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TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

LETTER FROM THE PRESIDENT

Dear Turkish Society of Gynecology and Obstetrics Family,

It gives me great pleasure to be with you through the September issue of our scientific journal, which is also the third issue of 2026. Building on the considerable momentum it has gained, our journal continues to advance and strengthen its scientific contribution with each passing day.

I would like to emphasize that the Turkish Society of Gynecology and Obstetrics is not merely a professional association; it is also a professional organization that closely follows the challenges faced by our physicians and responds to them swiftly. As the Executive Board of the Society, we have demonstrated some of the clearest examples of this over the past three months.

In recent months, following the Ministry of Health's implementation of a practice under which physicians were assigned without remuneration for six months on the basis of cesarean section rates, our society responded promptly and appropriately. We issued a press statement that attracted nationwide attention and subsequently met with the Minister of Health on August 13, 2026, resulting in the cancellation of these cesarean-related assignments.

Likewise, our society's justified response prevented an obstetrician and gynecologist who had experienced an unfortunate incident in Şanlıurfa from being subjected to an unlawful prosecution.

On August 24, 2026, our society participated, by invitation, in a meeting held by the General Directorate of Health Services of the Ministry of Health, at which a ten-point action plan in favor of our physicians was adopted.

As we have always stated, TJOD is a civil society organization that safeguards the interests of our physicians on every platform and whose responses and press statements are widely recognized and respected.

Dear colleagues, I extend my respectful regards and look forward to being with you again in the final issue of 2026.

Best Regards

Ismail Mete Itil, Prof. MD

President of TJOD

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

EDITORIAL

Dear Colleagues,

We are once again together with the September issue of the Turkish Journal of Obstetrics and Gynecology, the scientific publication of the Turkish Society of Gynecology and Obstetrics. In this third issue of 2026, prepared with tremendous dedication, we are pleased to present studies of high scientific merit.

According to Web of Science and Scopus data, our journal's impact factor has gained considerable momentum and reached 1.5. I am proud to state that it has become the most prestigious and respected scientific journal in its field in our country. I would like to congratulate each of our reviewers and section editors for their dedicated work.

I would also like to note that the final issue of 2026 will be published in December, and I wish all our colleagues health, peace, and success in their work.

Best Regards

Ercan Yilmaz, Prof. MD

Clinical Investigation / Araştırma

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Turk J Obstet Gynecol 2026;23(3):201-9

Prenatal diagnosis of hemoglobinopathies by chorionic villus sampling: A large single-center experience

Koryon villus örneklemesi ile hemoglobinopatilerin prenatal tanısı: Geniş ölçekli tek merkez deneyimi

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Abstract

Objective: Hemoglobinopathies are among the most common inherited disorders worldwide. Given the high prevalence and carrier rates in our region, this study aimed to evaluate the obstetric, genetic, and procedure-related outcomes of pregnancies undergoing chorionic villus sampling (CVS) for prenatal diagnosis.

Materials and Methods: This retrospective observational study included 1,330 pregnant women referred for hemoglobinopathy screening between January 2008 and December 2012. Data regarding gestational age, indication for CVS, placental location, number of insertions, complications, and genetic results were analyzed. Transabdominal CVS was primarily performed between 10 and 14 weeks of gestation.

Results: The mean gestational age at CVS was 12.4±1.03 weeks. Late referral (≥14 weeks) was observed in 8.8% of cases. Adequate sampling was achieved with a single insertion in 88.3% of procedures. The overall complication rate was 2.6%, with a fetal loss rate of 1.8% and an incidence of chorioamnionitis of 0.07%. Multiple insertions (p=0.036) and posterior placental location (odds ratio: 2.21; 95% confidence interval: 1.11-4.38; p=0.033) were significantly associated with an increased risk of complications. Genotype analysis showed that 50.2% of fetuses were carriers, 25.4% were normal, and 21% were affected. Pregnancy termination was performed in most of the affected cases (n=266), while 11 cases resulted in live births. Hemoglobin S (HbS) was the most frequent variant (31%), followed by HbD and HbE. The most common β-thalassemia mutation was IVS-I-110 (G>A), and the most frequent α-thalassemia mutation was -$\alpha^{3.7}$ deletion.

Conclusion: CVS is a reliable and effective method for early prenatal diagnosis of hemoglobinopathies. Procedure-related risks are influenced by technical factors, such as the number of insertions and placental location. Standardization of techniques and operator experience may reduce complications. In high-prevalence regions, the integration of carrier screening and early prenatal diagnosis is essential to reduce the disease burden.

Keywords: Chorionic villus sampling, hemoglobinopathies, prenatal diagnosis, β-thalassemia, sickle cell disease

Öz

Amaç: Hemoglobinopatiler dünya genelinde en sık görülen kalıtsal hastalıklar arasında yer almaktadır. Bölgemizdeki yüksek prevalans ve taşıyıcılık oranları göz önünde bulundurularak, bu çalışmada prenatal tanı amacıyla koryon villus örneklemesi (CVS) uygulanan gebeliklerin obstetrik, genetik ve işleme bağlı sonuçlarının değerlendirilmesi amaçlandı.

Gereç ve Yöntemler: Bu retrospektif gözlemsel çalışmaya, Ocak 2008-Aralık 2012 tarihleri arasında hemoglobinopati taraması amacıyla merkezimize başvuran ve CVS uygulanan 1330 gebe dahil edildi. Gebelik haftası, CVS endikasyonu, plasenta lokalizasyonu, giriş sayısı, komplikasyonlar ve genetik sonuçlara ait veriler analiz edildi. Transabdominal CVS işlemi ağırlıklı olarak 10-14. gebelik haftaları arasında uygulandı.

PRECIS: Chorionic villus sampling provides reliable prenatal diagnosis of hemoglobinopathies, while multiple needle insertions and posterior placental location increase procedure-related complications.

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Bulgular: CVS uygulama haftası ortalama 12,4±1,03 hafta idi. Olguların %8,8'inde geç başvuru (≥14 hafta) saptandı. Yeterli örnekleme işlemlerin %88,3'ünde tek giriş ile sağlandı. Toplam komplikasyon oranı %2,6, fetal kayıp oranı %1,8 ve koryoamniyonit insidansı %0,07 olarak bulundu. Giriş sayısının artışı (p=0,036) ve posterior plasenta yerleşimi (olasılık oranı: 2,21; %95 güven aralığı: 1,11-4,38; p=0,033) komplikasyon riski ile anlamlı olarak ilişkiliydi. Fetüslerin %50,2'si taşıyıcı, %25,4'ü normal ve %21'i etkilenmiş olarak saptandı. Etkilenmiş olguların çoğunda gebelik sonlandırıldı (n=266), 11'i canlı doğumla sonuçlandı. En sık hemoglobin varyantı hemoglobin S (HbS) (%31) olup, bunu HbD ve HbE izledi. En sık β-talasemi mutasyonu IVS-I-110 (G>A), en sık α-talasemi mutasyonu ise -$\alpha^{3.7}$ delesyonu idi.

Sonuç: CVS, deneyimli merkezlerde uygulandığında hemoglobinopatilerin erken prenatal tanısında güvenilir ve etkili bir yöntemdir. İşleme bağlı komplikasyonlar giriş sayısı ve plasenta lokalizasyonu gibi teknik faktörlerden etkilenmektedir. Yüksek prevalanslı bölgelerde taşıyıcı taraması ve erken prenatal tanının entegre edilmesi hastalık yükünün azaltılmasında kritik öneme sahiptir.

Anahtar Kelimeler: Koryon villus örneklemesi, hemoglobinopatiler, β-talasemi, orak hücre hastalığı

Introduction

Hemoglobin is the main component of erythrocytes and the primary protein responsible for oxygen transport to tissues. The predominant form of hemoglobin in adults, hemoglobin A (HbA, $\alpha_2\beta_2$), has a tetrameric structure encoded by two separate globin gene clusters located in different regions of the genome. Hemoglobinopathies, which are genetic disorders affecting hemoglobin synthesis, constitute the most common group of monogenic diseases worldwide. These disorders are caused by deoxyribonucleic acid (DNA) variants located in the genes encoding globin chains or in their regulatory regions[1]. Hemoglobinopathies may lead to quantitative or qualitative abnormalities in globin chain synthesis. Reduced production of α or β globin chains results in α- and β-thalassemia syndromes, whereas structural abnormalities due to amino acid substitutions in globin chains lead to sickle cell disease and other abnormal hemoglobin variants. Structural hemoglobinopathies alter the solubility, stability, and oxygen-binding properties of hemoglobin, leading to hemolytic anemia and clinical complications related to chronic anemia and tissue hypoxia. These disorders are mostly inherited in an autosomal recessive pattern and are commonly associated with anemia[1].

