuous flow Hopkins® hysteroscope with an 8.7 mm resectoscope sheath was used (Karl Storz SE&Co. KG, Germany). The uterine cavity was inflated with 0.9% normal saline solution at a pressure below 60 mm Hg, controlled by an electronic irrigation pump (Endomat, Karl Storz SE & Co. KG, Germany). Illumination was provided by a high-intensity cold light source provided by a fiber optic lead.

The hysteroscope was inserted into the lower vagina, then inflated with the distention medium for vaginoscopy. Fornixes and vaginal walls were examined. After the external cervical ostium was identified, the instrument was inserted into the cervical canal and directed towards the uterine cavity. The cervix, cervical canal, uterine cavity, tubal ostia and endometrium were examined. The patient was in communication with the surgeon and, could report any discomfort or pain during the office hysteroscopy. Patients were also encouraged to watch images of the procedure on the monitor. When the pain became unbearable for the patient, office hysteroscopy was stopped and postponed to be performed under anesthesia in the operating room at a later time. Pain was assessed on a 10 cm visual analog scale (VAS) (0 for no pain and 10 for the worst imaginable pain). Since the procedures are short and simple in office hysteroscopy, fluid deficit was not calculated. After the procedure, the patients were observed for any side effects for at least one hour and then discharged.

Every patient in group 2 was placed under anesthesia after standard aseptic preoperative conditions were provided. Once anesthetized, the speculum was inserted. The vagina and cervix were disinfected with a betadyn sponge. The cervix was grasped with a teneculum. Adequate cervical dilatation was provided with dilators according to the necessity of the procedure. The VAS scoring system was not applied to the patients in group 2. The fluid deficit was carefully studied during the operative hysteroscopic procedures. The patients were discharged after being observed in the hospital for 24 hours.

Pharmacological cervical preparation, urinary catheter and antibiotics were not used in any patient in either group. Patients who required antibiotic treatment due to urinary tract infection, vaginal discharge, fever, pelvic pain and fever above 38 °C within two weeks after the procedure were recorded. The primary study outcome was defined as infection due by office hysteroscopy with the vaginoscopic approach. The secondary study outcome was defined as other procedure-related complications.

Statistical Analysis

Mean standard deviation, median, and interquartile range were given for descriptive statistics for continuous data, and number and percentage values were given for discrete data. The Shapiro-Wilk test was used to examine the conformity of continuous data to a normal distribution. The Mann-Whitney U test was used for comparisons between groups of continuous variables. The chi-square test was used for comparisons of categorical variables between groups. The IBM SPSS Statistics 20.0 program was used in the evaluations and $p<0.05$ was accepted as the statistical significance limit.

Results

A total of 556 patients between the ages of 19 and 80 (mean 38.17±11.24) participated in the study. For their diagnoses, endometrial sampling and, where possible, therapeutic hysteroscopy without anesthesia with the vaginoscopic approach (group 1) in the office setting were performed on 302 of the patients. The hysteroscopy on every patient in group 1 was completed in approximately 15 minutes. There were 254 patients who did not accept the diagnostic office hysteroscopy procedure without anesthesia or if the operative hysteroscopy procedure was complicated enough to require cervical dilation and would take a long time were performed under regional or general anesthesia in operating room conditions (group 2). The main characteristics of each patient are shown in Table 1. The distributions of the patients diagnoses are shown in Table 2. A comparison between group 1 and group 2 shows that the group 1 patients were statistically younger ($p<0.001$), parity and the number of children was lower ($p<0.05$), and the nulliparity rate was higher ($p<0.05$) because the pathologies that will require operative hysteroscopy will be encountered more frequently as age progresses, and the mean age of group 2 was higher due to the atrophic cervixes of the menopausal patients.

Most patients (57.9%) in the office hysteroscopy group (group 1) reported moderate pain (4≤ VAS score <7), 30.8% mild (VAS score <4) and 11.3% severe (VAS score ≥7) (Table 3). Additionally, when the VAS scores of the patients in the reproductive period who underwent office hysteroscopy were compared with the patients in menopause, no statistically significant difference was found between the median values ($p<0.05$) (Table 4). The completion of the procedure was defined as complete hysteroscopy with an acceptable pain level for the patient without intraoperative complications. The procedure was completed in 96.4% (n=291) of the patients who underwent hysteroscopy without anesthesia with the vaginoscopic approach (group 1). An incomplete hysteroscopy was defined as the inability to enter the uterine cavity through the cervical canal in a sufficient time, failure to perform a full systematic examination of the uterine cavity, or the planned procedure. Since vasovagal reactions were observed in six patients (2%) in group 1, the procedure could not be completed and they recovered completely after bed rest and hydration. In group 1, five patients could incomplete the procedure due to pain, anxiety and poor imaging. There was no morbidity

Table 1. Comparison of main characteristics of the 556 patients

		Total (n=556)	Group 1 (n=302)	Group 2 (n=254)	p-value
Age (year)	Mean ± SD Median (Min-Max)	38.17±11.24 36 (19-80)	36.34±9.53 35 (19-80)	40.33±12.27 37 (20-80)	**<0.001***
Gravida	Median (Min-Max)	2 (0-9)	2 (0-9)	2 (0-9)	0.912*
Parity	Median (Min-Max)	1 (0-8)	1 (0-8)	2 (0-8)	**0.012***
Number of children		1 (0-7)	1 (0-5)	1 (0-7)	**0.013***
Period n (%)					
Premenopausal		464 (83.5)	270 (89.4)	194 (76.4)	**<0.001****
Postmenopausal		92 (16.5)	32 (10.6)	60 (23.6)	
Type of delivery n (%)					
Nulliparity		170 (30.9)	106 (35.5)	64 (25.5)	**0.034****
Vaginal delivery		258 (46.9)	134 (44.8)	124 (49.4)	
Cesarean section		122 (22.2)	59 (19.7)	63 (25.1)	

*Mann-Whitney U test, **chi-square test, SD: Standard deviation, Min: Minimum, Max: Maximum

Table 2. Distribution of patients' indications of hysteroscopy

	Total n (%)	Group 1 n (%)	Group 2 n (%)
INDICATIONS	556	302	254
Increased endometrial thickness	109 (19.6)	87 (28.8)	22 (8.7)
Recurrent pregnancy loss	104 (18.7)	86 (28.5)	18 (7.1)
Abnormal uterine bleeding	102 (18.4)	36 (11.9)	66 (26)
Endometrial polyp	80 (14.4)	27 (8.9)	53 (20.9)
Unexplained infertility	48 (8.6)	32 (10.6)	16 (6.3)
Submucous myoma	30 (5.4)	4 (1.3)	26 (10.2)
Postmenopausal bleeding	26 (4.7)	3 (1.0)	23 (9.1)
Removal of intrauterin device	20 (3.6)	10 (3.3)	10 (3.5)
Asherman syndrome	16 (2.9)	8 (2.6)	8 (3.1)
Uterine anomaly	13 (2.4)	4 (1.3)	9 (3.6)
Niche	5 (0.9)	2 (0.7)	3 (1.2)
Recurrent in vitro fertilization failure	2 (0.4)	2 (0.7)	0
Rectovaginal fistula	1 (0.2)	1 (0.3)	0

Table 3. VAS distributions of patients who underwent office hysteroscopy

VAS	n (%)
<4	93 (30.8)
≤4-<7	175 (57.9)
≥7	34 (11.3)

VAS: Visual analog scale

Table 4. Comparison of VAS scores of patients who underwent office hysteroscopy with those in the reproductive period and those in the menopause period

	Reproductive n (270)	Menopause n (32)	p-value
VAS Median (Min-Max)	4 (1-9)	4 (2-9)	0.846*

*Mann-Whitney U test, VAS: Visual analog scale, Min: Minimum, Max: Maximum

requiring hospitalization due to any complication in group 1. In group 2, uterine rupture developed during synechiolysis in two patients with diagnoses of Asherman's syndrome. Since the bleeding was self-limiting and did not develop sufficiently to require surgical intervention, the patients were discharged after two days of hospitalization with full recovery. Nine patients in group 1 (three urinary tract infections, five increased vaginal discharges, one fever and pelvic pain) and six patients in group 2 (one urinary tract infection, three vaginal discharge, two fever and pelvic pain) were followed up within two weeks after the procedure with diagnoses of infection presumed to be due to the procedure. Three patients with fever and pelvic pain were hospitalized and the others recovered completely with outpatient antibiotic treatment. However, when the two groups were compared in terms of infection and complication rates, no statistical difference was found (p>0.05) (Table 5).

Discussion

The results of our study showed that the incidence of infection, uterine rupture, or bleeding did not increase in office hysteroscopy performed with the vaginoscopic approach. In our department, we perform hysteroscopic procedures with a vaginoscopic approach, without analgesia and anesthesia in office settings for most patients who apply to the gynecology outpatient clinic and require intracavitary imaging and intervention. In numerous retrospective and randomized studies to date, the vaginoscopic "no-touch" technique during an office hysteroscopy procedure is successful, less painful and faster compared with traditional techniques using a vaginal speculum and cervical grasped[7,8]. Moreover, those who have not had sexual intercourse, who are nulliparous, and who have genital tract atrophy or vaginismus benefit most from the vaginoscopic approach.

The probability of complications after hysteroscopy is 1-2.7% [9-11]. Although some studies have shown that there are fewer surgical complications in vaginoscopy than in standard hysteroscopy[7], it has been shown that there is no statistical difference in terms of surgical complications in others[12]. Serious complications, such as uterine perforation or bleeding are rare in the office setting, but vasovagal reactions occur in 2.3 and 9.0%[7,13]. In this study, we observed a 2% vasovagal reaction in the group that underwent hysteroscopy with the vaginoscopic approach, consistent with these findings.

Routine antibiotic prophylaxis is not recommended because the ascending infections (endometritis and urinary tract infections) do not increase since the cervix can be sterilized by inserting a speculum in standard hysteroscopy[14]. There are few studies reporting that the rate of infectious complications after hysteroscopic surgery is 0.18-1.5%[13]. After retrospective operative hysteroscopy, consisting of 21,676 procedures, the infection rate was reported as 0.01%[9]. However, in a prospective study of 2,116 cases (endometritis 0.9%, urinary tract infection 0.6%), it was found to be 1.42%[15]. the risk of endometritis ranges between 0.85% and 2.7% in the literature[16]. However, there are concerns about the risk of ascending genital tract infection since adequate disinfection cannot be performed before hysteroscopy with the vaginoscopic approach. In a randomized controlled study by Smith et al.[7] in 1,597 women in 2019, vaginoscopic hysteroscopy without disinfection was compared with standard hysteroscopy showing that the rate of genital tract infection did not increase in the vaginoscopy group. Tien et al.[12] disinfected the vagina and cervix with betadine-soaked cotton swabs before vaginoscopy and found that there was no significant difference in the genital tract infection rate between the vaginoscopy and standard hysteroscopy groups.

The probability of bleeding requiring intervention after hysteroscopic procedures were found to be quite low (0-0.61%) in various studies[9-11,17]. One of the most common complications of hysteroscopy (0.12%) is uterine perforation, while the most common bleeding cause is uterine perforation. Depending on the type of operation, the perforation may be partial or complete. Even vital organ damage may develop due to blunt or electrosurgical damage[9-11]. Uterine perforation is most commonly seen in Asherman's syndrome cases (4.5%)[10]. In this study, uterine rupture developed in only two patients with a diagnosis of Asherman's syndrome (0.4%), which was consistent with the literature. No uterine ruptures developed in the office hysteroscopy group. All these findings are compatible with the literature.

The reasons for not completing the hysteroscopic procedure with the vaginoscopic approach include pain, anxiety, cervical stenosis, excessive flexion of the cervix, vasovagal reaction, a retroverted uterus and adhesions[5,7,18]. Between 83% and 98% of diagnostic procedures can be successfully performed with office hysteroscopy[7,8,13,19,20]. However, there are studies reported that the success of outpatient hysteroscopy varies between 44% and 99.5%[21-23]. In this study, the failure rate for all outpatient hysteroscopy procedures was 3.6%, which is lower than 10% reported in the previously[24]. The reason for such a good result may be that the procedures included in our study were performed by gynecologists highly experienced in hysteroscopy. In the study of Campo et al.[21], it was shown that the procedures performed by experienced surgeons are less painful.

Most studies till date have compared the pain scores and success rates of the vaginoscopic approach and standard hysteroscopy. In most of these studies, it has been shown that the vaginoscopic approach causes a lower VAS score than standard hysteroscopy[7,25-27]. However, Sharma et al.[28] showed no difference in pain scores between vaginoscopy and standard hysteroscopy. In our department, we do not perform hysteroscopy in the outpatient setting for patients who require direct standard hysteroscopy or who cannot tolerate the vaginoscopic approach. In the outpatient clinic, we only perform hysteroscopy with the vaginoscopic approach. Therefore, we could not compare the pain scores between the two groups. In

Table 5. Comparison of the groups in terms of success and complications

	Total n (%)	Group 1 n (%)	Group 2 n (%)	p-value
Process completion				
Yes	540 (97.1)	291 (96.4)	249 (98)	0.240**
No	16 (2.9)	11 (3.6)	5 (2.0)	
Vasovagal reaction				
No		296 (98)		-
Yes		6 (2.0)		
Infection				
No	541 (97.3)	293 (97)	248 (97.6)	0.654
Yes	15 (2.7)	9 (3.0)	6 (2.4)	
Uterine rupture or bleeding				
No	554 (99.6)	302 (100)	252 (99.2)	0.208
Yes	2 (0.4)	0	2 (0.8)	

**chi-square/Fisher's Exact test

fact, 88.7% of the patients in this study defined the pain scores of office hysteroscopy with the vaginoscopic approach as mild-moderate. Additionally, it was determined that the patients' being in the reproductive period or menopause did not change their pain scores.