It is estimated that 5-7% of the global population carry a structural variant that leads to defective hemoglobin synthesis. This results in approximately 300,000-500,000 affected newborns annually, the majority of whom have sickle cell disease, whereas a smaller proportion have transfusion-dependent β-thalassemia major. Although hemoglobinopathies are particularly prevalent in Africa, Asia, and Mediterranean countries, increasing migration has made them an important public health issue in Europe, the Americas, and Australia[2,3].

Structural hemoglobin variants arise from amino acid substitutions in globin chains. The most commonly encountered variants in clinical practice are HbS, HbD, HbE, and HbC. HbS is the most common and the first identified hemoglobin variant worldwide, with carrier rates reaching 10-40% in some regions of Africa[3]. In Türkiye, particularly in the Çukurova region, the carrier rate of HbS has been reported as 8.2%. The second most common structural hemoglobin variant in our country is HbD Los Angeles, with a carrier rate of 0.2%. HbE, the third most common variant, is predominantly observed in the Çukurova Region, with reported carrier rates ranging from 0.6% to 2.4%[4].

To date, 42 different structural hemoglobin variants have been identified in the Turkish population[4]. Due to the high prevalence and carrier rates of hemoglobinopathies in our region, the Hereditary Blood Diseases Application and Research Center established at Çukurova University in 1994 to prevent births affected by hemoglobinopathies and to conduct advanced diagnostic, therapeutic, and research activities. Chorionic villus sampling (CVS) has been performed for prenatal diagnosis since 1992, and the transabdominal single-needle technique is currently the standard method. Since the initiation of prenatal diagnostic services, approximately 5,000 cases have been evaluated. Due to the large caseload and archival limitations, this study included 1,330 cases diagnosed with hemoglobinopathies between January 1, 2008, and December 31, 2012.

Materials and Methods

Approximately 5,000 cases have undergone CVS for prenatal diagnosis at the Department of Obstetrics and Gynecology, Çukurova University Faculty of Medicine since 1992. Due to the large number of cases and archival limitations, a total of 1,330 cases evaluated for hemoglobinopathies between January 1, 2008, and December 31, 2012, were included in this study.

All pregnant women scheduled for CVS for prenatal diagnosis received genetic counseling prior to the procedure. They were informed about the procedure technique, possible complications, and their individual genetic disease risk relative to the population risk. In patients who accepted the procedure, blood groups and routine laboratory tests were evaluated, and gestational age, indication for CVS, placental location, pregnancy complications, and CVS results were recorded in the medical history and procedural records.

CVS was performed via the transabdominal route between 10 and 21 weeks of gestation. Sterile conditions were ensured prior to the procedure; during the intervention, villus samples were aspirated using a 20-gauge needle and a syringe containing 5 mL of heparinized saline solution.

Ultrasonographic evaluation was performed using a 3.5 MHz convex probe (Voluson 730 Pro, GE Healthcare). Routine analgesia was not administered during the procedure, and prophylactic anti-D immunoglobulin was given to patients at risk of Rh incompatibility.

Fetal DNA was isolated from the obtained samples in the Molecular Biology and Gene Laboratory of the Department of Medical Biochemistry, Çukurova University Faculty of Medicine. Mutation analysis was carried out using amplification-refractory mutation system (ARMS), restriction fragment length polymorphism (RFLP), or both. On average, the results were reported within two weeks. Follow-up of pregnancies with normal mutation analysis results continued at different centers. In fetuses with high-risk parental genotype combinations, pregnancy termination was performed with the family's consent and in accordance with the decision of the relevant ethics committee.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Çukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (approval number: 13, date: 04.04.2013). Written informed consent was obtained from all participants.

Statistical Analysis

Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as numbers and percentages, while continuous variables were expressed as mean ± standard deviation or median (minimum-maximum). The chi-square test was used to compare categorical variables. Normality of continuous variables was assessed using the Kolmogorov-Smirnov test; depending on the distribution, either the independent samples t-test or the Mann-Whitney U test was applied. A p-value <0.05 was considered statistically significant.

Results

A total of 1,330 cases undergoing CVS for prenatal diagnosis were included in the study. The mean gestational age at presentation to the clinic was 10 weeks; 8.8% of cases presented at or after 14 weeks of gestation (Table 1).

The earliest gestational age at which CVS was performed was 10 weeks, and the latest was 21 weeks and 5 days. The mean gestational age at the time of the procedure was 12.4±1.03 weeks, with a median of 12 weeks and 2 days. The distribution of cases according to gestational age at the time of the procedure is presented in Table 2.

Genotypic characteristics related to hemoglobinopathies in pregnant women presenting to our clinic are shown in Table 3. Accordingly, the most frequently detected maternal genotype was heterozygous HbS carrier (HbAS) (n=812), followed by heterozygous IVS-I-110 (G>A) mutation, consistent with β-thalassemia carrier status (n=216).

Table 1. Gestational week at admission of cases scheduled for chorionic villus sampling

Gestational weeks	n	%
4	1	0.1
5	1	0.1
6	21	1.6
7	76	5.7
8	124	9.3
9	226	17
10	274	20.6
11	245	18.4
12	166	12.5
13	80	6
14	54	4.1
>14	62	4.7
Total	1330	100

Table 2. Distribution of cases undergoing chorionic villus sampling according to gestational age

Gestational weeks	n	%
10	1	0.1
11	65	5
12	480	36
13	435	32.8
14	194	14.6
>14	152	11.5
Total	1330	100

Table 3. Genotypic characteristics of pregnant women presenting to our clinic

Genotype	n	%
HbAS (heterozygous HbS carrier)	812	61.1
β-thalassemia carrier: IVS-I-110 (G>A)	216	16.2
β-thalassemia carrier: IVS-I-6 (T>C)	31	2.3
Indeterminate genotype	30	2.3
α-thalassemia: -$\alpha^{3.7}$ deletion	27	2
HbAS + α-thalassemia (-$\alpha^{3.7}$)	24	1.8
β-talasemi: Codon 39 (C>T)	19	1.4
HbAD (heterozygous HbD)	16	1.2
HbAE (heterozygous HbE)	14	1.1
β-thalassemia: -30 (promoter mutation)	14	1.1
HbSS (homozygous HbS)	14	1.1
β-thalassemia: Codon 8 (-AA)	11	0.8
Other	102	2.7
Total	1330	100

Table 4 shows the genotypic characteristics of partners of pregnant women presenting to our clinic with respect to hemoglobinopathies. Accordingly, the most frequently detected genotype was the heterozygous HbAS (n=821), followed by the heterozygous IVS-I-110 (G>A) mutation, consistent with β-thalassemia carrier status (n=180).

The number of insertions performed to obtain adequate samples in cases undergoing CVS is presented in Table 5.

Table 4. Genotypic characteristics of partners of pregnant women presenting to our clinic

Genotype	n	%
HbAS (heterozygous HbS carrier)	821	61.7
β-thalassemia carrier: IVS-I-110 (G>A)	180	13.5
β-thalassemia carrier: IVS-I-1 (G>A)	39	2.9
α-thalassemia: -$\alpha^{3.7}$ deletion	32	2.4
Indeterminate genotype	27	2.0
β-thalassemia carrier: IVS-I-6 (T>C)	26	2.0
β-thalassemia: Codon 39 (C>T)	22	1.7
β-thalassemia: Codon 8 (-AA)	20	1.5
HbAS + α-thalassemia (-$\alpha^{3.7}$)	18	1.4
HbAE (heterozygous HbE)	17	1.3
β-thalassemia: -30 (promoter mutation)	12	0.9
HbAD (heterozygous HbD)	11	0.8
HbSS (homozygous HbS)	8	0.6
Other	97	7.3
Total	1330	100.0

Table 5. Number of insertions performed to obtain adequate material in cases undergoing chorionic villus sampling

Number of insertions	n	%
Single insertion	1175	88.3
Two insertions	134	10.1
Three insertions	19	1.4
Four insertions	1	0.1
Five insertions	1	0.1
Total	1330	100

Adequate material was obtained with a single insertion in 1,175 cases (88.3%), while two insertions were required in 134 cases (10.1%) and three insertions were required in 19 cases (1.4%).

Adequate samples were obtained and successfully analyzed in 1,284 of 1,330 pregnancies (96.6%); no diagnostic result was obtained in 46 cases (3.4%). Among these, 38 cases with inconclusive results are presented in detail in Table 6. In these cases, maternal contamination was identified in 5 cases, the mutation was not detected in 16 cases, and samples were insufficient in 17 cases. Considering the gestational age, repeat CVS was performed in 17 cases, whereas cordocentesis was carried out in 21 cases.

Among the 38 cases that required a repeat procedure, a single insertion was performed in 32 cases, two insertions were performed in 5 cases, and three or more insertions were performed in 1 case. Of the 17 cases with insufficient sample acquisition, 13 underwent a single insertion (76.4%), 3 required two insertions (17.8%), and 1 required three or more insertions (5.8%). Among the 5 cases with maternal contamination, 4 underwent a single insertion (80%) and 1 required two insertions (20%). Of the 16 cases in which the mutation could not be detected, 15 underwent a single insertion (93.7%), and 1 required two insertions (6.3%).

Complications occurring after CVS are presented in Table 7. Of the 1,330 cases undergoing CVS, 34 developed complications within the first two weeks. Post-procedural complications included missed abortion in 20 cases (1.5%), amniotic fluid leakage in 6 cases (0.5%), vaginal bleeding in 4 cases (0.3%), spontaneous abortion in 3 cases (0.2%), and sepsis in 1 case (0.1%). The overall complication rate was determined to be 2.6%.

The overall fetal loss rate following the procedure was determined to be 1.8%. A statistically significant increase in the risk of complications was observed with an increasing number of insertions (p=0.036). The incidence of chorioamnionitis after CVS was 0.07%.

The relationship between placental location and the risk of complications is presented in Table 8. The risk of complications was significantly higher in cases with posterior placental localization than in those with non-posterior localization (odds ratio: 2.21; 95% confidence interval: 1.11-4.38; p=0.033).

Table 6. Distribution of repeat procedure indications according to the number of insertions in chorionic villus sampling

Number of insertions	Failure to detect mutation	Contamination	Insufficient material	Total
1	15 (46.9)	4 (12.5)	13 (40.6)	32
2	1 (20.0)	1 (20.0)	3 (60.0)	5
≥3	0 (0.0)	0 (0.0)	1 (100.0)	1
Total	16 (42.1)	5 (13.2)	17 (44.7)	38

The relationship between the number of insertions performed during CVS and the development of complications is presented in Table 9. A statistically significant increase in the risk of complications was observed with an increasing number of insertions (p=0.036).

The mean turnaround time for CVS results among cases presenting to our clinic was 9 days. The genotypic characteristics of the fetuses, according to CVS results, are presented in Table 10. Accordingly, the most frequently detected structural hemoglobin variant was HbS (31%), while HbD and HbE were observed at lower frequencies.

When thalassemia mutations were evaluated, the most common mutation associated with β-thalassemia was IVS-I-110 (G>A) (7.9%), whereas the most frequent mutation for α-thalassemia was the -$\alpha^{3.7}$ deletion (2%).

Mutational characteristics detected in cases undergoing CVS are presented in Table 11. Accordingly, heterozygous mutations were identified in approximately 50.2% of cases, while no mutations were detected in 25.4% of cases. Homozygous mutations were observed in 21% of cases, and genotype determination could not be performed in 3.4%.

Pregnancy outcomes of women undergoing CVS are presented in Table 12. A total of 636 infants (47.8%) were born heterozygous carriers of hemoglobinopathies, whereas 329 infants (24.8%) had no detectable structural hemoglobin variant and were healthy at birth. Of the 279 fetuses diagnosed with homozygous mutations by CVS, pregnancy termination was performed in 266 cases (20.2%). Despite the presence of homozygous mutations, pregnancies were continued in 11 cases, all of which resulted in live births, while 2 cases ended in missed abortions.

In 46 cases (3.4%), no diagnostic result could be obtained. All 6 cases resulting in stillbirths were found to have heterozygous mutations. In one case resulting in preterm delivery, no mutation was detected. Among the 9 cases ending in spontaneous abortion, 5 had heterozygous mutations, whereas no mutation was identified in the remaining 4 cases.

The comparison between pregnancy outcomes and maternal education levels is presented in Table 13. Notably, among infants born with an affected genotype, 10 out of 11 (90.8%) had mothers who were either illiterate or had completed only primary education.

Among cases undergoing CVS for hemoglobinopathies, two pregnancies were diagnosed with trisomy 21 following amniocentesis and cytogenetic karyotype analysis, which were performed because an increased risk was identified on

Table 7. Complications observed following chorionic villus sampling

Outcome	n	%
No complication	1296	97.4
Complication (+)	34	2.6
Missed abortion	20	1.5
Amniotic fluid leakage (PROM)	6	0.5
Vaginal bleeding	4	0.3
Spontaneous abortion	3	0.2
Sepsis	1	0.1
Total	1330	100

PROM: Premature rupture of membranes

Table 8. Placental location in cases with complications following chorionic villus sampling

Placental location	Complication (+)	Complication (-)	Total	p	Odds ratio
Posterior	16 (4.1)	372 (95.9)	388 (29.2)	0.033	2.21
Others	18 (1.9)	924 (98.1)	942 (70.8)		
Total	34 (2.6)	1296 (97.4)	1330 (100)		

Table 9. Relationship between the number of insertions and complications in chorionic villus sampling

Number of insertions	Complication (+)	Complication (-)	Total	p
1	26 (2.2)	1149 (97.8)	1175 (100)	
>1 (multiple)	8 (5.2)	147 (94.8)	155 (100)	0.036
Total	34 (2.6)	1296 (97.4)	1330 (100)	

Table 10. Genotypic characteristics of fetuses according to CVS results

Genotype	n	%
HbAS (heterozygous HbS)	412	31.0
HbAA (normal)	337	25.3
HbSS (homozygous HbS)	177	13.3
β-thalassemia carrier: IVS-I-110 (G>A)	105	7.9
Indeterminate genotype	46	3.4
α-thalassemia: -$\alpha^{3.7}$ deletion	26	2.0
β-thalassemia: IVS-I-1 (G>A)	20	1.5
HbAS + α-thalassemia (-$\alpha^{3.7}$)	15	1.1
β-thalassemia: IVS-I-6 (T>C)	13	1.0
β-thalassemia: Codon 8 (-AA)	12	0.9
HbAD (heterozygous HbD)	9	0.7
β-thalassemia: Codon 39 (C>T)	9	0.7
HbAE (heterozygous HbE)	8	0.6
β-thalassemia: -30 (promoter mutation)	7	0.5
Other	134	10.1
Total	1330	100.0

CVS: Chorionic villus sampling

second-trimester triple screening. Both pregnancies were subsequently terminated. In one case, a cystic hygroma was detected, and the pregnancy resulted in intrauterine fetal demise at 23 weeks' gestation. In another case, the pregnancy was terminated following the diagnosis of a tracheoesophageal fistula.

The CVS genotypes of the cases diagnosed with trisomy 21 were HbAS and heterozygous IVS-I-110 (G>A) mutation, respectively. In both cases, which presented with cystic hygroma and tracheoesophageal fistula, CVS results revealed HbAS.

Table 11. Fetal mutation characteristics in cases undergoing chorionic villus sampling

Mutation status	n	%
Heterozygous genotype (carrier)	668	50.2
No mutation detected (normal genotype)	337	25.4
Homozygous or compound heterozygous genotype (affected)	279	21.0
Genotype undetermined	46	3.4
Total	1330	100

Table 12. Pregnancy outcomes of women presenting to our clinic

Outcome	n	%
Heterozygous carrier newborn	636	47.8
Newborn with normal genotype	329	24.8
Pregnancy termination	269	20.3
Cases without diagnostic result	46	3.4
Missed abortion	23	1.8
Newborn with affected genotype	11	0.8
Spontaneous abortion	9	0.6
Stillbirth (intrauterine fetal demise)	6	0.4
Preterm delivery	1	0.1
Total	1330	100.0

Discussion

In this study, obstetric, genetic, and procedure-related outcomes in a large case series of patients undergoing CVS for the prenatal diagnosis of hemoglobinopathies were evaluated. The findings indicate that CVS is a reliable and effective prenatal diagnostic method when performed in experienced centers. Hemoglobinopathies are inherited disorders caused by DNA variants affecting globin genes and constitute one of the most common groups of monogenic diseases worldwide[1,5]. E arly prenatal diagnosis is critically important not only for preventing affected births but also for enabling families to make informed reproductive decisions. In a series of 5,500 CVS procedures conducted by Podobnik et al.[6] in Croatia, the fetal loss rate was reported as 1.19%. Martins et al.[7] reported a rate of 0.2% in a study including 1,523 pregnancies. Similarly, Gil et al.[8] demonstrated that the risk of fetal loss following CVS is approximately 1%. In our study, the overall fetal loss rate was 1.8%, which, although slightly higher than rates reported in the literature, can still be considered within acceptable limits.