Fluid overload, another early but rare complication (<5%) of operative hysteroscopy, did not develop in any patient in this study[9-11]. No severe complications, such as infectious shock[29] or pelvic abscesses[30] were noted in our study.

It appears that hysteroscopy with the vaginoscopic approach does not show a statistically significant increase in the risk of complications, including the worrisome risk of infection. However, after rare cases of septic shock have been reported, we now prefer to perform vaginoscopy after cleaning the vaginal entrance with betadyn swabs.

The strengths of our study are the large number of patients from different age groups, the fact that all procedures were performed using the same devices and by a small group of surgeons. However, pain assessment was only possible in patients in the vaginoscopy group. The limitation of the study was that it was retrospective and only patients with registered infections were considered positive. Patients who could not be reached were considered negative for infection. Unfortunately, our groups were not similar in terms of diagnosis and patient characteristics as we did not perform the standard hysteroscopy that would require us to hold and dilate the cervix in the office.

Conclusion

Office hysteroscopy with a vaginoscopic approach is increasingly used in daily practice as technological developments and surgical experience increases. Although our results do not indicate that there would be an increased risk of infection and complications compared with standard hysteroscopy, care should be taken in terms of the risk of serious infection considering the rare cases in the literature. Vaginoscopy should be a routine method for outpatient hysteroscopy. In this way, the number of procedures performed under anesthesia will decrease, the duration of hospital stays will be shortened and the cost will decrease. Therefore, clinicians accustomed to standard hysteroscopy will also require training to become proficient in this technique. Women should be informed that there is a low risk of genital tract infections, which may necessitate antibiotic treatment within two weeks following the procedure. We believe that more accurate data can be obtained on this subject with well-planned, prospective studies with more cases in the future.

Ethics

Ethics Committee Approval: This study was conducted retrospectively at a tertiary referral university hospital. Ethical approval was obtained from the institutional ethics committee (approval number: 2020.07.2.07.108).

Informed Consent: Informed consent of the patients was obtained for the procedure.

Peer-review: Externally peer-reviewed.

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Review / Derleme

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Fertility preservation strategies for cancerous women: An updated review

Kanseri olan kadınlarda doğurganlığı koruma stratejileri: Güncellenmiş bir derleme

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Abstract

Due to the increase in cancer among young women, the risk of premature ovarian insufficiency with subsequent infertility has been raised. Fertility preservation restores reproductive potential along with increasing life expectancy in these patients. Given the articles on new options for treating cancerous women, we searched the keywords, including fertility preservation, in vitro maturation (IVM), and ovarian cryopreservation. This review focuses on the currently available procedures, including in (IVM) of retrieved immature oocytes, oocyte, embryo, and ovarian tissue cryopreservation (OTC). OTC is a helpful procedure that restores ovarian function and natural pregnancy. Also, we summarized the literature that reported the qualification of using the abovementioned procedures, comparing the cryopreservation methods including vitrification and slow freezing. Due to the impressive clinical development of OTC in cancerous patients, it is recommended as a standard treatment in cryopreservation strategies.

Keywords: Fertility preservation, in vitro maturation, ovarian tissue cryopreservation, ovarian tissue transplantation

Öz

Genç kadınlar arasında kanserin artması nedeniyle, erken yumurtalık yetmezliği ve ardından kısırlık riski artmıştır. Doğurganlığın korunması, bu hastalarda yaşam beklentisinin artmasıyla birlikte üreme potansiyelini geri kazandırmıştır. Kanserli kadınların tedavisinde yeni seçeneklerle ilgili makaleler göz önüne alındığında, doğurganlığın korunması, in vitro olgunlaştırma (IVM) ve yumurtalık kriyoprezervasyonu gibi anahtar kelimeleri aradık. Bu derleme, elde edilen immatür oositlerin IVM ve oositin, embriyonun ve yumurtalık dokusunun kriyoprezervasyonu (OTC) dahil olmak üzere şu anda mevcut olan prosedürlere odaklanmaktadır. OTC, yumurtalık fonksiyonunu ve doğal hamileliği geri kazanmaya izin veren faydalı bir prosedür olduğunu gördük. Ayrıca, vitrifikasyon ve yavaş dondurma dahil kriyoprezervasyon yöntemlerini karşılaştırarak, yukarıda bahsedilen prosedürleri kullanmanın yeterliliğini bildiren literatürü özetledik. OTC'nin kanserli hastalardaki etkileyici klinik gelişimi nedeniyle, OTC kriyoprezervasyon stratejisinde standart bir tedavi olarak önerilmektedir.

Anahtar Kelimeler: Doğurganlığın korunması, in vitro olgunlaştırma, yumurtalık dokusu kriyoprezervasyonu, yumurtalık dokusu nakli

Introduction

Cancer incidence in different age groups, especially in adolescents and young women, has shown a slight increase since the 1970s[1]. Although the survival rates from cancer have improved, cancer is still one of the leading health concerns, especially in young people[2]. Fertility preservation is an approach used to protect cancer patients from the risk of infertility due to medical treatments, as radiotherapy, chemotherapy, and surgery. Cancer therapies are, in fact, harmful to reproductive function. The treatments used in these patients correlated with a high percentage of losing follicular numbers, especially in young women[3]. Nowadays, this treatment allows us to maintain the reproductive potential

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of these patients using methods including, cryopreservation of oocytes, embryos[4], and ovarian tissue (OT)[5] transposition of the ovaries before radiation[6], or in vitro culture (IVC) of ovarian follicles[7]. As recommended by the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology, embryo/oocyte cryopreservation a known technique in fertility preservation[8]. Nevertheless, embryo cryopreservation needs a sperm source, it is not a suitable option for single women. Also, there are other numerous limitations to embryo production, cryopreservation, and storage due to ethical, religious, and social reasons[4]. However, vitrification of oocytes recovered from stimulated in vitro fertilization (IVF) cycles causes a delay in cancer treatment, due to the time necessary for controlled ovarian hyperstimulation (COH)[9]. Other fertility preservation techniques, such as ovarian tissue cryopreservation (OTC) and in vitro maturation (IVM) of immature oocytes, can be implemented immediately in cancer therapy, even in underage girls[9]. The purposes of this review are to explain the up-to-date knowledge about current developments of IVM, the clinical employment of OTC, and transplantation in cancerous women.

Methodology

MEDLINE-PubMed (http://www.ncbi.nlm.nih.gov/PubMed), Google Scholar (https://scholar.google.com/), Scopus (https://www.scopus.com) and ISI web of science (http://apps.lib.wosg.ir/WOS) databases were applied for extracting available human original and review studies, from 2010-August 2021. The keywords were used: "fertility preservation," "in vitro maturation," "ovarian cryopreservation" and "ovarian transplantation." We mentioned 75 pieces of scientific literature consisting of reviews, original, guidelines, and recommendations, which have addressed the issue of fertility cryopreservation recently.

IVM in Reproductive Medicine

Indications of IVM

In the IVM process, the immature germinal vesicle (GV) and metaphase I (MI) stage oocytes (Table 1) were retrieved with a minor or no gonadotropin stimulation[9]. IVM was first applied in patients with polycystic ovarian syndrome with gonadotropin stimulation to avoid ovarian hyperstimulation syndrome[10]. Furthermore, IVM is a useful technique for patients who are concerned about the long periods of hormonal stimulation, and in cycles of recurrent oocyte maturation arrest, poor embryo quality, or IVF failures[11]. IVM may also be suitable for women who cannot have sufficient time to obtain fully mature oocytes before cancer therapy. IVM avoids the increased estrogen levels in women with hormone-sensitive tumors, which are seen in COH cycles. Retrieving immature oocytes and cryopreservation of them is a way for these women to preserve their reproductive ability in the future[12].

Oocyte Maturation

Nuclear and cytoplasmic maturation are two essential processes for oocyte maturation. The steps of nuclear maturation can be referred to as the meiotic resumption, indicated by the germinal vesicle breakdown, chromatin aggregation, the organization of the meiotic spindle, chromosome separation with the extrusion of the first polar body, progression to MII, and meiotic re-arrest before fertilization[13]. Cytoplasmic maturation is necessary to obtain a capacity for insemination, and early embryogenesis, subsequently, it provides conditions for implantation, pregnancy, and normal fetal development. This process includes numerous metabolics such as the accumulation of mRNA, proteins and substrates which all are needed to achieve the oocyte developmental competence and structural variations in the organelle typology and distribution for the proper fertilization and early embryo development[14].

Molecular Mechanism of *in vivo* Oocyte Maturation

Oocyte maturation *in vivo* is an intricate mechanism regulated through hormonal pathways, interactions with circumambient somatic cells, and gene expression, which is regulated by transcription factors. The elevation of cyclic adenosine 3', 5'-monophosphate (cAMP) levels can prevent oocyte maturation. The high intra-oocyte cAMP concentration inactivates the meiosis promoting factor (MPF), thus blocking meiotic development. A drop in the cAMP levels stimulates the luteinizing hormone (LH) surge, resulting in the oocytes being released from the inhibitory milieu of the follicle, and maturation occurs. There are three sources of high cAMP levels within the oocyte. It includes the oocyte itself via G-protein coupled receptors on the oolemma[15], cumulus cells (CCs) through the gap junctions, which is necessary for connecting cytoplasm and nuclear maturation[10], and guanosine 3,5-cyclic monophosphate (cGMP), which is produced in the mural and CCs, crosses through gap junctions into the oocyte and inhibits cAMP hydrolysis by the oocyte-specific phosphodiesterase 3A[16]. Is mentioned that cAMP and cGMP, are the main molecules that play a key role in controlling mammalian oocyte meiosis. After the LH surge, another factor induced by mural granulosa cells is the epidermal growth factor (EGF). LH activation of mural granulosa cells induces the expression of the EGFs binding to their receptors in CCs; thus, mitogen-activated protein kinase (MAPK) is activated. The increased activation of MAPK may lead to the synthesis of meiosis resumption-inducing factor (s) and the blocking of gap junctions via a gap junction protein[17]. Also, hyaluronan is synthesized by hyaluronan synthase (HAS2) in the plasma membrane and directly extends into the mucous-elastic extracellular matrix (ECM). After that, the COCs are interrupted, which cessation the transportation of cAMP and resulting in the activation of MPF. Furthermore, oocytes secret soluble factors, such as growth differentiation factor-9 (GDF-9), bone morphogenetic protein 15 (BMP-15), and BMP-6. These growth factors stimulate the *HAS2* gene expression and

cumulus expansion in the presence of the follicle-stimulating hormone (FSH)[18]. In standard IVM cycles, the immature COCs are isolated from antral follicles and subsequently saturated in a culture medium without cAMP-modulating agents. Standard IVM mediums typically include FSH or other additives such as EGF, EGF-p, and/or LH/hCG. In this system, FSH significantly improves MII rates, intra-oocyte cAMP levels decrease, and stimulation of the meiotic process begins.

IVM Laboratory Procedures

The laboratory procedure for IVM cycling is time-consuming and technically challenging. First, the COCs were collected from a follicular environment by searching them into a Petri dish under a stereomicroscope or using a cell strainer composed of nylon mesh with 70-µm pores to collect more oocytes with a small number of CCs. All handling procedures should be performed in optimal conditions such as warm stages or plates at 37 °C. In the IVM cycles treated with human chorionic gonadotropin (hCG) priming, *in vivo* matured oocytes may be retrieved at oocyte collection. However, in the IVM cycles without hCG priming, *in vivo* matured oocytes cannot be recovered on the day of retrieval. The retrieved COCs are usually transferred to an IVM culture medium supplemented with hormones and growth factors. They were cultured for 24-30 h (day 1) to 48 h (day 2) then, the matured oocytes were cryopreserved or inseminated with partner spermatozoa[19,20].

IVM Culture Medium

Special culture media as the essential IVM media have been applied for both research and clinical purposes[9,19]. The human IVM medium is typically supplemented with serum albumin and gonadotropins[9]. Some studies reported that the use of a patient's serum is more effective and led to significantly

Table 1. IVM studies in which human immature oocytes were cultured in different media or cryopreserved with different methods

Oocyte stage	IVM medium	Cryopreservation method	Type of COH	Results	Ref
GV/MI	IVM medium supplemented with 0.075 IU/mL FSH and 0.075 IU/mL LH for 24-48 hr.	Vitrification	IVF cycle	IVM is effective strategy, if done before oocyte vitrification.	[62]
GV/MI	Hams F10 supplemented with 0.75 IU LH, 0.75 IU FSH and 40% FF.	Vitrification	IVF cycle	GV-stage vitrification followed by IVM is superior to that performed in MI.	[63]
GV/MI	Ham's F10 supplemented with 0.75 IU FSH, 0.75 IU LH and 40% HFF.	Vitrification	IVF cycle	IVM of fresh immature oocytes is better than of vitrified ones, with higher maturation and viability.	[64]
GV/MI	Ham's F10 supplemented with 0.75 IU LH, 0.75 IU FSH and 40% FF.	-	IVM cycle	In patients with gynaecological diseases, oocytes maturation after IVM from unstimulated ovaries showed good developmental competence.	[65]
GV	Ham's F10 medium supplemented with 0.75 IU of LH and 0.75 IU of FSH with 40% HFF.	-	IVF cycle	IVM with blastocyst medium was superior in ICSI cycles.	[21]
GV	IVM medium supplemented with 75 mIU/mL FSH, 75 mIU/mL LH and 10% SSS for 24-48 hr.	Vitrification	IVF cycle	Vitrification of MII oocytes after IVM was superior.	[66]
GV/MI	Simple IVM system (S-IVM), autologous follicular fluid (AFF-IVM), HFF (HFF-IVM), and HFF with CCs isolated from non PCOS women.	-	IVF cycle	In PCOS patients, HFF/CGC-IVM protocol significantly increased IVF outcomes.	[67]
GV	Blastocyst medium (G2) supplemented with 75 IU/L of HMG.	-	IVF cycle	Rescue IVM had negative effects on embryo morphokinetics.	[68]
GV/MI	IVM medium supplemented with 75 mIU/mL FSH, 100 mIU/mL hCG, and 20% HSA with GDF9.	Vitrification	IVM cycle	In cancer patients, vitrification impaired oocyte maturation, viability, subcellular quality after IVM.	[69]
GV	IVM medium supplemented with 75 mIU/mL FSH and 75 mIU/mL LH.	-	IVM cycle	IVM medium supplemented with GDF9 and CCs increased fertilization, embryo development and blastocyst viability rates.	[70]

IVM: In vitro maturation, COH: Controlled ovarian hyperstimulation, IVF: In vitro fertilization, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, GV: Germinal vesicle, MI: Metaphase I

higher rates of maturation and pregnancy compared to the use of a donor's FF and serum substitute supplement. Serum may have some relevant factors for oocyte maturation, such as EGF. Commercial IVM media, such as SAGE (Cooper surgical) IVM medium, MediCult IVM medium (MediCult, Origio, Måløv, Denmark)[21], and tissue culture medium 199 (TCM199, Invitrogen, Carlsbad, CA)[21] have the advantage of immature oocytes culture. Recently, improved culture systems to mimic the in vivo maturation process, such as the use of 3-D culture systems[22], the supplementation with C-Type Natriuretic Peptide to retain gap junctions for a specific time before starting oocyte maturation in vitro[23], with EGF-like growth factors or oocyte secreting factors (GDF-9 and BMP-15), are used[24].

IVM Oocyte Cryopreservation

Oocytes retrieved from IVF/IVM cycles can be cryopreserved using the slow-cooling or vitrification approaches. In theory, there are two methods for immature oocyte cryopreservation before IVM (at GV or MI-stage) or after IVM (MII-stage) (Table 1). The first successful pregnancy and live birth were reported after slow-cooling of immature human oocytes[25], but further successful items were from vitrification of MII-stage oocytes after the IVM procedure[26].

Clinical Outcomes in IVM Cycles

Limited studies have reported live births after the cryopreservation of IVM oocytes. The first live birth was achieved using the slow-cooling method at the GV stage oocytes recovered from IVM cycles[25]. One study reported the first live birth after vitrification of immature oocytes[27]. Later, the McGill reproductive center reported five gravidities with live births after vitrification at MII-stage after IVM of immature oocytes collected from hCG-primed IVM cycles. In their study, MII oocytes obtained from IVM cycles had significantly lower recovery and fertilization rates after vitrification than *in vivo* MII oocytes generated from IVF cycles. Additionally, implantation, clinical pregnancy, and live birth rates were lower in IVM-oocytes vitrification groups[26]. Nevertheless, in cancer patients, there are limited studies of successful pregnancies or live births after cryopreservation of IVM oocytes, employing either slow-cooling or vitrification methods. Previous studies reported that the mean oocyte maturation rate of 39% ±23% was achieved after the collection of 1.220 COCs from 77 patients who were done oophorectomy for OTC (maturation rate of 22% in pre-menarche children and 42% in adult patients)[28]. Only three live births from OT oocyte in vitro maturation (OTO-IVM) in women have been reported in this literature. The rate of live births from OTO-IVM per embryo transfer was 43% in the aforementioned study.

OTC

Indications for OTC

OTC and ovarian tissue transplantation (OTT) is the best choice for women undergoing chemoradiotherapy who cannot delay the start of these therapies or are ineligible for ovarian stimulation[29]. Also, it could be used by women in postponing their first pregnancy and menopause[30]. This approach described some diseases, including genetic abnormalities such as Turner's syndrome[31], gynecological diseases[32], systemic and endocrine disorders, autoimmune disease[33], and endometriosis leading to premature ovarian insufficiency[34]. It is the only option available for pre-pubertal girls and women with estrogen-sensitive malignancies[35,36]. However, OTC is a useful procedure that allows for restoring ovarian function and natural pregnancy[37]. It is an important evaluation of cumulative factors, such as adequate ovarian reservation, level of AMH hormone, age of patients, and previous treatment regimens for performing this technique[38]. This approach was suggested in some guidelines, such as ASCO 2018, which mentioned OTC as a standard treatment for these cases, due to the rapid improvement of the OT freezing technique[39].

Transplantation to the Patient

Cortical OTT

For the cryopreservation of cortical OT, strips of the ovarian cortex removed throughout the menstrual period by laparoscopy or laparotomy. The OT contains many primordial and immature follicles numbers that can be protected by freezing. However, it is more complicated than embryo and oocyte freezing due to different cell types and permeability to water and cell volum[40]. After removal of the medulla, the cortex is divided into several strips of approximately equal size for grafting (10x5 with 1 mm-thick piece) or slices (4x2 with 1 mm-thick piece) which allow penetrating the cryoprotectant agents (CPAs) into the thin layer of the cortex[36]. Using larger pieces of the cortex (5x5x1 mm) may prepare better conditions for OTT. It is worth mentioning that too small pieces of the cortex are difficult to fixed to the underlying surface and subsequently oxygenation and normal re-vascularization were disturbed[41]. Ovarian cortical pieces could be transplanted into patients after treatment of the disease or could be done IVM of obtained follicles from OTs. Almost all the healthy live births were achieved following this method. According to a previous meta-analysis study, the reestablishment of ovarian activity rate was 63.9%, and live birth was reported 57.5% by autotransplantation method in women younger than 30 years at the time of OTT[42]. A survival rate of 84% was reported in follicles after frozen-thawed OTs. However, up to 72% of the follicles are disrupted due to ischemia and reperfusion injury after OTT[43].

Orthotropic Transplantation of Cortical Tissue

Orthotropic transplantation involves transplanting strips of OT into the remaining portion of the ovary or the peritoneum of the ovarian fossa. The advantage of this procedure becomes possible by natural conception and has provided a suitable environment for follicular development[44]. However, the number of grafted fragments limited by the remaining ovarian size, also, it may increase ischemia and follicle atresia after grafting due to avascular condition. However, the first pregnancy was reported in 2004 using this method, and so far most live births have been from this transplantation[44].

Heterotopic Transplantation of Cortical Tissue

Heterotopic transplantation refers to the grafting of cortical OT into extra-pelvic sites such as the forearm, abdominal, and chest wall. The transplanted tissue can easily removed or replaced when necessary. Contrary to the orthotropic method, it avoids major abdominal surgery and, has no limit to the number of grafted fragments. Although this technique is less invasive than orthotropic, although, spontaneous pregnancy is impossible; therefore, subsequent ovarian stimulation and IVF must be performed[44].

Transplantation of the Whole Ovary

In theory, the transplantation of thawed whole ovaries can lead to vascular anastomosis in the ovarian pedicle, however, the ischemia and follicle atresia is reduced due to vascular grafting. As a result, it may provide a more significant follicular reserve and a longer lifetime for an organ transplant. Although, it had some problems, such as a large mass of OT, creating a non-homogeneous cooling rate between different layers of OT. Problems associated with mass and cold transfer eventually increase the probability of ice formation. The multi-thermal gradient technique provides a possible way to overcome the ischemic damage to the whole ovary[45].

Ovarian Tissue Freezing Techniques

There are two standard methodologies that have been introduced for cryopreservation procedure, including conventional slow freezing and vitrification[45].

Conventional Slow Freezing

In this technique, a controlled cooling machine is used to OT slowly until -140 °C at low rates (~1 °C/min) before plunging it into liquid nitrogen (LN2)[46]. Ovarian sample as a complex tissue has different types of cell and ECM. The slow freezing method can prepare a higher equilibration period to allow the release CPA release into the inner complex tissue areas. Slow freezing helps osmotic adjustments between extra and intracellular fluids with CPAs during freezing/thawing procedures. Most times the combination of permeating CPAs, such as glycerol, dimethyl sulfoxide, ethylene glycol, and 1,2-propanediol, and non-permeating CPAs as sucrose, trehalose, and raffinose, was used to protect against cell damage caused by the production of ice crystals and hypertonicity during cryopreservation[47]. More than 130 live birth was reported from this method; however, its disadvantages are time-consuming and require costly equipment[48].

Vitrification

The vitrification procedure was introduced with an ultrafast cooling rate (~20,000 °C/min) by direct plunging into LN2 and a high concentration of CPAs. The concentration of CPA was the most critical cause of cell damage; however, it is recommended to use a combination of two or more CPAs[47]. Vitrification is a considerable method due to its quickness, ease, and cost-effectiveness without using special and expensive equipment[30]. It has been reported in a low risk of ice crystal formation in the vitrification method[29]. Nevertheless, there is still no optimal protocol for vitrification. As a result, data about the vitrification technique in human OT is still limited, and some centers may prefer to perform slow freezing for OTC. Thus, the superiority between vitrification and slow freezing for OTC remains unresolved. Some studies showed a lower percentage of apoptotic cells and higher viability of primordial follicles after the slow freezing procedure. Additionally, the frozen-thawed cortical tissue could produce a higher number of hormones AMH in tissue culture after the slow freezing method[49]. However, others found no differences in the percentage of apoptotic cells and follicle viability and density between these procedures[21,50]. The viability rate of primordial follicles should be assessed after different cryopreservation methods. This assessment is performed using staining assays such as hematoxylin-eosin and trypan blue solution. By staining, the state of primordial follicle quality, including intact nucleolus, clear cytoplasm, and round shape, density, and viability, can be examined[50]. A meta-analysis of 14 studies in 2017 suggested that less primordial follicular DNA damage and better conditions for the preservation of stromal cells after vitrification[51]. A disadvantage of vitrification is the direct contact of the sample with nitrogen, therefore, it can lead to viral cross-contamination. Sugishita et al.[20] recently introduced a new closed vitrification system. According to this study, none of the cryopreservation methods, including slow freezing, conventional vitrification, and closed vitrification didn't show any significant difference in terms of DNA damage and apoptosis pathway in both primordial and primary follicles compared with a fresh baseline control group[21]. Nevertheless, only three live births have been reported from the vitrification procedure. A summary of the main properties and outcomes regarding the comparison of vitrification and slow freezing are presented in Table 2.

Table 2. Basic characteristics and outcomes of studies in terms of comparison between vitrification and slow freezing procedures

Method	Basic medium (BS)	Cryoprotectants agent	Equilibrium/Cooling and warming rate	Results	Ref
Vit	Leibovitz L-15 (BM1)	ES: 5.58% (1M) EG, 3.55% (0.5M) DMSO, 0.125 M sucrose and 2.50% SSS, VS1: 11.16% (2M) EG, 7.10% (1M) DMSO, 0.25 M sucrose with 5.00% SSS VS2: 22.32% (4M) EG, 14.20% (2M) DMSO, 0.5 M sucrose and 10% SSS. WS1: BM1 with 0.8 M sucrose WS2: BM1 with 0.4 M sucrose WS3: sucrose-free BM1	Equilibration solution: 5 min at RT, VS1: 7 min at RT, VS2: 4 °C for 10 min, then plunged into LN2. Warming: each solution for 5 min at RT.	More intact follicles showed after vitrification and the apoptotic primordial follicles were no significant between 2 groups. Vitrification led to gene overexpressed (gene expression of granulosa cells, oocytes and cellular cycle) compared to slow freeing.	[71]
SF	Leibovitz L-15 (BM1)	FS: 2 M DMSO +10% sss TS: BM1	Cooling: 10 min at RT. from 20 °C to -35 °C at -2 °C/min, semi-automatic seeding at -11 °C. cooling to -150 °C at 25 °C/min. Thawing: shaking in the water bath at 37 °C for one min, then placed in TS for 5 min at RT.		
Vit	TCM199 with 20% SSS	ES: 7.5% EG, 7.5% DMSO in VS: 20% EG, 20 % DMSO and 0.5 M sucrose TS: 55 mL HEPES, 20 mL SSS, and 34.24 g sucrose DS: 65 mL HEPES, 20 mL SSS, and 17.12 g sucrose Washing solution: (40 mL HEPES and 10 mL SSS)	ES: 25 min at RT VS: 15 min at RT, then plunged into LN2 Warming: TS: 5 min at RT, DS: 5 min at RT, Washing: 5 min at RT (twice).	Slow freezing was superior to vitrification in terms of primordial follicle preservation, vascularization, follicular cell proliferation, DNA damage, and AMH expression.	[72]
SF	TCM199 with 5% SSS	FS: Ascending percentage of DMSO (7.5,10 and 12.5) Washing solution: 5% DMSO	Cooling: from 4 °C to -7.0 °C at a rate of -2.0 °C/min, manual seeding, cooling to -40.0 °C at a rate of -0.3 °C/min and -140 °C at a rate of -10 °C/min. Thawing: shaking in the water bath at 37 °C, then placed in washing solution for 10 min at RT.		
Vit	Basic cryoprotectants solution with 20% HAS	ES: 7.5% EG and 7.5% DMSO VS: 15% EG,15 % DMSO and 0.25M sucrose WS1: 1 M sucrose, WS2: 0.5 M sucrose, WS3: 0.25 M sucrose, and WS4: 0.125 M sucrose.	ES: 15 min on ice VS: 10 min on ice, then plunged into LN2. Warming: each solution for 5 min.	Slow freezing showed better preservation regardless of the type of follicle. Expression of apoptotic genes was significantly decreased in slow-frozen samples.	[73]
SF	Basic cryoprotectants solution with 2% HAS	FS: 10% DMSO	Cooling: from 0 °C to -8 °C at -2 °C/min, manually seeding, cooling to -40 at 0.3 °C, and -70 °C at 5 °C/min. Thawing: shaking in the water bath at 37 °C for 2 min, then placed in basic cryoprotectants solution for 5 min at RT (three times).		