The increased risk of complications observed in cases with posterior placental localization may be related to the technical challenges of CVS. In such cases, a longer needle trajectory and increased manipulation may prolong the procedure and increase tissue trauma. International guidelines emphasize that CVS should be performed under ultrasound guidance, with the minimum number of insertions possible, and in experienced centers[6,9,10]. In this context, our findings support current guideline recommendations. In particular, the significant association between an increased number of insertions and a higher risk of complications highlights the technical sensitivity of the procedure and the importance of operator experience. This observation is also supported by the multicenter retrospective study by Navaratnam et al.[11], which reported that multiple insertions are associated with increased risks of fetal loss and infection[6]. These findings are consistent with previous studies. Recent studies have further demonstrated that the procedure-related risk of fetal loss following CVS is lower than previously estimated, particularly when performed in experienced centers.

Table 13. Comparison of pregnancy outcomes according to maternal education level

Outcome	Illiterate	Primary education	High school	University	Total	p
Heterozygous carrier newborn	16 (3.0)	407 (64.0)	148 (23.0)	65 (10.0)	636 (100)	
Newborn with normal genotype	15 (3.0)	191 (60.0)	83 (25.0)	40 (12.0)	329 (100)	
Pregnancy termination	6 (2.0)	161 (60.0)	75 (28.0)	27 (10.0)	269 (100)	
Newborn with affected genotype	4 (36.3)	6 (54.5)	1 (9.2)	0 (0.0)	11 (100)	
Other pregnancy outcomes	85	-	-	-	85 (100)	
Total	1330					**<0.001***

* Statistical significance was evaluated using the chi-square test; p<0.05 was considered statistically significant

Contemporary evidence suggests that CVS-related pregnancy loss is comparable to that of amniocentesis and may be close to the background risk in low-risk populations. Advances in ultrasound guidance and operator experience have contributed to improved safety profiles of invasive prenatal diagnostic procedures[12]. However, our cohort represents clinical practice between 2008 and 2012. Therefore, the reported complication rates and diagnostic performance should be interpreted within the context of ultrasound technology, procedural experience, and molecular diagnostic approaches available during that period. Continuous improvements in imaging quality, invasive procedure techniques, and genetic testing platforms may have further optimized current outcomes. These findings support the continued role of CVS as a first-line diagnostic tool, especially during early gestation, when timely diagnosis is essential for clinical decision-making.

Evaluation of the fetal genotype distribution revealed that 21% of cases had homozygous or compound heterozygous (affected) genotypes, reflecting the high carrier frequency of hemoglobinopathies in our region. Globally, approximately 5-7% of the population are carriers of a pathogenic hemoglobin variant, and 300,000-500,000 affected neonates are born annually. The majority of these cases consist of sickle cell disease and transfusion-dependent β-thalassemia[3,13]. In our study, the majority of pregnancies with fetuses carrying affected genotypes were terminated, highlighting the significant role of prenatal diagnosis in reducing the burden of hemoglobinopathies.

The most frequently detected structural hemoglobin variant in our study was HbS, which is consistent with expectations for the Mediterranean basin and Middle Eastern populations. Sickle cell disease is one of the most common monogenic disorders worldwide, affecting approximately 7.7 million individuals, resulting in an estimated 515,000 affected births annually. The more than 40% increase in global prevalence between 2000 and 2021 is largely attributed to population growth in high-burden regions such as sub-Saharan Africa[3,12,14]. Similar experiences from Mediterranean hemoglobinopathy prevention programs have demonstrated the effectiveness of combining population-based carrier screening, genetic counseling, and early prenatal diagnosis. National programs in countries with a high prevalence of β-thalassemia and sickle cell disease, including Cyprus, Italy, Greece, and Türkiye, have substantially reduced the number of affected births through systematic screening and CVS-based molecular diagnosis. Our findings are consistent with these programs and support the role of early invasive prenatal diagnosis in high-risk populations[15,16]. Among β-thalassemia mutations, the most frequently detected mutation was IVS-I-110 (G>A), which is consistent with the mutation spectrum reported in Türkiye and neighboring countries[17,18]. This finding underscores the importance of considering regional mutation distributions when planning prenatal diagnostic strategies.

Another important consideration is the molecular diagnostic methodology used in this study. Mutation analysis was performed using ARMS and RFLP, which were standard approaches during the study period. Although these methods are reliable for detecting known mutations, they may have limitations in identifying rare or novel variants compared to current high-throughput techniques such as next-generation sequencing (NGS). Nevertheless, given that the mutation spectrum in our region is well characterized and largely consists of common variants, these methods remain clinically adequate for targeted prenatal diagnosis. Future studies incorporating advanced molecular techniques may further improve diagnostic accuracy.

The success of prenatal diagnosis programs depends not only on the implementation of invasive diagnostic procedures but also on the integration of preconception screening, identification of carriers, and effective genetic counseling services. The World Health Organization and other international health authorities recommend an integrated approach combining carrier screening and prenatal diagnosis for hemoglobinopathies[15,16]. CVS offers a significant advantage over amniocentesis by allowing earlier prenatal diagnosis, thereby enabling families to make timely decisions.

Study Limitations

One of the major strengths of this study is the inclusion of a large case series reflecting extensive single-center experience accumulated over many years. However, several limitations should be acknowledged. First, the retrospective design of the study may introduce inherent bias. Although several factors such as gestational age at CVS, maternal characteristics, placental accessibility, and procedural variables may influence complication risk, a multivariable logistic regression model could not be reliably performed because of the limited number of adverse events. Performing such an analysis with a small number of outcomes could result in unstable estimates. Second, the limited availability of long-term neonatal outcomes restricts the comprehensive evaluation of the clinical impact. Additionally, the study period (2008-2012) may limit the generalizability of the findings to current clinical practice.

Another important limitation is the use of conventional molecular techniques, such as ARMS and RFLP, for mutation analysis. Although these methods were standard at the time of data collection, they have lower sensitivity for detecting rare or novel mutations than current high-throughput techniques such as NGS.

Despite these limitations, the findings provide valuable contributions to both clinical practice and the development of regional prenatal diagnostic policies. Since this study

included only high-risk pregnancies referred to a tertiary prenatal diagnosis center, the observed mutation frequencies and genotype distributions should not be interpreted as representative of the general population. Referral bias may have influenced the frequency of specific hemoglobin variants and mutations. Overall, our results support the conclusion that CVS is a reliable, effective, and feasible method for the prenatal diagnosis of hemoglobinopathies. Particularly in regions with a high prevalence of hemoglobinopathies, the expansion of comprehensive prenatal diagnostic and genetic counseling programs conducted at experienced centers remains a cornerstone strategy for reducing disease burden.

Conclusion

This study demonstrates that CVS is a reliable and effective method for prenatal diagnosis of hemoglobinopathies. Findings derived from a large case series indicate that CVS provides an accurate genetic diagnosis during early gestational weeks with acceptable complication rates. The identification of technical factors such as the number of insertions and placental location as determinants of complication risk underscores the importance of performing CVS in experienced centers and in accordance with standardized protocols.

The distribution of fetal genotypes further indicates that severe hemoglobinopathies, particularly sickle cell disease and β-thalassemia, remain a significant public health concern in our region. The ability to detect affected fetuses at an early stage through prenatal diagnosis provides important advantages in pregnancy management and genetic counseling. In regions with a high prevalence of hemoglobinopathies, the integration of preconception screening, carrier detection, and prenatal diagnostic services represents a key strategy for reducing disease burden. When performed by experienced practitioners and with appropriate patient selection, CVS remains an effective and reliable method for the prenatal diagnosis of hemoglobinopathies.

Ethics

Ethics Committee Approval: This study was conducted in accordance with the Declaration of Helsinki and was approved by the Çukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (approval number: 13, date: 04.04.2013).

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

Footnotes

Authorship Contributions

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

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Predictive value of fasting bile acids and liver enzymes for perinatal outcomes in intrahepatic cholestasis of pregnancy

Gebelik kolestazında açlık safra asitleri ve karaciğer enzimlerinin perinatal sonuçları öngörmedeki değeri

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Abstract

Objective: To investigate the value of fasting bile acid (FBA) levels and liver enzymes [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] in predicting adverse perinatal outcomes in intrahepatic cholestasis of pregnancy (ICP).

Materials and Methods: This retrospective study included 296 pregnant women diagnosed with ICP between 2020 and 2025. Patients were classified as having mild (FBA 10-40 µmol/L, n=223) or severe ICP (FBA >40 µmol/L, n=73). Clinical, laboratory, and neonatal outcomes were compared; receiver operating characteristic (ROC) analysis was performed to evaluate the predictive value of FBA for meconium passage; and patients who received ursodeoxycholic acid (UDCA) therapy were compared with those who did not.