Table 2. Continued

Method	Basic medium (BS)	Cryoprotectants agent	Equilibrium/Cooling and warming rate	Results	Ref
Vit	"Medium A" supplemented with 0.5% HSA, HEPES (21.8 mM) and glycine (50.0 mM)	solution A: 0.37 M PrOH, 0.37 M EG solution B: 0.75 M PrOH, 0.75 M EG solution C: 1.5 M PrOH, 1.5 M EG supplemented with 0.5 M raffinose WS1: 0.75 M PrOH, 0.75 M EG WS2: 0.37 M PrOH, 0.37 M EG WS3: Medium A	Solution A: 5min at RT, Solution B: 5 min at RT, Solution C: 10 min at +4 °C, then dropped into LN2. Warming: 37 °C water bath for 2 min, WS1: 5 min at 37 °C, WS2: 5 min at 37 °C, WS3: 5 min at 37 °C.	Vitrification preserves follicle and stroma morphology as well as the slow-freezing method and did not increase the rates of follicles and stroma cells with DNA fragmentation	[48]
SF	"Medium A" supplemented with 0.5% HAS with 21.8 mM HEPES and glycine (50.0 mM)	FS1: 3.0 M PrOH and 0.05 M raffinose FS2: 1.5 M PrOH and 0.025 M raffinose TS: Medium A +0.5% HSA	Cooling: Equilibration at 4 °C for 15 min, from 4 °C to -11 °C at -2 °C/min, then lowered to -40 °C at -2 °C/min and from -40 °C to -150 °C at -10 °C/min Thawing: water bath at 37 °C for 2 min, TS: for 5 min at 37 °C (twice).		
Vit	Ham's F10 with 20% HAS	ES: 7.5% EG and 7.5% DMSO VS: 15% EG ,15% DMSO and 0.5 M sucrose WS1: 1 M sucrose, WS2: 0.5 M glucose, Washing solution: BS	ES: 15 min at 38 °C VS: 2 min at RT, then plunged into LN2. Warming: each solution at 38 °C for 5 min.	Slow-freezing and vitrification results showed similar morphological integrity and rates of follicular proliferation and apoptosis.	[74]
SF	Ham's F10 with 20% HAS	FS: 1.5 M DMSO and 0.1 M sucrose	Cooling: from 4 °C to -8 °C at a rate of -2 °C/min, manual seeding, cooling to -40 °C at a rate of -0.3 °C/min, and -30 °C/min to -150 °C Thawing: water bath at 38 °C for 2 min, then washed in Ham's F10 at 38 °C (three times)		
Vit	Leibovitz medium (L-15) with 20% FBS	ES: 7.5% EG and 7.5% DMSO VS: 13.5% EG,13.5% DMSO and 0. 5M sucrose	ES: 10 min at RT VS: 2 min at RT, then plunged into LN2.	Morphologically abnormal primordial follicles and the rates of TUNEL-positive in these cells were lower in vitrification than in slow freezing group.	[75]
SF	Leibovitz medium (L-15) with 10% FBS	FS: 1.5 M DMSO with 0.1 M sucrose	From 4 °C to -7.0 °C at a rate of -2.0 °C/min, held for 5 min manual seeding, held for 10 min, cooling to -40.0 °C at a rate of -0.3 °C/min and -140 °C at a rate of -10 °C/min		

Vit: Vitrification, SF: Slow freezing, ES: Equilibration solution, VS: Vitrification solution, WS: Warming solution, TS: Thawing solution, DS: Diluent solution, FS: Freezing solution, EG: Ethylene glycol, DMSO: Dimethyl sulphoxide, PrOH: 1,2-propanediol, LN2: Liquid nitrogen

Clinical Outcomes

The first successful human live birth from orthotropic transplantation was reported in 2004, and Meirow et al.[52] reported a second live birth in 2005[53]. Up until now, due to the impressive development of the OT freezing technique, specially, the ovarian cortex implantation method, more than 130 healthy babies have been born since, 2017 worldwide[54,55]. This statistic has been mentioned in 200 cases until 2021[56]. Andersen et al.[43] investigated the clinical outcome rate of the 3 largest cohort studies in Belgium, Denmark, and Israel. They reported that pregnancy rates varied from 3.9% to 19.3% and live birth rates from 3.9% to 14% per cycle[43]. In other studies, the live birth rate was reported from 25.4% to 30.6%[56]. Also, the cumulative clinical pregnancy and the cumulative live birth and clinical ongoing pregnancy rates were 57.5% and 37.7%, respectively[42]. The lack of consensus could be due to the timing of initiation of ART from OTT, patient's age, type of ovarian stimulation protocol, and

overall the strategy of centers regarding providing services to these patients. A systematic review showed that clinical outcomes were considerably lower in women undergoing OTT than in IVF cycles[43]. However, there are few reports on the prevalence of pregnancy in pre-pubertal girls. Recent literature reported only two cases of live births who underwent OTC. One case was 14 years old with sickle cell disease that was underwent autologous tissue transplantation for her at the age of 24 years, and pregnancy was achieved spontaneously. Another case was a girl at the age of 9 years old with beta-thalassemia. After the treatment process, she returned for OTT and achieved a live birth undergoing the IVF program[57].

Future Perspectives on Eliminating the Risk of Malignant Cell Transmission

Alternative approaches have been introduced for the deletion of malignant cells in certain types of cancer with high metastasis potential, such as leukemia, Burkitt's lymphoma, neuroblastoma, and ovarian tumors. The artificial ovary technique is one of the new approaches that are known, as primordial follicles isolated from OTs and transferred onto a scaffold-like ovarian organ. The development of human pre-antral follicles was seen after grafting of primordial follicles inside a fibrin scaffold and, respectively, xenografted in nude mice[58]. Future studies attempt to find a three-dimension-printed artificial ovary to restore both endocrine and reproductive function in animals[59]. Another approach is to isolate immature oocytes and perform IVM in the ART lab[59]. The main challenge is maintaining the interaction between the oocytes and the somatic cells that surround them[60]. The acquisition of meiotic, s developmental conditions, and genome imprinting are important factors that should be considered for this issue. Oocytes differentiated from ovarian stem cells (OSCs) may be another option for the mentioned conditions. Studies have shown that OSCs have been retrieved from mice that it are suitable for fertilization and implantation, as well as embryo development and live births in an animal model[61]. However, due to the scarcity of OSCs and their ethical issues use of these cells in the clinical application was insufficient, for this reason, this technique is not currently used in clinical practice, especially in cancer patients[29]. These aforementioned techniques are still in a research setting and can be used for female fertility preservation in the near future.

Conclusion

Due to the impressive clinical development of OTC in cancerous patients, it is recommended as a standard treatment in cryopreservation strategies. However, OTC was a useful procedure that allows for restore ovarian function and natural pregnancy. However, IVM treatment does not require high gonadotropin stimulation and it is not necessary to take more than 48 h for the decision to perform. Therefore, when patients are unable to delay the chemotherapy, retrieving immature oocytes from the antral follicles and the IVM method may be a good approach. A combination of IVC of isolated OSCs, small follicles, and an artificial ovary technique could eliminate the risk of malignant cell transmission. These approaches could be a good fertility preservation strategy for cancer patients in future studies.

Ethics

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

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

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Review / Derleme

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Ondansetron versus metoclopramide for managing hyperemesis gravidarum: A systematic review and meta-analysis of randomized controlled trials

Hiperemezis gravidarum tedavisinde ondansetron ve metoklopramidin karşılaştırılması: Randomize kontrollü çalışmaların sistematik bir incelemesi ve meta-analizi

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Abstract

This investigation examined the efficacy of ondansetron (intervention) versus metoclopramide (control) in managing parturient females with hyperemesis gravidarum (HG), by pooling data from randomized controlled trials (RCTs) using a meta-analysis approach. From inception until January 2022, five information sources were screened: Cochrane Central Register of Controlled Trials, Google Scholar, Scopus, PubMed and Web of Science. Quality assessment was done through the Cochrane Risk of Bias (version 2) assessment tool. The mean difference (MD) with 95% confidence interval (CI) was used to summarize the continuous data in a fixed- or random-effects model, depending on the extent of between-study heterogeneity. Five RCTs were included, comprising a total of 695 patients (355 and 340 females were assigned to ondansetron and metoclopramide, respectively). Four RCTs had an overall "low" risk of bias, whereas one RCT had an overall "some concerns" due to lack of sufficient information about randomization. There was no significant difference between both groups regarding the pregnancy-unique quantification of emesis and nausea score [MD=0.23, 95% CI (-0.42, 0.88), p=0.49], length of hospital stay [MD=-0.17 days, 95% CI (-0.35, 0.02), p=0.08], the number of doses of drug received [MD=0.45, 95% CI (-0.08, 0.98), p=0.10], and duration of intravenous fluids [MD=-1.73 hours, 95% CI (-5.79, 2.33), p=0.40]. Among parturient females with HG, there was no substantial difference in efficacy between both agents. Nevertheless, ondansetron is favored over metoclopramide in view of its trending therapeutic efficacy and better safety profile.

Keywords: Ondansetron, metoclopramide, hyperemesis gravidarum, nausea, vomiting

Öz

Randomize kontrollü çalışmaların (RKÇ) bu sistematik derleme ve meta-analizi, hiperemezis gravidarumlu (HG) gebe kadınların tedavisinde ondansetronun (müdahale) metoklopramide (kontrol) karşı etkinliğini incelemeyi amaçlamıştır. PubMed, Scopus, Web of Science, Cochrane Central Register of Controlled Trials ve Google Akademik veritabanları, başlangıçtan Ocak 2022'ye kadar tarandı. Dahil edilen çalışmaların yanlılık riski Cochrane Collaboration aracına (versiyon 2) göre değerlendirildi. Çalışma sonuçları, sabit veya rastgele etkiler modeli altında %95 güven aralığı (GA) ile ortalama fark (MD) olarak özetlendi. Toplam 695 hastadan oluşan beş RKÇ dahil edildi (355 katılımcı ondansetron ve 340 katılımcı metoklopramid ile tedavi edildi). Dört RKÇ'nin genel olarak "düşük" yanlılık riski varken, bir RKÇ için randomizasyon hakkında yeterli bilgi vermemesi nedeniyle genel olarak "bazı endişeler" mevcuttu. Pregnancy Unique Qualification of Emesis skoru [MD=0,23, %95 GA (-0,42, 0,88), p=0,49], hastanede kalış süresi [MD=-0,17 gün, %95 GA (-0,35, 0,02), p=0,08], alınan ilaç doz sayısı [MD=0,45, %95 GA (-0,08, 0,98), p=0,10] ve intravenöz sıvıların süresi [MD=-1,73 saat, %95 GA (-5,79, 2,33), p=0,40] açısından her iki grup arasında anlamlı bir fark yoktu. HG'li hastalarda ondansetron ve metoklopramid arasında etkililik açısından anlamlı bir fark yoktu. Bununla birlikte, terapötik etkililik trendi ve daha iyi güvenlik profili göz önüne alındığında, ondansetron metoklopramide göre tercih edilir.

Anahtar Kelimeler: Ondansetron, metoklopramid, hiperemezis gravidarum, bulantı, kusma

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Introduction

Nausea and vomiting (N&V) impact close to 90% of parturient females. They tend to begin at 6-8 weeks of gestation. The severity of the condition becomes higher around nine weeks of pregnancy, and subsequently lesson at the end of the first trimester. Notably, symptoms may persist until 20 weeks of pregnancy in a slight fraction of females[1,2].

Hyperemesis gravidarum (HG) is a serious type of N&V of pregnancy, which impacts up to 3% of parturient females. This causes dehydration, weight loss, and electrolyte disturbance. Additionally, it carries a hazard of problems for the mother and her fetus, for instance, maternal Wernicke's syndrome and fetal intrauterine growth retardation[3].

Pregnant women with HG can be treated with oral antiemetics at home if they are hemodynamically stable and can tolerate oral intake to avoid unnecessary hospitalization[4]. However, if they cannot tolerate oral intake, ambulatory parenteral fluids, multivitamins, B-complexes, and antiemetics are considered[4]. Women who have nutritional deficiencies and electrolyte imbalances should be treated as inpatients[4]. If one antiemetic drug is not effective alone, the additional second line antiemetics are used for a synergistic effect such as metoclopramide and ondansetron.

Metoclopramide (a dopamine antagonist) and ondansetron (a serotonin receptor antagonist) are two common antiemetics used to manage HG[4]. Multiple randomized controlled trials (RCTs) compared the superiority of metoclopramide or ondansetron in treating pregnant women with HG[5-9]. But, small sample sizes and conflicting findings are a few limitations. Additionally, these results have not been yet systematically summarized.

Consequently, this systematic review and meta-analysis aims to establish evidence from RCTs that comparing metoclopramide with ondansetron in treating pregnant women with HG.

Methods

Research Protocol

We followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines[10] and the steps of the Cochrane Handbook for Systematic Reviews of Interventions[11].