Results: Compared with the mild ICP group, patients with severe ICP had earlier gestational ages at diagnosis and at delivery, and lower birth weight and neonatal pH (all p<0.05). FBA levels were significantly associated with fetal growth restriction (p=0.042), preterm birth (p=0.003), neonatal intensive care unit (NICU) admission (p<0.001), and meconium passage (p<0.001). AST was associated only with NICU admission and meconium passage, whereas ALT showed no significant association with perinatal complications. The median FBA level was significantly higher in patients with meconium passage (105 vs. 20 µmol/L; p<0.001). ROC analysis identified an FBA cut-off of 88.45 µmol/L (area under the curve=0.694), with 57.1% sensitivity and 81.3% specificity.

Conclusion: FBA is the strongest predictor of adverse perinatal outcomes in ICP. AST provides complementary prognostic information, whereas ALT has limited predictive value. Clinical management should be guided by FBA-based risk stratification and individualized timing of delivery.

Keywords: Intrahepatic cholestasis of pregnancy, fasting bile acid, perinatal outcomes, meconium passage, aspartate aminotransferase

Öz

Amaç: Bu çalışmanın amacı, gebelik kolestazında (ICP) açlık safra asidi (FBA) düzeyleri ile karaciğer enzimlerinin [aspartat aminotransferaz (AST) ve alanin aminotransferaz (ALT)] advers perinatal sonuçları öngörmedeki değerini araştırmaktır.

Gereç ve Yöntemler: Bu retrospektif çalışmaya 2020-2025 yılları arasında ICP tanısı alan 296 gebe dahil edildi. Olgular hafif (FBA 10-40 µmol/L; n=223) ve şiddetli (FBA >40 µmol/L; n=73) ICP olarak sınıflandırıldı. Klinik, laboratuvar ve neonatal sonuçlar karşılaştırıldı, FBA'nın mekonyum pasajını öngörmedeki performansı alıcı işletim karakteristiği (ROC) analizi ile değerlendirildi. Ayrıca, ursodeoksikolik asit (UDCA) tedavisi alan ve almayan hastalar karşılaştırıldı.

Bulgular: Hafif ICP grubuyla karşılaştırıldığında, şiddetli ICP grubunda tanı ve doğum haftası daha erken, doğum ağırlığı ve neonatal pH ise daha düşüktü (tümü p<0,05). FBA düzeyleri; fetal büyüme kısıtlılığı (p=0,042), preterm doğum (p=0,003), yenidoğan yoğun bakım ünitesine (NICU)

PRECIS: In pregnancies complicated by intrahepatic cholestasis, fasting bile acid levels accurately identify women at increased risk of adverse perinatal outcomes and may guide individualized surveillance and timing of delivery.

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yatış (p<0,001) ve mekonyum pasajı (p<0,001) ile anlamlı ilişki gösterdi. AST yalnızca NICU yatışı ve mekonyum pasajı ile ilişkiliyken, ALT perinatal komplikasyonlarla anlamlı ilişki göstermedi. Mekonyum pasajı gelişen olgularda ortanca FBA düzeyi daha yüksekti (105'e karşı 20 µmol/L; p<0,001). ROC analizinde FBA için 88,45 µmol/L kesme değeri saptandı (eğri altındaki alan=0,694); duyarlılık %57,1, özgüllük ise %81,3 olarak bulundu.

Sonuç: FBA, ICP'de advers perinatal sonuçların en güçlü öngörücüsüdür. AST tamamlayıcı prognostik bilgi sağlarken, ALT'nin öngörü değeri sınırlıdır. Klinik yönetimde FBA temelli risk sınıflandırması kullanılmalı ve doğum zamanlaması hastanın risk profiline göre bireyselleştirilmelidir.

Anahtar Kelimeler: İntrahepatik gebelik kolestazı, açlık safra asidi, perinatal sonuçlar, mekonyum pasajı, aspartat aminotransferaz

Introduction

Intrahepatic cholestasis of pregnancy (ICP) is a hepatic disorder occurring in the second and third trimesters of pregnancy, characterized primarily by generalized pruritus that typically begins on the palms and soles, and is defined by elevated serum fasting bile acid (FBA) levels[1]. Although it is clinically considered a benign condition for the mother, ICP may lead to significant fetal and neonatal complications in addition to maternal symptoms[2]. The disease increases the risk of fetal cardiac arrhythmias, meconium passage, fetal distress, preterm birth, and, in rare cases, intrauterine fetal demise, due to the transplacental passage of bile acids to the fetus[3].

The incidence of ICP varies by geographic and ethnic factors, but it is reported to range from 0.5% to 2% in the general population; higher rates have been observed in certain regions, such as South America and Scandinavia[4]. Diagnosis is established by the presence of typical pruritus, a FBA level exceeding 10 µmol/L, and exclusion of other hepatic disorders[1]. Disease severity is classified according to FBA levels: the mild form is generally defined as 10-40 µmol/L, whereas the severe form is considered ≥40 µmol/L; in some studies, levels ≥100 µmol/L are additionally categorized as a "very severe" subgroup, representing a distinct high-risk category[2].

Higher FBA levels have consistently been reported to be strongly associated with adverse perinatal outcomes. In particular, patients with FBA levels >40 µmol/L demonstrate a significantly increased frequency of adverse outcomes such as preterm birth, meconium-stained amniotic fluid, neonatal intensive care unit (NICU) admission, and low birth weight[5-8]. At an FBA threshold of ≥100 µmol/L, the risk of intrauterine fetal demise increases markedly (approximately 3-7%), which has led to the consideration of early delivery planning—typically between 35 and 37 weeks of gestation—in management strategies[6].

Liver enzymes [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] are also frequently elevated in ICP, and some studies have reported that these enzymes, particularly AST, correlate with adverse perinatal outcomes, including meconium passage and NICU admission[9]. However, FBA levels are generally considered stronger predictors than liver enzyme levels. Elevated FBA levels increase fetal morbidity through mechanisms involving fetal myocardial involvement and placental dysfunction[10]. In treatment, ursodeoxycholic acid (UDCA) improves maternal symptoms and liver enzyme levels and may provide limited benefit in reducing the risk of preterm birth; however, it has been emphasized that it does not definitively prevent perinatal mortality[11].

This study aims to retrospectively evaluate the predictive value of FBA and liver enzymes for perinatal outcomes in patients with ICP, including gestational age at delivery, birth weight, Apgar scores, meconium passage, fetal growth restriction (FGR), NICU admission, and intrauterine fetal demise. In particular, the study aims to contribute to risk stratification by disease severity through comparisons of mild and severe groups, analysis of biochemical parameters in patients with and without complications, and receiver operating characteristic (ROC) analysis of FBA for meconium passage.

Materials and Methods

This study was a retrospective single-center investigation. Patients aged 18-44 years who were diagnosed with ICP and managed at the Perinatology Department of University of Health Sciences Türkiye, Ankara Bilkent City Hospital between January 2020 and December 2025 were included in the study. Ethical approval for the study was obtained from the University of Health Sciences Türkiye, Ankara Bilkent City Hospital Medical Research Scientific and Ethical Review Board (approval number: TABED 2-26-2291, date: 13.05.2026). The study was conducted in accordance with the principles of the Declaration of Helsinki at all stages. Patient data were retrieved from the hospital database. For each patient, the following data were recorded: maternal age, gravidity, parity, gestational age at disease onset, receipt of UDCA therapy, pregnancy complications, routine liver function test results and FBA levels at the time of diagnosis, gestational age at delivery, neonatal birth weight, 1- and 5-minute Apgar scores, umbilical cord pH, and NICU admission. The diagnosis of ICP was established in the presence of pruritus unexplained by any other liver disease and a FBA level of ≥10 µmol/L. At our institution, serum AST, ALT, and FBA levels are routinely measured in all patients with suspected ICP. FBA levels were measured in venous blood samples collected after an overnight fast of at least 8 hours. The laboratory parameters used in this study were the initial measurements obtained at the time of diagnosis, before the initiation of treatment. At our institution, patients diagnosed with ICP were initiated on UDCA therapy after consultation with a gastroenterologist as part of routine clinical practice. The decision to initiate UDCA therapy was based on a

comprehensive evaluation of clinical symptoms, FBA levels, and liver function test results. UDCA was initiated at a dose of 10-15 mg/kg/day[12]. At our institution, the timing of delivery was determined according to disease severity, gestational age, maternal and fetal clinical status, and current international guideline recommendations[2].

Patients with ICP were classified into two groups according to FBA levels: mild ICP (FBA 10-40 µmol/L) and severe ICP (FBA >40 µmol/L). The reference ranges for the laboratory parameters used in this study were as follows: FBA, <10 µmol/L; AST, 10-35 U/L; and ALT, 7-35 U/L. Patients with acute or chronic hepatitis, autoimmune hepatitis, hypertensive disorders of pregnancy, multiple gestations, a history of organ transplantation, known fetal chromosomal or major structural anomalies, and those with incomplete medical records were excluded from the study.