Literature Search Strategy

From inception until January 2022, five information sources were screened: Cochrane Central Register of Controlled Trials, Google Scholar, Scopus, PubMed and Web of Science. The exact query search comprised (ondansetron OR "ondansetron hydrochloride" OR "ondansetron monohydrochloride" OR "ondansetron dihydrate" OR GR38032F OR SN307 OR Zofran) AND (metoclopramide OR maxolon OR rimetin OR "metoclopramide hydrochloride" OR "metoclopramide monohydrochloride" OR primperan OR reglan OR cerucal OR "metoclopramide dihydrochloride") AND ("HG" OR "pregnancy pernicious vomiting"). Moreover, the references of the obtained studies were read to complement the broad search.

Eligibility Criteria

The inclusion criteria comprised parturient females with a diagnosis of HG who received either ondansetron or metoclopramide treatments in an RCT setting. The exclusion criteria comprised all non-RCT studies, parturient females without a diagnosis of HG, or drug interventions other than ondansetron and metoclopramide.

Study Selection

The titles and abstracts of the articles were examined for initial eligibility. This next step involved full-text reading of the potential articles. Two authors independently completed the study selection process, and disagreements were rectified by dialogue.

Quality Assessment

Quality assessment was performed through the Cochrane Risk of Bias (version 2) assessment tool[12]. Two authors independently completed the quality assessment process, and disagreements were rectified by dialogue.

Data Extraction and Outcome Measurements

Much data were collected, including a summary of the characteristics of the included studies, as well as a summary of the baseline characteristics of the included patients. The primary efficacy endpoints comprised the pregnancy-unique quantification of emesis (PUQE), duration of hospitalization, the quantity of doses of drug received, and duration of intravenous fluids.

Data Analysis

The mean difference (MD) with 95% confidence interval (CI) was used to summarize the continuous data in a fixed- or random-effects model, depending on the extent of between-study heterogeneity. Significant heterogeneity was established according to $p<0.1$ and $I^2>50\%$[13], whereas statistical significance was based on p-value <0.05. Publication bias was not done because of the small number of studies[14]. Statistical analysis was accomplished by the Review Manager Software.

Results

Summary of Literature Search

Overall, five studies (comprising 695 patients, ondansetron=355 and metoclopramide=340) met the inclusion criteria (Figure 1)[5-9]. Table 1 and Table 2 show the summary of the meta-analyzed RCTs and baseline characteristics of the patients, respectively.

Quality Assessment

An overall "low" risk of bias was found in four out of the five included RCTs (Figure 2)[5-7,9]. Shaheen et al.[8] did not provide satisfactory information about randomization; therefore, a

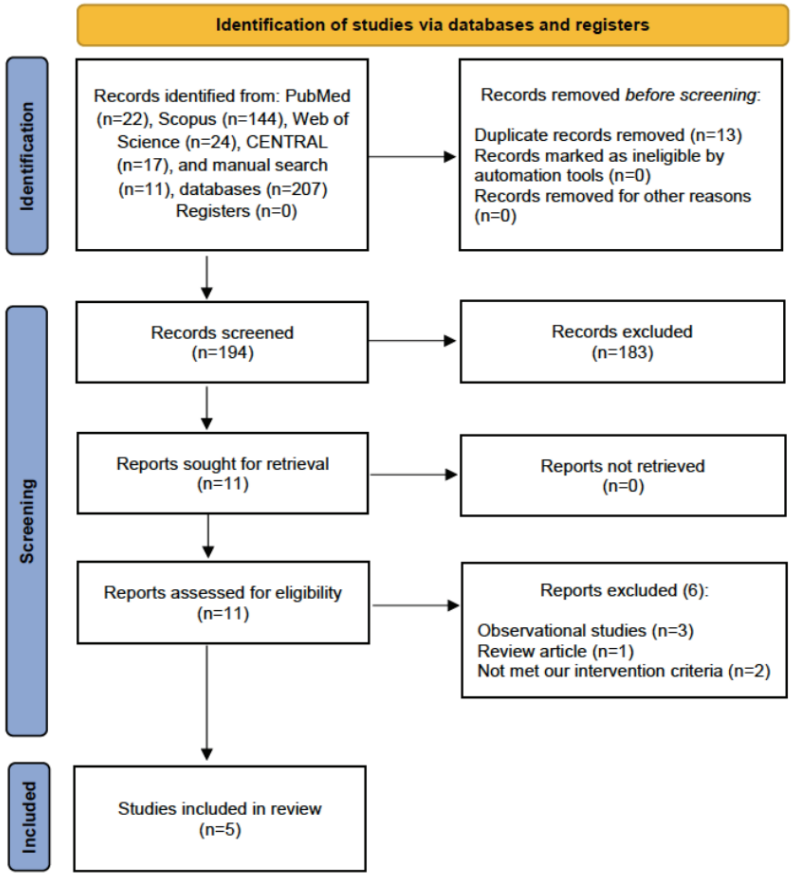

Figure 1. The Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flowchart for literature search

grading of "some concerns" was assigned to the randomization bias domain.

Efficacy Outcomes

A. PUQE

Two RCTs with 219 patients reported the outcome[7,9]. No significant difference between the groups was noted [MD=0.23, 95% CI (-0.42, 0.88), p=0.49] and the results were heterogeneous (p<0.001, I^2=93%). On subgroup analysis, no significant difference between both groups was noted on day 1 [MD=0.27, 95% CI (-0.79, 1.33), p=0.62], day 2 [MD=-0.04, 95% CI (-0.64, 0.56), p=0.9], and day 3 [MD=0.43, 95% CI (-1.68, 2.53), p=0.69]. All results of the subgroup analysis were heterogeneous (p<0.1, I^2>50%) (Figure 3).

B. Length of Hospital Stay

Three RCTs with 379 patients reported the outcome[5,7,9]. No significant difference between the groups was noted [MD=-0.17 days, 95% CI (-0.35, 0.02), p=0.08], and the results were homogeneous (p=0.83, I^2=0%) (Figure 4).

C. Number of Doses of Drug Received

Two RCTs with 219 patients reported the outcome[7,9]. No significant difference between the groups was noted [MD=0.45, 95% CI (-0.08, 0.98), p=0.10], and the results were homogeneous (p=0.27, I^2=18%) (Figure 5).

D. Duration of Intravenous Fluid

Two RCTs with 219 patients reported the outcome[7,9]. No significant difference between the groups was noted [MD=-1.73 hours, 95% CI (-5.79, 2.33), p=0.40], and the results were homogeneous (p=0.94, I^2=0%) (Figure 6).

Discussion

Summary of Findings

This study examined the efficacy of ondansetron versus metoclopramide for the management of HG. Five RCTs were included, encompassing a sum of 695 parturient females (355 and 340 patients were apportioned to ondansetron and metoclopramide, respectively). Four of the included RCTs had an overall "low" risk of bias, whereas one RCT had an overall "some concerns" evaluation. The findings displayed insignificant variance between both groups regarding all outcomes, including PUQE score, length of hospital stay, the number of doses of drug received, and duration of intravenous fluid treatment.

Interpretation of Findings and Clinical Implications

Hyperemesis represents the second ranked source of hospitalization during gestation and is the first ranked source of hospitalization during the first trimester[15]. Other sources of nausea and vomiting during gestation must be excluded before concluding HG[16].

The results of Kashifard et al.[6] showed that women who were allocated to ondansetron had potentially less severe nausea, fewer vomiting episodes, and overall better nausea scores at

Table 1. The summary of the included studies

Study ID	Country	Duration	Total sample size, n (intervention/ control)	Study arms		Conclusion
				Intervention	Control	
Kashifard et al. 2013[6]	Iran	From June 2011 to March 2012	n=83 (49/34)	OND (4 mg)	MET (10 mg)	OND was able to diminish vomiting treatment more rapidly than MET
Abas et al. 2014[5]	Malaysia	From November 2011 to August 2012	n=160 (80/80)	OND (4 mg)	MET (10 mg)	OND and MET demonstrated similar antiemetic and antinauseant effects in HG
Chhetry et al. 2014[7]	Nepal	From April 2011 to March 2012	n=68 (34/34)	OND (4 mg)	MET (10 mg)	OND and MET appeared to be equally effective to treat HG
Shaheen et al. 2021[8]	Pakistan	From August 2015 to January 2016	n=230 (115/115)	OND (4 mg)	MET (10 mg)	Efficacy and tolerability of OND is better as compared to MET in HG
Moradiha et al. 2022[9]	Iran	From June 2019 to September 2019	n=154 (77/77)	OND (4 mg)	MET (10 mg)	OND revealed more efficacy than MET on the HG management

HG: Hyperemesis gravidarum, MET: Metoclopramide, OND: Ondansetron

Table 2. The baseline characteristics of the included studies

Study ID	Group	Age (years)	Gestational age (week)	Gravidity	Parity	BMI (kg/m^2)	Serum sodium (mmol/L)	Serum potassium (mmol/L)	Route of drug administration
Kashifard et al. 2013[6]	OND	25.3±5.5	8.7±2.6	NR	NR	NR	NR	NR	Orally three times/ week, then twice/ three days, then once/ four days
	MET	25.2±4.9	8.7±2.6						
Abas et al. 2014[5]	OND	29.7±4.7	9.6±2.3	2±1.50	1±1.50	23.5±4.3	13±62	3.9±0.4	Intravenously every 8 hours for at least 24 hours and then switched into oral if patients can tolerate
	MET	29.2±4.5	9.4±2.5	2±1.50	1±1.50	23.1±3.9	13±62	3.9±0.4	
Chhetry et al. 2014[7]	OND	24.06±4.4	8.56±2.12	NR	1.88±1.20	NR	NR	NR	Intravenously every 8 hours for at least 24 hours and then switched into oral if patients can tolerate
	MET	24±4.15	9.29±2.49		1.74±0.99				
Shaheen et al. 2021[8]	OND	29.43±6.48	7.93±3.11	NR	NR	NR	NR	NR	Intravenously every 8 hours for 24 hours
	MET	29.12±6.07	7.88±3.21						
Moradiha et al. 2022[9]	OND	25.43±5.42	11.32±3.63	165±1.14	NR	23.7±2.54	138±2.67	3.73±0.30	Intravenously every 8 hours for at least 24 hours and then switched into oral if patients can tolerate
	MET	28.44±6.45	10.19±2.35	198±1.16		23.16±3.32	139±2.24	3.76±0.38	

BMI: Body mass index, MET: Metoclopramide, NR: Not reported, OND: Ondansetron

the third and fourth days of therapy in contrast with those allocated to metoclopramide. Moradiha et al.[9] documented a substantial variance between the two arms on the third day of therapy as women in the ondansetron group had better PUQE scores contrasted with the metoclopramide arm. Moreover, the findings by Shaheen et al.[8] depicted that ondansetron had higher efficacy in terminating nausea and vomiting than metoclopramide (89.6% versus 77.4%, respectively, p=0.013). However, Abas et al.[5] and Chhetry et al.[7] conveyed an insignificant change between ondansetron and metoclopramide therapies in terms of efficacy. Overall, the results suggest a trending better therapeutic benefit for ondansetron over metoclopramide in treating patients with HG.

Ondansetron is a serotonin receptor antagonist that is effective in treating HG, however, its use should be done with caution owing to potential concerns to both the mother and fetus[17,18]. An updated recent meta-analysis of 12 comparative studies revealed that exposure to ondansetron during the first trimester correlated with higher significant risks for ventricular septal defects (n=6 studies, odds ratio=1.11) and cleft palate (n=5 studies, odds ratio=1.48). However, no substantial connection was identified for various cardiac-related defects and craniofacial anomalies. Moreover, Dormuth et al.[18] executed a large, multicentric, cohort investigation comprising 456963 pregnancies. This study compared various pregnancy endpoints among females who received ondansetron or alternative antiemetic agents. Overall, the study by Dormuth et al.[18] concluded no correlation between ondansetron intake during gestation and higher threats of increased major hereditary malformations, fetal demise, stillbirth, and spontaneous abortion compared with exposure to alterative antiemetic agents. All in all, the findings suggest that ondansetron is largely safe, and its use is highly recommended after the first trimester. The risk of cleft palate upon exposure to ondansetron remains a point of conflict across large cohort studies[19,20].

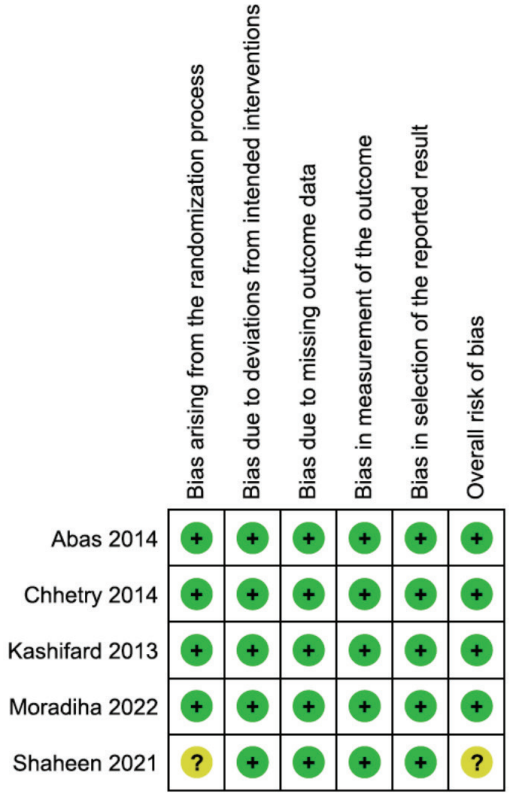

Figure 2. The baseline characteristics of the included studies

On the other hand, metoclopramide is a dopamine pharmacologic competitor that is equally active in managing HG with no hazard of major hereditary defects based on a high-quality meta-analysis of six cohort studies with 33.374 patients[21]. However, it can have some serious potential side effects, such as extrapyramidal manifestations[22,23]. Abas et al.[5] found no single event of involuntary muscle movement (dystonia) in 80 HG patients treated with metoclopramide. However, in the same RCT by Abas et al.[5], the authors found that the metoclopramide group had significantly higher rates of drowsiness (30% vs 12.5%, p=0.011) and xerostomia (23.8% vs 10%, p=0.03) compared with the ondansetron group. Nevertheless, Kashifard et al.[6] found no major side effects between both groups.