Study Groups

Initially, patients were classified into two groups according to disease severity: mild and severe ICP; comparisons were performed between the two groups. Subsequently, laboratory parameters were compared between patients with and without obstetric and neonatal complications. In addition, comparisons were made between patients who received UDCA therapy and those who did not.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). Normality of data distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous variables were summarized as the median (25^{th}-75^{th} percentile), whereas categorical variables were expressed as frequencies and percentages. Associations between categorical variables were analyzed using the chi-square test. As the data were not normally distributed, differences between groups were analyzed using the Mann-Whitney U test. The predictive performance of FBA levels for meconium passage was evaluated using ROC curve analysis, including the area under the curve (AUC), sensitivity, and specificity. A two-sided p-value <0.05 was considered statistically significant.

Results

A total of 296 pregnant women who met the study eligibility criteria were included. Of these, 223 (75.3%) were classified as having mild ICP and 73 (24.7%) as having severe ICP.

The clinical and demographic characteristics, as well as the obstetric, delivery, and neonatal outcomes of patients with mild and severe ICP are presented in Table 1.

Patients with FBA levels >40 µmol/L had significantly higher AST and ALT levels, and lower gestational age at diagnosis and at delivery, birth weight, and neonatal pH than those with FBA levels of 10-40 µmol/L (Table 1).

The associations of maternal AST, ALT, and FBA levels with FGR, NICU admission, preterm birth, and meconium passage are presented in Table 2.

FBA levels were significantly higher in patients who experienced FGR, NICU admission, preterm birth, or meconium passage than in patients without these complications. AST levels were significantly higher only in patients who required NICU admission or who experienced meconium passage. In contrast, ALT levels did not differ significantly between patients with and without obstetric or neonatal complications (Table 2).

ROC curve analysis was performed to evaluate the predictive value of FBA levels for meconium passage in pregnancies complicated by ICP. An FBA cut-off of 88.45 µmol/L predicted

Table 1. Comparison of clinicodemographic characteristics, laboratory findings, and neonatal outcomes between the study groups

	FBA 10-40 µmol/L (n=223) median (25^{th}-75^{th} percentile)	FBA >40 µmol/L (n=73) median (25^{th}-75^{th} percentile)	p-value
Age	28 (25-32)	28 (24-32.5)	0.737
Gravida	2 (1-3)	2 (1-3)	0.842
Parity	0 (0-1)	0 (0-1)	0.412
Gestational age at diagnosis	**34 (32-36)**	**32 (28-34)**	**0.001***
AST	**51 (27.5-75)**	**75 (54-120)**	**0.001***
ALT	**73 (29-119)**	**102 (44.5-201)**	**0.004***
Birth weight (g)	**2935 (2660-3180)**	**2660 (2510-2960)**	**<0.001***
1^{st}-minute Apgar score	7 (7-8)	7 (7-8)	0.270
5^{th}-minute Apgar score	9 (9-9)	9 (8-9)	0.351
Gestational age at delivery	**37 (36-37)**	**37 (35.5-37)**	**0.021***
Neonatal pH	**7.35 (7.35-7.39)**	**7.33 (7.26-7.36)**	**0.009***

* p<0.05, continuous variables are presented as median (25^{th}-75^{th} percentile), and categorical variables as n (%)
FBA: Fasting bile acid, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase

Table 2. Association of maternal liver function parameters and FDAs with obstetric and neonatal outcomes

		AST median (25^{th}-75^{th} percentile)	ALT median (25^{th}-75^{th} percentile)	FBA median (25^{th}-75^{th} percentile)
FGR	Absent, n=255 (86.1%)	53 (31-84.5)	78 (32-140.5)	20 (14.9-46.35)
	Present, n=41 (13.9%)	50 (28-82)	62 (29-128)	24 (17.75-46.35)
	p-value	0.652	0.340	0.042*
NICU admission	Absent, n=236 (79.7%)	**53 (29-83)**	74 (29-131)	**18 (19.5-66.9)**
	Present, n=60 (20.3%)	**69 (45.5-94.5)**	78 (44-200)	**26 (19.25-67.35)**
	p-value	**0.026***	0.137	**<0.001***
Preterm birth	Absent, n=237 (80.1%)	52 (29-80)	73 (31.5-124.5)	**20 (14.95-28)**
	Present, n=59 (19.9%)	68 (32-115)	105 (40-205)	**28 (16.2-65)**
	p-value	0.089	0.102	**0.003***
Meconium passage	Absent, n=275 (92.9%)	**52 (29-82)**	74 (31-132)	**20 (14.95-28.2)**
	Present, n=21 (7.1%)	**75 (43.5-125)**	97 (56.5-215.5)	**105 (44.65-115.5)**
	p-value	**0.026***	0.161	**<0.001***

* p<0.05, continuous variables are presented as median (25^{th}-75^{th} percentile), and categorical variables as n (%)
AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, FBA: Fasting bile acid, NICU: Neonatal intensive care unit, FGR: Fetal growth restriction

meconium passage with a sensitivity of 57.1% and a specificity of 81.3% (AUC=0.694; p<0.05) (Figure 1).

Comparisons of laboratory parameters at the time of diagnosis, as well as obstetric and neonatal outcomes, between patients who received UDCA therapy and those who did not are presented in Table 3.

The proportion of patients with severe ICP was significantly higher among those who received UDCA therapy (p=0.024). In addition, patients who received UDCA therapy had significantly lower gestational age at diagnosis, gestational age at delivery, birth weight, and 5-minute Apgar score; conversely at diagnosis were significantly higher. In addition, the rate of preterm birth was significantly higher in the UDCA-treated group (Table 3).

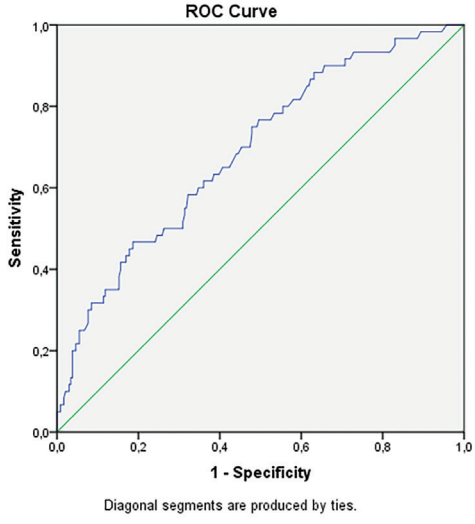

Figure 1. ROC curve of fasting bile acid levels for predicting meconium passage

ROC: Receiver operating characteristic

Only three patients (1.0%) included in the study experienced intrauterine fetal death (IUFD). One patient had mild biochemical disease (FBA: 22 µmol/L; AST: 40 U/L; ALT: 42 U/L), whereas the other two patients had severe biochemical disease (FBA: 44 and 162 µmol/L; AST: 28 and 76 U/L; and ALT: 61 and 62 U/L; respectively). The patient, with an FBA level of 162 µmol/L, also had severe FGR and meconium passage.

Discussion

In this retrospective study, we systematically evaluated the predictive value of FBA levels and liver enzymes, including AST and ALT, for adverse perinatal outcomes in 296 pregnancies complicated by ICP. Our findings demonstrate that FBA is a robust biochemical marker for distinguishing mild from severe ICP, and that it is significantly associated with adverse perinatal outcomes, including meconium passage, NICU admission, preterm birth, and FGR. Similarly, a 2024 study from our country, which referenced the SMFM guideline, recommended FBA as the primary biomarker for clinical monitoring and emphasized that the timing of delivery should be individualized according to disease severity[13].