Multiple investigations have explored the antiemetic efficacy and tolerability of ondansetron and metoclopramide in non-obstetric conditions. Pitts[24] showed that the degree of nausea and vomiting was not affected by either ondansetron or metoclopramide among patients in the emergency department. However, Patanwala et al.[25] suggested using ondansetron as a first-line treatment in emergency settings to alleviate nausea and vomiting due to its fewer side effects than metoclopramide. Zamani et al.[26] also confirmed that ondansetron had fewer side effects and was safer to use in patients with minor head trauma than metoclopramide. A network meta-analysis of RCTs showed ondansetron was one of the five single agents that reduced postoperative nausea and vomiting with high-certainty evidence[27].

Comparison with Previous Investigations

In 2018, Boelig et al.[28] published a meta-analysis of RCTs that scrutinized various pharmacologic interventions for treating HG and included only one RCT[5] that directly compared ondansetron with metoclopramide. In 2020, Sridharan and Sivaramakrishnan[29] performed a related network meta-analysis and included only two RCTs[5,6]. Hence, the previous meta-analyses were limited by the reduced number of analyzed articles.

Study Strengths

This article has some strengths. Most outstandingly, this is the first ever meta-analysis that specifically and comprehensively examined the efficacy of ondansetron and metoclopramide in treating patients with HG. We included only RCTs to generate high-quality conclusions. Almost all the endpoints

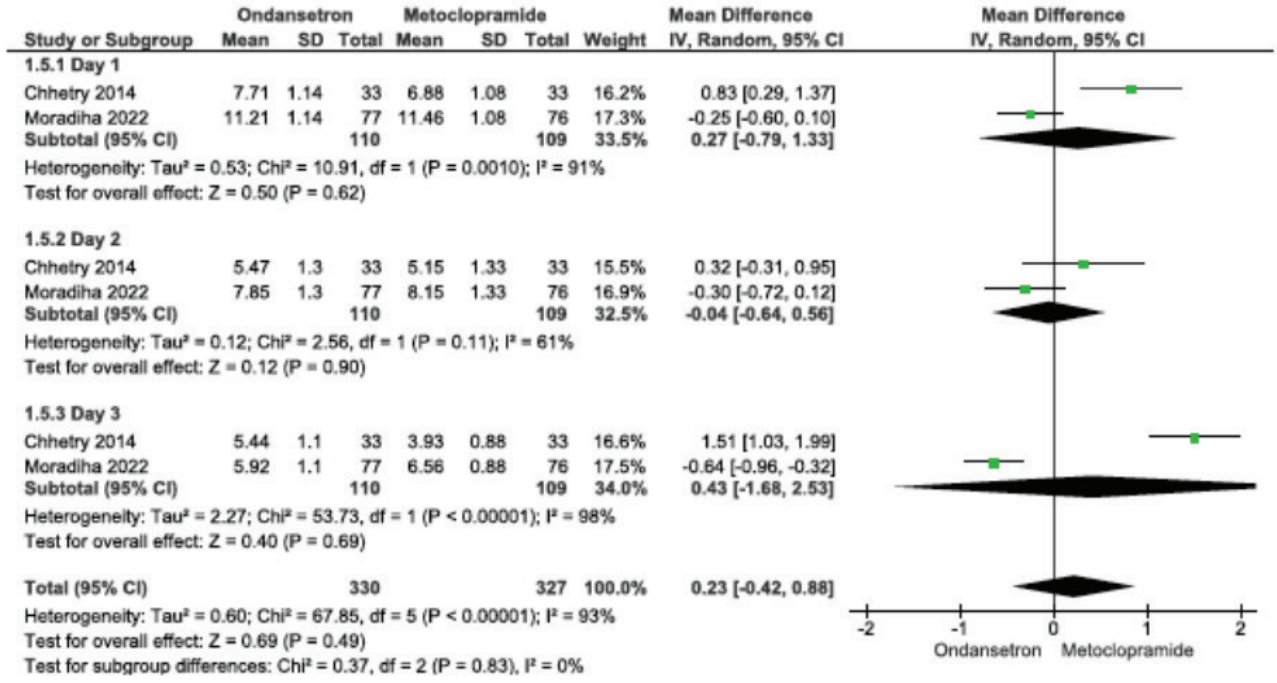

Figure 3. Meta-analysis of the pregnancy-unique quantification of emesis and nausea (PUQE)

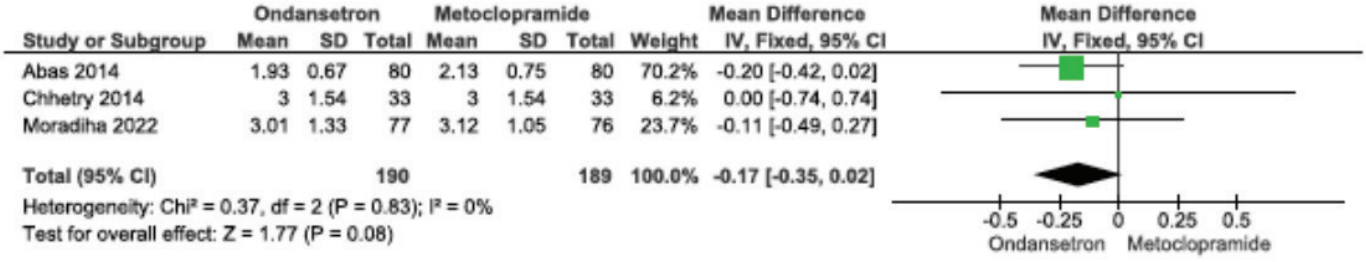

Figure 4. Meta-analysis of the length of hospital stay

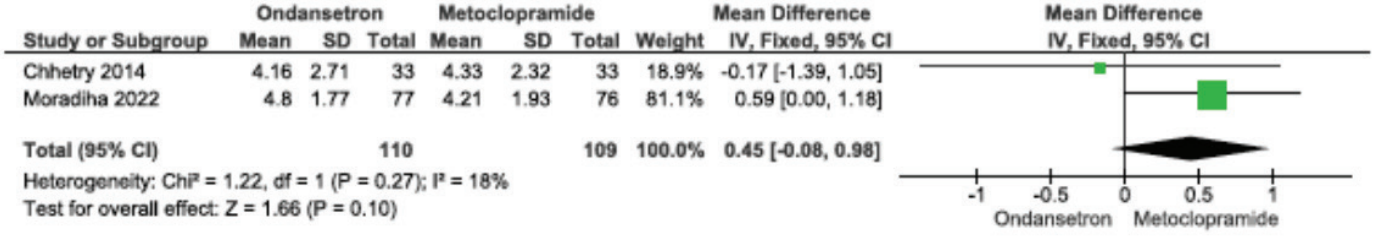

Figure 5. Meta-analysis of the number of doses of drug received

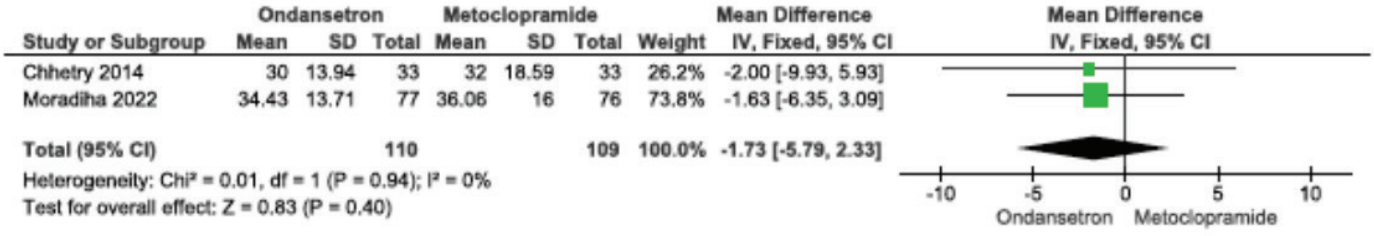

Figure 6. Meta-analysis of the duration of intravenous fluids

were homogeneous, highlighting the truthfulness of the data. Moreover, the data of the included studies are generalizable as they originated from dissimilar countries.

Study Limitations

Nonetheless, this meta-analysis has several limitations. The small number of included studies and matching small sample sizes represent the major limitations. Additional shortcomings comprise the dearth of reporting of the primary endpoints (i.e., PUQE and length of hospital stay) by all eligible RCTs. Moreover, further weaknesses include the absence of reporting comprehensive safety outcomes concerning the mother and fetus.

Future Directions

Future directions comprise the need for additional, well-planned, and large RCTs that must carefully report the primary efficacy outcomes of interest, such as PUQE score, duration of hospitalization, and safety profile. Further studies may examine the additive efficacy and tolerability of combinational treatment (i.e., ondansetron and metoclopramide) versus monotherapy alone among patients with HG.

Conclusion

Among parturient females with HG, this meta-analysis of RCTs indicated no substantial difference between ondansetron and metoclopramide regarding all outcomes, including PUQE score, length of hospital stay, the number of doses of drug received, and duration of intravenous fluid treatment. Nevertheless, ondansetron is favored over metoclopramide in view of its trending therapeutic efficacy and better safety profile.

Ethics

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: E.A., A.A., Design: E.A., L.A., F.A., A.A., Data Collection or Processing: E.A., L.A., F.A., D.S., W.A., R.A., S.B., Analysis or Interpretation: E.A., A.A., Literature Search: E.A., L.A., F.A., D.S., W.A., R.A., S.B., A.A., Writing: E.A., L.A., A.A.

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

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Review / Derleme

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Superior hypogastric plexus (SHP) block during minimally invasive hysterectomy: A systematic review

Minimal invaziv histerektomi sırasında superior hipogastrik pleksus (SHP) bloğu: Sistematik bir derleme

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Abstract

To systematically summarize the efficacy and safety of superior hypogastric plexus (SHP) block versus no SHP block among patients undergoing minimally invasive hysterectomy (MIH). Five information sources were screened from inception until 04.04.2022 and comprised the Cochrane Central Register of Controlled Trials, PubMed, Embase, Scopus, and Web of Science. The inclusion criteria comprised (i) patients: individuals undergoing MIH, (ii) intervention: SHP block, (iii) Comparator: no SHP block, (iv) Outcomes: postoperative pain, postoperative opioid consumption, operation time, estimated intraoperative blood loss, hospital stay, and complications/toxicities, and (v) Study design: randomized controlled trials (RCTs) and non-randomized comparative trials published in peer-reviewed journals. Owing to the insignificant number of available studies, methodologic heterogeneity, and procedural variances, it was impossible to carry out a quantitative meta-analysis. Hence, the results of the included studies were only reported qualitatively (descriptively). Three studies (2 RCTs and 1 cohort study), comprising 210 patients (SHP=107 and non-SHP=103) were included in the qualitative synthesis. Overall, the included studies had a low risk of bias. The results showed that SHP block appeared largely safe and could reduce postoperative pain and opioid consumption. However, SHP block did not offer clinical benefits in terms of reduced operation time, intraoperative blood loss, and hospital stay compared with non-SHP block. Among patients undergoing MIH, this first ever systematic review showed that SHP block was safe and exhibited potential analgesic and opioid-sparing effects postoperatively. Additional RCTs are needed to carry out a powered meta-analysis and validate the findings.

Keywords: Superior hypogastric plexus, hysterectomy, minimally invasive surgery, postoperative pain, systematic review

Öz

Minimal invaziv histerektomi (MİH) uygulanan hastalarda superior hipogastrik pleksus (SHP) bloğunun etkinliğini ve güvenliğini SHP bloğu uygulanmaması ile karşılaştırarak sistematik olarak özetlemektir. Başlangıçtan 04.04.2022'ye kadar beş bilgi kaynağı tarandı ve Cochrane Central Register of Controlled Trials, PubMed, Embase, Scopus ve Web of Science'dan oluşuyordu. Dahil etme kriterleri şunlardan oluşuyordu: (i) Hastalar: MİH uygulanan bireyler, (ii) Müdahale: SHP bloğu, (iii) Karşılaştırıcı: SHP bloğu yok, (iv) Sonuçlar: Postoperatif ağrı, postoperatif opioid tüketimi, ameliyat süresi, tahmini intraoperatif kan kaybı, hastanede kalış ve komplikasyonlar/toksisiteler ve (v) Çalışma tasarımı: randomize kontrollü çalışmalar (RCT) ve hakemli dergilerde yayınlanan randomize olmayan karşılaştırmalı çalışmalar. Çok az sayıda mevcut çalışma, metodolojik heterojenlik ve prosedürel farklılıklar nedeniyle nicel bir meta-analiz yapmak mümkün olmadı. Bu nedenle, dahil edilen çalışmaların sonuçları yalnızca nitel (tanımlayıcı) olarak rapor edilmiştir. Kalitatif senteze 210 hastayı (SHP=107 ve SHP olmayan=103) içeren üç çalışma (2 RCT ve 1 kohort çalışması) dahil edildi. Genel olarak, dahil edilen çalışmaların bias hatası riski düşüktü. Sonuçlar, SHP bloğunun büyük ölçüde güvenli göründüğünü ve potansiyel olarak postoperatif ağrı ve opioid tüketimini azaltabileceğini gösterdi. Bununla birlikte, SHP bloğu, SHP olmayan bloğa kıyasla daha kısa operasyon süresi, intraoperatif kan kaybı ve hastanede kalış açısından klinik fayda sağlamadı. MİH uygulanan hastalar arasında, bu ilk sistematik derleme, SHP bloğunun güvenli olduğunu ve postoperatif dönemde potansiyel analjezik ve opioid koruyucu etkiler sergilediğini gösterdi. Güçlü bir meta-analiz yürütmek ve bulguları doğrulamak için ek RCT'lere ihtiyaç vardır.

Anahtar Kelimeler: Superior hipogastrik pleksus, histerektomi, minimal invaziv cerrahi, postoperatif ağrı, sistematik inceleme

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Introduction

Minimally invasive hysterectomy (MIH) can be performed via various routes, including vaginally, laparoscopically, and robotically. Generally, MIH is favored over abdominal hysterectomy for patients with benign gynecologic conditions[1]. Key advantages of MIH comprise lower complication rates, shorter hospitalization, and better quality of life[2,3]. Nevertheless, severe postoperative pain remains a significant complaint that often warrants postoperative opioid consumption[4].

The origin of postoperative pain following MIH can be ascribed to somatic and visceral pain sources[5]. The somatic pain source originates from nociceptive receptors found in the skin and deep tissue (i.e., fascia, muscle, and subcutaneous tissue) of the abdominal wall. Conversely, the visceral pain source originates from a principal autonomic innervation to the pelvis via the superior hypogastric plexus (SHP)[6]. Hence, blockade or neurectomy of the SHP has been advocated as a plausible strategy to mitigate chronic pelvic pain secondary to cancerous and non-cancerous causes[7].

Few studies have examined the efficacy of SHP block on reducing postoperative pain and opioid consumption among patients undergoing MIH[8-10]. However, the results have been limited by the small sample size of participants, contradictory findings, and different study designs. To our understanding, no study thus far has been conducted to systematically assemble evidence on the topic and synthesize solid conclusions. Such research is pivotal to informing evidence-based clinical decisions, highlighting the literature gaps, and pinpoint the future directions.

Therefore, the objective of this investigation was to conduct a systematic review and meta-analysis of all controlled studies that examined the efficacy and safety of SHP block versus no SHP block among patients undergoing MIH.

Methods

Study Protocol and Registration

This investigation was conducted in compliance with the guidelines underlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[11] and the Cochrane Handbook for Systematic Reviews of Interventions[12]. Moreover, the protocol of this investigation was not retrospectively recorded in the International Prospective Register of Systematic Reviews (PROSPERO). Additionally, ethical approval was not warranted as this investigation used only published literature.

Inclusion and Exclusion Criteria

The inclusion criteria comprised (i) patients: individuals undergoing MIH, (ii) intervention: SHP block, (iii) Comparator: no SHP block, (iv) outcomes: postoperative pain, postoperative opioid consumption, operation time, estimated intraoperative blood loss, hospital stay, and complications/toxicities, and (v) study design: randomized controlled trials (RCTs) and non-randomized comparative trials published in peer-reviewed journals. The exclusion criteria comprised non-original studies (i.e., reviews, editorials, and abstracts) and studies involving patients undergoing abdominal (open) hysterectomy.

Search Strategy, Information Sources, and The Study Selection Process

The following query search was used in all databases: (superior hypogastric plexus OR SHP OR presacral plexus OR presacral nerve) AND (block OR neurolysis OR neurectomy) AND (hysterectomy). No filters were used during the search for information sources. Supplemental Table 1 shows the precise query search strategy used in all information sources.

Five information sources were screened from inception until 04.04.2022 and comprised the Cochrane Central Register of Controlled Trials, PubMed, Embase, Scopus, and Web of Science.

Table 1. The baseline characteristics of the included studies

Study ID (Author, year)	Trial registration	Country	Study design	Groups	n	Age in years	BMI in kg/m^2	Route of MIH	Details of SHP intervention
Aytuluk et al.[8]	NCT # NCT03427840	Turkey	Non-randomized cohort study	SHP	30	52.73±8.54	28.81±3.96	Laparoscopic	Performed at the end of the MIH with 30 mL of 0.25% bupivacaine
				Non-SHP	30	49.03±5.34	29.11±3.04		
Clark et al.[9]	NCT # NCT03283436	United States	Randomized controlled trial	SHP	50	44 (8.0)	28.7 (9.0)	Laparoscopic	Performed at the start of the MIH with 10 mL of 0.25% bupivacaine
				Non-SHP	50	45 (8.0)	30.5 (10.2)		
De Silva et al.[10]	ACTRN # 12620000242921	Australia	Randomized controlled trial	SHP	27	43±6.4	26.6±6	Laparoscopic, robotic	Performed at the end of the MIH with 10 mL of 0.75% ropivacaine
				Non-SHP	23	43.1±8.6	27±6.7		

ACTRN: Australian New Zealand Clinical Trials Registry, BMI: Body mass index, MIH: Minimally invasive hysterectomy, NCT: National Clinical Trial, SHP: Superior hypogastric plexus, Age and BMI were reported as mean ± standard deviation or median (interquartile range)

For the study selection process, after the removal of duplicate citations, the remaining ones were screened for potential eligibility based on reading of titles and abstracts, and the irrelevant ones were omitted. Afterward, the remaining citations were screened for potential eligibility via full-text evaluation, and the irrelevant ones were omitted. Besides, the reference lists of all eligible studies and recent reviews were manually screened for potential inclusion of other relevant studies. Two investigators completed the search of information sources and study selection process independently, and inconsistencies were resolved by consensus.

Data Items, Risk of Bias Assessment, and The Data Collection Process

The following baseline characteristics of the included studies were extracted: last author's name, date of publication, trial registration identifier, country of publication, study arms, sample size of patients, the age of patients, body mass index of patients, the route of MIH, and details of SHP block. The outcomes of this investigation comprised postoperative pain [according to the 10-point visual analogue scale (VAS) scoring system], postoperative opioid consumption [according to the morphine milligram equivalent (MME) unit], operation time (min), estimated intraoperative blood loss (mL), length of hospital stay (d), and complications (e.g., mechanical injury to anatomical structures) or toxicities (e.g., local anesthetic-related side effects such as bradycardia and hypotension) of the SHP block.

The quality of included studies was appraised according to the Cochrane risk of bias assessment tool for RCTs[13] and the Newcastle-Ottawa scale for nonrandomized comparative trials with cohort study designs[14].

All the data items were collected according to a predetermined form. Two pairs of investigators extracted the data items independently, and inconsistencies were resolved by consensus among the investigators of each pair.

Synthesis of Data

A quantitative meta-analysis was initially planned. However, owing to the insignificant number of available studies, methodologic heterogeneity (i.e., different study designs), and procedural variances (i.e., different routes of MIH), it was impossible to carry out a quantitative meta-analysis. Hence, the results of the included studies were only reported qualitatively (descriptively).

Results

Summary of The Literature Search and Baseline Characteristics of The Included Studies

Figure 1 displays the PRISMA flowchart. Overall, 112 citations were retrieved from the information sources, of which 52 citations were excluded from the duplication. Of the remaining 60 citations, 53 citations were excluded after reading the titles and abstracts. The remaining seven citations were subjected to full-text reading, of which four citations were excluded

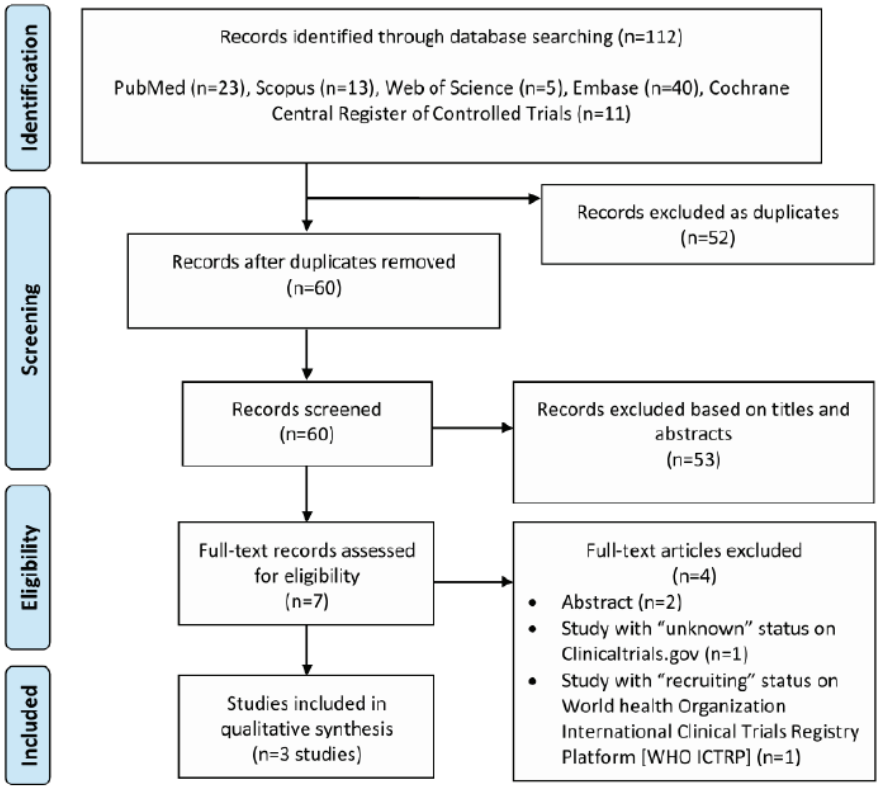

Figure 1. The PRISMA flowchart for literature search

with reasons: abstract (n=2), study with "unknown" status on Clinicaltrials.gov (n=1), and study with "recruiting" status on World Health Organization International Clinical Trials Registry Platform [WHO ICTRP] (n=1). Finally, three studies, comprising 210 patients (SHP=107 and non-SHP=103) were included in this systematic review[8-10]. These studies were published during 2019-2022 and conducted in Turkey (n=1) [8], United States of America (n=1)[9], and Australia (n=1)[10]. One study was a nonrandomized comparative trial (i.e., cohort study)[8] whereas the remaining two studies were RCTs[9,10]. The routes of MIH were laparoscopic in two studies[8,9] and mixed laparoscopic/robotic in one study[10]. The SHP block was performed at the start of MIH in one study[9] and at the end of the MIH in two studies[8,10]. The type of local anesthetic comprised 0.25% bupivacaine in two studies (amount ranging from 10-30 mL)[8,9] and 0.75% ropivacaine (10 mL) in one study[10]. Table 1 summarizes the baseline characteristics of the included studies.

Summary of Risk of Bias of The Included Studies

Figure 2 shows the risk of bias summary of the two RCTs. Both RCTs[9,10] were single-blinded and hence, the domain of performance bias was scored as high risk. Otherwise, all other domains were scored as low risk. Supplemental Table 2 shows the risk of bias assessment for the nonrandomized comparative trial (cohort study)[8]. The overall Newcastle-Ottawa scale score was 8 stars, suggesting "high-quality" and corresponding to "good quality" according to the Agency for Healthcare Research and Quality (AHRQ) standards[15].

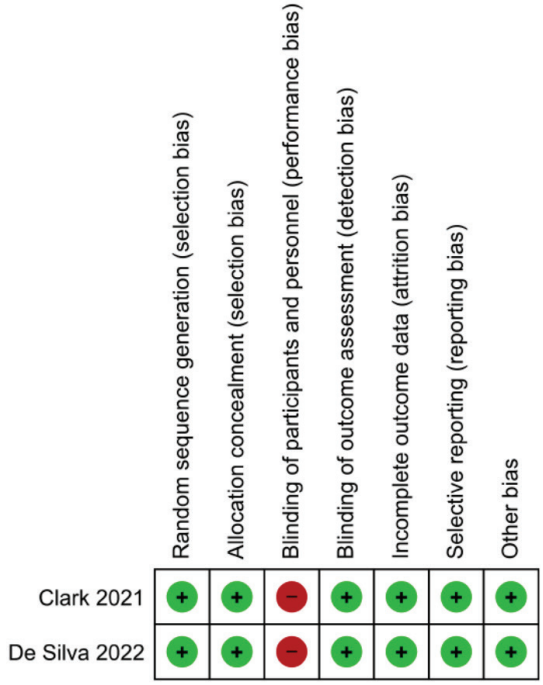

Figure 2. Quality assessment according to the Cochrane risk of bias tool for randomized controlled trials

Qualitative Synthesis of Outcomes

Table 2 details the main outcomes of the three systematically reviewed studies. Postoperative pain score at 24 h was reported in three studies[8-10]. Two studies[8,10] found that VAS scores were significantly lower in favor of the SHP group compared with the non-SHP group. However, while the RCT by Clark et al.[9] showed lower VAS scores in favor of the SHP group compared with the non-SHP group, the difference was not statistically significant.

Postoperative opioid consumption in 24 h was reported in three studies[8-10]. Aytuluk et al.[8] showed that the total MME consumption at both post-anesthesia care unit and surgical ward was significantly reduced in favor of the SHP group compared with the non-SHP group. Similar results were reported by De Silva et al.[10]. While the RCT by Clark et al.[9] showed lower MME in the recovery unit and surgical ward in favor of the SHP group compared with the non-SHP group, the difference was not statistically significant.

The mean operation time was reported in three studies[8-10], all of which showed no significant difference between the groups. Moreover, the estimated intraoperative blood loss was reported in two studies[9,10], both of which showed no significant difference between the groups. Furthermore, the length of hospital stay was reported in two studies[8,9], both of which showed no significant difference between the groups.