In our study, patients with severe ICP (FBA >40 µmol/L) had significantly higher AST and ALT levels than those with mild ICP. This finding is consistent with the results of large-scale meta-analyses. In particular, Ovadia et al.[6] demonstrated in their Lancet meta-analysis that FBA levels are independently

Table 3. Comparison of laboratory findings at diagnosis, obstetric outcomes, and neonatal outcomes between the UDCA and non-UDCA groups

	UDCA group (n=220)	Non-UDCA group (n=76)	p-value
Continuous variables - median (25th-75th percentile)			
Gestational age at diagnosis	**33 (30-34)**	**36 (35-37)**	**0.001***
FBA	**22 (15.6-44.9)**	**18 (13.55-25.95)**	**0.007***
AST	**59 (31-92)**	**38 (27-64)**	**0.003***
ALT	**87 (38-151)**	**51 (23-82)**	**0.001***
Gestational age at delivery	**37 (36-37)**	**37 (37-38)**	**0.001***
Birth weight (g)	**2800 (2542-3100)**	**2990 (2715-3232)**	**0.001***
1st-minute Apgar score	7 (7-8)	7 (7-8)	0.140
5th-minute Apgar score	**9 (8-9)**	**9 (9-9)**	**0.007***
Neonatal pH	7.34 (7.29-7.39)	7.35 (7.31-7.38)	0.646
Categorical variables, n (%)			
Meconium passage	16 (7.3%)	5 (6.6%)	0.537
FBA: 10-40	**159 (72.3%)**	**64 (84.2%)**	**0.024***
FBA: >40	**61 (27.7%)**	**12 (15.8%)**	
Preterm birth	**51 (23.2%)**	**8 (10.5%)**	**0.011***
FGR	33 (15%)	8 (10.5%)	0.220
NICU	46 (20.9%)	14 (18.4%)	0.388

* p<0.05, continuous variables are presented as median (25th-75th percentile), and categorical variables as n (%)
AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, FBA: Fasting bile acid, NICU: Neonatal intensive care unit, FGR: Fetal growth restriction, UDCA: Ursodeoxycholic acid

and linearly associated with adverse perinatal outcomes. Geenes et al.[14] also demonstrated, in a prospective population-based study, that severe ICP is strongly associated with an increased risk of preterm birth, NICU admission, and IUFD. In our cohort, patients with severe ICP were diagnosed and delivered at significantly earlier gestational ages and had lower birth weights and neonatal pH values than patients with mild ICP, which supports the association between earlier disease onset, greater biochemical severity, and poorer perinatal outcomes. Similarly, Glantz et al.[5] reported a marked increase in the rate of fetal complications among pregnancies with FBA levels exceeding 40 µmol/L. Recent clinical practice guidelines have likewise retained the 40 µmol/L FBA threshold and recommend closer fetal surveillance and individualized timing of delivery for pregnancies complicated by severe ICP[2].

Our comparison of laboratory parameters between patients with and without complications demonstrated that patients who developed FGR, required NICU admission, experienced preterm birth, or had meconium passage. In contrast, AST was significantly associated only with NICU admission and meconium passage, whereas ALT showed no significant association with any of the evaluated obstetric or neonatal complications. Similarly, Juusela et al.[15] reported a significant association between AST levels and both meconium passage and NICU admission, while FBA remained an independent predictor of adverse perinatal outcomes in multivariable analysis. These findings are also consistent with the meta-analysis by Cui et al.[16], which demonstrated that elevated serum bile acid levels are associated with an increased risk of adverse perinatal outcomes, including preterm birth, meconium-stained amniotic fluid, and neonatal respiratory complications. Collectively, these findings suggest that ALT alone has limited value in predicting perinatal risk, whereas FBA remains the most robust biochemical marker for risk stratification in ICP. The association between AST and specific neonatal complications suggests that this enzyme may provide complementary prognostic information in pregnancies complicated by ICP. Arthuis et al.[17], in their eight-year case-control study, also reported an increased risk of adverse perinatal outcomes in pregnancies complicated by ICP and emphasized the importance of a comprehensive clinical approach that integrates biochemical markers with clinical and obstetric findings.

The association between FBA levels and meconium passage was particularly noteworthy. In our study, the median FBA level was significantly higher in patients with meconium passage than in those without (105 vs. 20 µmol/L, p<0.001).

ROC curve analysis identified an FBA cut-off value of 88.45 µmol/L with an AUC of 0.694, indicating a moderate discriminatory ability for predicting meconium passage. This finding is consistent with the study by Brouwers et al.[18], who reported that increasing FBA levels were associated with a higher risk of meconium passage and fetal distress, with the risk becoming particularly pronounced at FBA levels exceeding 100 µmol/L. Gregorc et al.[19] likewise reported in their 2025 retrospective study that the rate of meconium passage reached 43.3% among patients with severe ICP and FBA levels ≥100 µmol/L. Similarly, the systematic review by Di Mascio et al.[8] comprehensively documented the increased risk of perinatal mortality in pregnancies complicated by severe ICP. The relatively high FBA cut-off value identified in our study may be attributable to the heterogeneous nature of our cohort, particularly with respect to the proportion of severe ICP cases and the incidence of meconium passage. Nevertheless, this threshold may remain clinically useful for identifying patients who require closer fetal surveillance.

In our study, the significantly lower neonatal pH observed in the severe ICP group (7.33 vs. 7.35; p=0.009) suggests that elevated FBA levels may contribute to fetal acidemia. This finding supports the proposed pathophysiological mechanism whereby elevated bile acid levels may exacerbate fetal hypoxia through fetoplacental vasoconstriction, cardiotoxicity, and impaired oxygenation. The 2024 systematic review by Xin et al.[20] also comprehensively demonstrated a positive association between the severity of ICP and the risk of neonatal asphyxia. The 2024 review by Hague et al.[21] likewise emphasized the critical role of FBA-based risk stratification and individualized timing of delivery in reducing neonatal morbidity in pregnancies complicated by ICP.

Comparison between patients who did or did not receive UDCA therapy revealed that the UDCA-treated group had a higher proportion of severe ICP, earlier gestational age at diagnosis, and more pronounced biochemical abnormalities. This finding likely reflects confounding by indication, as UDCA therapy was more frequently initiated in patients with more severe disease; therefore, any conclusions regarding the effects of UDCA should be interpreted with caution. In the PITCHES trial, Chappell et al.[7] demonstrated that UDCA did not significantly improve the composite perinatal outcome, including perinatal death, preterm birth, and NICU admission, despite modest improvements in maternal symptoms and biochemical parameters. In the individual participant data meta-analysis, however, UDCA was associated with a significant reduction in the risk of spontaneous preterm birth (adjusted odds ratio, 0.46), suggesting that it may confer perinatal benefits in selected patient subgroups[11]. In the 2024 prospective observational study by Iqbal et al.[22], UDCA therapy was associated with significant improvements in maternal pruritus and transaminase levels; however, its effect on perinatal mortality remained inconclusive. The 2024 Canadian guideline likewise recommends UDCA as the first-line pharmacological treatment for ICP while acknowledging the persistent uncertainty regarding its effect on perinatal outcomes[12].

A total of three cases (1.0%) of IUFD were observed in our cohort. One patient had a mildly elevated FBA level (22 µmol/L), whereas the other two had markedly elevated FBA levels (44 and 162 µmol/L), consistent with severe ICP. In the patient with an FBA level of 162 µmol/L, severe FGR and meconium passage occurred concurrently, suggesting that markedly elevated FBA levels may be associated with multiple adverse obstetric complications and an increased cumulative perinatal risk. Similarly, Di Mascio et al.[8], in their systematic review, demonstrated that the risk of perinatal death increases substantially in pregnancies with FBA levels of ≥100 µmol/L. Nevertheless, the occurrence of IUFD despite a relatively low FBA level underscores the need for risk assessment in ICP to incorporate the overall clinical context in addition to FBA concentration. Nevertheless, the 2024 Canadian guideline recommends considering delivery at 35-36 weeks of gestation in patients with serum bile acid levels ≥100 µmol/L and emphasizes that the timing of delivery should be individualized based not only on bile acid concentrations but also on the overall obstetric and fetal clinical condition[12]. The individual participant data meta-analysis by Ovadia et al.[6] also confirmed that increasing serum bile acid levels are associated with adverse perinatal outcomes, particularly stillbirth, and supported the use of high bile acid thresholds for clinical risk stratification. Lin et al.[23], using an experimental animal model, demonstrated that maternal ICP induces alterations in the neonatal gut microbiota and increases susceptibility to inflammatory responses in the offspring.

Study Limitations

Several limitations of this study should be acknowledged. The retrospective design precludes establishing causal relationships, and the single-center nature of the study may limit the generalizability of our findings to the broader population of pregnancies complicated by ICP. In addition, variability in the timing of delivery, which was determined according to institutional protocols and the attending clinicians' judgment, may have influenced the observed outcomes. Conversely, this study has several notable strengths, including a relatively large sample size, homogeneous diagnostic criteria, and a standardized laboratory protocol, all of which enhance the reliability of our findings. Future studies should focus on evaluating individual bile acid fractions, particularly cholic acid and chenodeoxycholic acid, and validating FBA thresholds in prospective multicenter studies. Prospective studies should evaluate dynamic changes in serial FBA measurements rather than rely solely on a single baseline value, as temporal trends may further improve risk stratification in ICP.