Intraoperative and postoperative complications were reported in three studies[8-10]. Aytuluk et al.[8] showed there was no difference in the rate of postoperative nausea and vomiting between the groups. All the three studies[8-10] showed no adverse events in the SHP group, such as mechanical injury to anatomical structures or toxicities arising from local anesthetic injection (e.g., bradycardia and hypotension).

Discussion

Summary of Findings

This systematic review was carried out to summarize the analgesic efficacy of SHP block versus no SHP block among patients undergoing MIH. Three studies, comprising 210 patients (SHP=107 and non-SHP=103) were included in the qualitative synthesis. Overall, the included studies had a low risk of bias. The results showed that SHP block appeared largely safe and could reduce postoperative pain and opioid consumption. However, SHP block did not offer clinical benefits in terms of reduced operation time, intraoperative blood loss, and hospital stay compared with non-SHP block.

Interpretation of Findings and Clinical Implications

Adequate control of postoperative pain following MIH is an important endpoint. This is because poor control of postoperative pain is disadvantageously connected to many adverse consequences. Such adverse consequences comprise long-term opioid addiction, reduced quality of life, acknowledged mobilization, extended hospitalization, and

higher healthcare costs[16]. Notably, prolonged postoperative analgesia with opioid is not without its adverse aftermath, such as nausea, vomiting, constipation, drowsiness, respiratory depression, and possibly chronic addiction if postoperative pain is not adequately controlled[17]. Hence, opioid-free multimodal analgesic approaches to decrease postoperative pain lessen opioid intake, and accelerate recovery are badly warranted[16]. Our systematic review revealed that SHP block was correlated with a substantial reduction in postoperative pain. Moreover, the favorable analgesic effects of SHP block were further corroborated by the decreased consumption of postoperative opioids.

Conventionally, SHP block is performed by injecting a local anesthetic agent (e.g., ropivacaine or bupivacaine) near the SHP (i.e., presacral region) by using anatomic landmarks to determine the injection site. Thus, this procedure

Table 2. Summary of the main outcomes

Study ID (Author, year)	Main outcomes
Aytuluk et al.[8]	• Mean VAS pain score at PACU was significantly lower in favor of the SHP compared with the non-SHP group (SHP=3.2±1.35, non-SHP=6.59±1.94, p<0.001) • Mean VAS pain score at 1 hour was significantly lower in favor of the SHP compared with the non-SHP group (SHP=2.17±1.12, non-SHP=5.47±2.26, p<0.001) • Mean VAS pain score at 24 hours was significantly lower in favor of the SHP compared with the non-SHP group (SHP=0.47±0.68, non-SHP=1.37±1.59, p=0.021) • Mean rescue analgesic time (min) was significantly delayed in favor of the SHP compared with the non-SHP group (SHP=825±322.86, non-SHP=325±180.19, p<0001) • Mean opioid consumption (MME) at PACU was significantly lower in favor of the SHP compared with the non-SHP group (SHP=0.6±2.5, non-SHP=1.3±3.4, p<0.001) • Mean opioid consumption (MME) at surgical ward was significantly lower in favor of the SHP compared with the non-SHP group (SHP=0±0, non-SHP=1.3±3.5, p=0.04) • Mean operation time (min) did not significantly differ between both groups (SHP=114.5±42.19, non-SHP=115.83±35.67, p=0.835) • Mean estimated intraoperative blood loss (mL) was not reported • Mean hospital stay (d) did not significantly differ between both groups (SHP=2.6±0.67, non-SHP=2.5±0.68, p=0.501) • Rate of postoperative nausea and vomiting did not significantly differ between both groups (SHP=10%, non-SHP=10%) • No complications (e.g., mechanical injury to anatomical structures) or toxicities (e.g., sympatholytic effects of bradycardia or hypotension) occurred in the SHP group
Clark et al.[9]	• Median VAS pain score at 2 hours did not significantly differ between both groups [SHP=3.9 (IQR=4.7), non-SHP=4.7 (IQR=2.9), p=0.45] • Median VAS pain score at 24 hours did not significantly differ between both groups [SHP=5 (IQR=5.5), non-SHP=6 (IQR=2.8), p=0.42] • Median opioid consumption (MME) at PACU did not significantly differ between both groups [SHP=5 (IQR=14.2), non-SHP=7.5 (IQR=12.5), p=0.22] • Median opioid consumption (MME) at 24 hours did not significantly differ between both groups [SHP=5 (IQR=13.8), non-SHP=10.2 (IQR=12.5), p=0.1] • Median operation time (min) did not significantly differ between both groups [SHP=110 (IQR=56), non-SHP=130.5 (IQR=55), p>0.05] • Median estimated intraoperative blood loss (mL) did not significantly differ between both groups [SHP=50 (IQR=50), non-SHP=50 (IQR=50), p>0.05] • Median hospital stay (d) did not significantly differ between both groups [SHP=0 (IQR=0), non-SHP=0 (IQR=0), p=0.78] • No complications (e.g., mechanical injury to anatomical structures) or toxicities (e.g., sympatholytic effects of bradycardia or hypotension) occurred in the SHP group
De Silva et al.[10]	• Mean VAS pain score at 24 hours was significantly lower in favor of the SHP compared with the non-SHP group (SHP=1.8, 95% CI: 1.5-2.1, non-SHP=2.6, 95% CI: 2.3-2.9) • Mean opioid consumption (MME) at 24 hours was significantly lower in favor of the SHP compared with the non-SHP group (SHP=33.1±4.3, non-SHP=54.9±6.8, p=0.0077) • Mean operation time (min) did not significantly differ between both groups (SHP=127±48, non-SHP=128.6±58.9, p=0.92) • Mean estimated intraoperative blood loss (ml) did not significantly differ between both groups (SHP=141.9±82.2, non-SHP=156.5±80.2, p=0.53) • No complications (e.g., mechanical injury to anatomical structures) or toxicities (e.g., sympatholytic effects of bradycardia or hypotension) occurred in the SHP group

CI: Confidence interval, IQR: Interquartile range, MME: Morphine milligram equivalent, PACU: Post-anesthesia care unit, SHP: Superior hypogastric plexus, VAS: Visual analogue scale, Aytuluk 2018 and De Silva 2022 reported findings as mean ± standard deviation, whereas Clark 2021 study reported findings as median [interquartile range]

necessitates the guidance of an imaging-based modality, such as fluoroscopy, ultrasonography, or computed tomography. However, during MIH, the abdominal and pelvic anatomical structures are well exposed intraoperatively, allowing for easy and direct access to the SHP[8]. Hence, intraoperative SHP block could be done rather simply and rapidly, without an obligatory need for imaging-based guidance. Since the SHP is anatomically situated close to key structures (e.g., somatic nerves, vertebral column, urinary bladder, and intestines), the SHP block procedure may be associated with potential intraoperative iatrogenic complications. Moreover, note that hemodynamic instability such as hypotension and bradycardia is possible, yet very rare, aftermath of the SHP block with ropivacaine or bupivacaine[8-10,18]. Overall, our systematic review confirmed that SHP block during MIH was technically feasible, quick to perform without extending operation time, and largely safe without adverse events.

SHP block has been depicted to improve the management of chronic pelvic pain arising from various cancer- and non-cancer-related etiologies[7]. Cancer-related etiologies comprise gynecologic and non-gynecologic pelvic malignancies, such as uterine, ovarian, cervical, bladder, prostatic, and rectal cancers. However, non-cancer-related etiologies comprise dysmenorrhea, endometriosis, pelvic malignant pain, pelvic inflammatory disease, and interstitial cystitis. Here, our systematic review expands the utility horizon of SHP block to include a gynecologic indication for postoperative analgesia following MIH. SHP block has been previously illustrated to successfully manage postoperative pain among patients undergoing abdominal hysterectomy[5,19] and cesarean section[20,21].

The RCT by Clark et al.[9] concluded that among patients undergoing MIH (laparoscopic route) with enhanced recovery after surgery (ERAS) protocol, SHP block failed to substantially reduce postoperative pain score and opioid consumption at different time points. The authors highlight several elucidation for these findings. Most notably, all patients were enrolled in an ERAS protocol, which decreases physiologic procedural stress, reduce hospitalization, minimize postoperative pain, and accelerate overall recovery[22,23]. All patients in this trial received SHP block and incisional analgesia, hence the patients benefited from alleviation of both visceral pelvic pain[24] and somatic pain (i.e., skin and deep tissue of abdominal wall), respectively. An additional reason was ascribed to the timing and volume of the SHP block. The SHP block was administered early at the beginning of the procedure, which might not have been adequate enough to produce sufficient postoperative analgesia. Moreover, the volume of the SHP block was relatively small (10 mL. It has been reported that the injection of higher volumes (15 to 18 mL) of local anesthetic or neurolytic agents during SHP block is associated with better analgesic responses than lower volumes[24,25]. Therefore, important clinical implications comprise the administration of SHP block at the end of the procedure with higher volumes, administration of incisional analgesia to lessen somatic pain, and enrollment of patients in ERAS perioperative protocols.

Strengths and Limitations

This investigation had several strengths that ought to be emphasized. To our understanding, we performed the first ever systematic review to examine the efficacy of SHP block for the management of postoperative pain following MIH. We included both nonrandomized comparative studies and RCTs in our investigation to increase the power of the pooled conclusions, which is a recommendation that is highly endorsed[26,27]. Additionally, we performed a PRISMA-complaint research investigation and reported as many outcomes as possible.

Nonetheless, this investigation equally harbors several limitations that ought to be underlined. The major limitation includes the small number of eligible studies and the corresponding small sample size of analyzed patients. An additional limitation includes the between-study heterogeneity, including variances in surgical procedures (e.g., route of MIH and type/dose/volume of the injected local anesthetic) and study designs (i.e., RCTs vs. nonrandomized comparative trials). These factors may also have somehow impacted the power of the conclusions. Because of the small number of included studies and clinical/methodologic heterogeneity, the results were only summarized systematically without a quantitative meta-analysis. Lastly, although the eligible studies were not double-blind, the measured outcomes (i.e., VAS score and opioid consumption) were less likely to be significantly impacted by this lack of blinding.

Future Directions

Taking into consideration the limited number of systematically reviewed studies, into additional large-sized RCTs are needed to validate the results of this investigation. As the origin of postoperative pain following MIH can arise from somatic and visceral sources[5], it will be worthwhile to examine the combined additive efficacy of incisional analgesia or abdominal wall plane block in addition to SHP block to alleviate somatic and visceral pain, respectively. Additional research may examine the ideal local anesthetic agent (ropivacaine versus bupivacaine) for the SHP block during MIH. Also, it is equally important to conduct dose-response analysis to identify the dose/volume that is associated with maximal efficacy and minimal toxicity. Lastly, it is meaningful to identify the cohorts of patients (i.e., stratified based on route of MIH, patient demographics, or clinical indications) who are more likely to benefit the most from postoperative analgesia with SHP block.

Conclusion

Among patients undergoing MIH, this systematic review showed that SHP block appeared largely safe and could reduce postoperative pain and opioid consumption. However, SHP block did not offer clinical benefits in terms of reduced intraoperative blood loss, operation time, and hospital stay

compared with non-SHP block. In view of the limitations of this systematic review, additional RCTs are needed to carry out a meta-analysis and validate the findings.

Ethics

Peer-review: Internally and externally peer-reviewed.

Authorship Contributions

Concept: M.A., A.A.Z., Design: M.A., A.A.Z., Data collection or processing: M.A., O.A., İ.A.A.B., H.S., Analysis or interpretation: M.A., A.A.Z., Literature search: M.A., A.A.Z., O.A., İ.A.A.B., H.S., Writing: M.A., A.A.Z., Reviewing manuscript for editorial and intellectual contents: M.A., A.A.Z., O.A., İ.A.A.B., H.S., Approval of manuscript for submission: M.A., A.A.Z., O.A., İ.A.A.B., H.S.

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Supplemental Table 1. The precise query search strategy used in all information sources

PubMed All Fields: (superior hypogastric plexus OR SHP OR presacral plexus OR presacral nerve) AND (block OR neurolysis OR neurectomy) AND (hysterectomy)
Scopus TITLE-ABS-KEY {Superior hypogastric plexus} OR SHP OR {presacral plexus} OR {presacral nerve} AND (block OR neurolysis OR neurectomy) AND (hysterectomy)
Web of Science All Fields: (superior hypogastric plexus OR SHP OR presacral plexus OR presacral nerve) AND (block OR neurolysis OR neurectomy) AND (hysterectomy)
Embase Quick search: ('superior hypogastric plexus'/exp OR 'superior hypogastric plexus' OR (superior AND hypogastric AND ('plexus'/exp OR plexus)) OR shp OR 'presacral plexus' OR (presacral AND ('plexus'/exp OR plexus)) OR 'presacral nerve' OR (presacral AND ('nerve'/exp OR nerve))) AND (block OR 'neurolysis'/exp OR neurolysis OR 'neurectomy'/exp OR neurectomy) AND ('hysterectomy'/exp OR hysterectomy)
Cochrane Central Register of Controlled Trials (CENTRAL) Title Abstract Keyword: (superior hypogastric plexus OR SHP OR presacral plexus OR presacral nerve) AND (block OR neurolysis OR neurectomy) AND (hysterectomy)

Supplemental Table 2. Quality assessment according to the Newcastle-Ottawa scale for cohort studies

	Selection				Comparability		Outcome			
Items	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	The study controls for demographics	The study controls for randomization	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow-up of cohorts	Overall score
Aytuluk et al.[8]	*	*	*	*	*		*	*	*	8/9