Conclusion

This study demonstrates that FBA levels are the strongest biochemical predictors of adverse perinatal outcomes in pregnancies complicated by intrahepatic cholestasis, whereas AST provides complementary prognostic information. The significant associations between FBA levels, disease severity, meconium passage, NICU admission, preterm birth, and FGR further support the central role of FBA in risk stratification, clinical surveillance, and individualized timing of delivery. Patients who received UDCA therapy were, as expected, more likely to have severe ICP; however, recent individual-participant data meta-analyses suggest that UDCA may reduce the risk of spontaneous preterm birth. Overall, our findings support the combined use of FBA and AST in the clinical management of ICP. Risk stratification and the timing of delivery should be individualized in accordance with current clinical guidelines, and these findings should be validated in future prospective multicenter studies.

Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from the University of Health Sciences Türkiye, Ankara Bilkent City Hospital Medical Research Scientific and Ethical Review Board (approval number: TABED 2-26-2291, date: 13.05.2026).
Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.Ç., Z.A., D.Ş., Concept: A.Ç., Z.A., D.Ş., Design: A.Ç., Z.A., D.Ş., Data Collection or Processing: A.Ç., Analysis or Interpretation: A.Ç., Z.A., Literature Search: A.Ç., Z.A., Writing: A.Ç., Z.A., D.Ş.
Conflict of Interest: Dilek Şahin Assoc. Prof. is Section Editor in Turkish Journal of Obstetrics and Gynecology. She had no involvement in the peer-review of this article and had no access to information regarding its peer-review. The other authors declared no conflict of interest.
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Combined triple screening and BMI models for gestational diabetes prediction

Gebelik diyabetinin tahmininde üçlü tarama ve VKİ modellerinin birleştirilmesi

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Abstract

Objective: This study investigated whether routinely used second-trimester triple screening markers (alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol) could help identify pregnancies at increased risk of gestational diabetes mellitus. In addition, several hematological inflammation indices were evaluated as potential adjunctive predictors.

Materials and Methods: Medical records of 405 singleton pregnancies were retrospectively reviewed. All participants underwent triple screening during gestational weeks 15-20 and subsequently underwent oral glucose tolerance testing during gestational weeks 24-28. Patients were categorized, according to oral glucose tolerance test findings, as either having gestational diabetes or as normoglycemic controls. Receiver operating characteristic analyses were performed to assess both the isolated and combined predictive capacities of measurements of alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol. The impact of maternal body mass index on model performance was also analyzed.

Results: Alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol levels showed poor discriminatory ability for identifying pregnancies complicated by gestational diabetes, both individually and in combined models. However, adding maternal body mass index to the combined biomarker model substantially improved predictive accuracy (area under the curve=0.809; p<0.001). None of the evaluated inflammatory indices demonstrated significant predictive performance for gestational diabetes mellitus.

Conclusion: Routine second-trimester triple screening markers appear to have limited standalone value for predicting gestational diabetes. Nevertheless, incorporating maternal body mass index considerably enhances model performance and may improve clinical risk stratification.

Keywords: Second trimester, gestational diabetes mellitus, AFP, hCG, estriol, inflammation markers

Öz

Amaç: Bu çalışmanın amacı, gebelik diyabetini öngörmede ikinci trimester üçlü tarama testi parametrelerinin (alfa-fetoprotein, insan koryonik gonadotropin ve konjuge olmayan estriol) performansını değerlendirmekti. Ayrıca, sistemik enflamasyon indeksleri ile gebelik diyabeti arasındaki ilişki de incelenmiştir.

Gereç ve Yöntemler: Bu retrospektif gözlemsel çalışmaya, gebeliğin 15. ve 20. haftaları arasında ikinci trimester üçlü tarama testi ve 24. ve 28. haftaları arasında oral glikoz tolerans testi yapılan 405 hamile kadın dahil edilmiştir. Oral glikoz tolerans testi sonuçlarına göre olgular, gebelik diyabeti ve kontrol gruplarına ayrılmıştır. Alfa-fetoprotein, insan koryonik gonadotropin ve konjuge olmayan estriol düzeylerinin bireysel ve kombine prediktif

PRECIS: Combining maternal body mass index with second-trimester triple screening markers significantly improves the prediction of gestational diabetes mellitus compared with biochemical markers alone.

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performansı alıcı işletim karakteristiği analizi kullanılarak değerlendirilmiştir. Ayrıca, vücut kitle indeksinin modele katkısı da incelenmiştir. Bunun yanı sıra obstetrik ve neonatal sonuç verileri hastane doğum kayıtları kullanılarak retrospektif olarak ayrıntılı ve istatistiksel açıdan karşılaştırmalı biçimde değerlendirilmiştir.

Bulgular: Alfa-fetoprotein, insan koryonik gonadotropin ve konjuge olmayan estriolün, tek başına veya kombinasyon halinde, gebelik diyabeti için anlamlı bir öngörü performansı sergilemediği saptanmıştır. Buna karşın, vücut kitle endeksinin üçlü belirteç modeline dahil edilmesi, ayırt edici performansı önemli ölçüde iyileştirmiştir (eğri altındaki alan=0,809; p<0,001). Enflamasyon indeksleri ile ilgili olarak anlamlı bir ilişki gözlemlenmemiştir.

Sonuç: İkinci trimester üçlü tarama parametrelerinin tek başına gebelik diyabetini öngörmede değeri sınırlıdır. Ancak, modele vücut kitle indeksinin eklenmesi, öngörü performansını önemli ölçüde iyileştirir.

Anahtar Kelimeler: İkinci trimester, gebelik diyabeti, AFP, hCG, estriol, enflamasyon belirteçleri

Introduction

Gestational diabetes mellitus (GDM) represents one of the most frequently encountered metabolic disorders during pregnancy and is associated with adverse maternal and neonatal outcomes[1]. Reported prevalence rates differ among populations; however, the global burden of GDM has increased steadily in parallel with rising obesity rates and delayed childbearing[2]. Beyond pregnancy-related complications, GDM has also been linked to long-term metabolic consequences affecting both mothers and their offspring[3].

Maternal complications such as hypertensive disorders, operative delivery, and obstetric trauma occur more frequently in pregnancies complicated by GDM, while neonatal risks include macrosomia, hypoglycemia, and future metabolic dysfunction[4]. Therefore, identifying women at increased risk before routine diagnostic testing remains an important clinical objective.

In current clinical practice, GDM is generally diagnosed using oral glucose tolerance testing performed during the late second trimester[5]. Because conventional screening occurs relatively late in gestation, considerable interest has focused on markers capable of identifying high-risk pregnancies earlier. Consequently, routine biochemical and clinical markers have been increasingly investigated for early GDM risk assessment.

Alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG), and unconjugated estriol (uE3), which are routinely measured during second-trimester triple screening, primarily serve in fetal anomaly assessment but may also reflect placental and metabolic alterations occurring during pregnancy[6]. Accordingly, these biomarkers have attracted interest as possible indicators of metabolic complications including GDM. Previous studies evaluating their predictive utility have yielded inconsistent findings, and their clinical usefulness remains uncertain[7].

Increasing evidence suggests that low-grade chronic inflammation contributes to the pathophysiology of gestational diabetes[8]. Accordingly, systemic inflammation indices derived from complete blood counts—particularly the systemic immune-inflammation index (SII), the systemic inflammatory response index (SIRI), and the aggregate systemic inflammation index (AISI)—have been used in recent years to predict various metabolic and cardiovascular diseases[9]. However, available evidence regarding the predictive value of these indices during pregnancy remains limited and inconsistent, particularly for GDM[10].

The aim of this study was to evaluate the performance of second-trimester triple-screening parameters (AFP, hCG, and uE3), alone and in combination, in predicting GDM. Additionally, the potential contribution of the SII, SIRI, and AISI indices—which reflect inflammation and immune response—to the prediction of GDM was examined in an exploratory analysis. Additionally, we aimed to assess whether routinely available biochemical and hematological parameters could provide clinically useful information for early assessment of GDM risk.

Materials and Methods

A retrospective review was conducted in the Clinic of Obstetrics and Gynecology at Samsun Training and Research Hospital, based on records collected between July 2023 and January 2026. Pregnancies that were both followed and delivered at the same institution were identified through the institutional digital archive and reviewed. Approval for the study was obtained from the Samsun University Clinical Research Ethics Committee (approval number: 2023/15/8, date: 23.08.2023). Biochemical parameters of pregnant women who underwent the triple screening test during the second trimester were recorded; subsequently, the results of the oral glucose tolerance test (OGTT) administered to the same patients between the 24th and 28th weeks of gestation were evaluated. Participants were classified as either GDM cases or normoglycemic controls according to OGTT findings, and the predictive performance of second-trimester parameters was subsequently evaluated between groups. Delivery outcomes and neonatal characteristics were additionally assessed using archived obstetric records. Because the study was hospital-based and included only women with complete records for triple-screening, OGTT, delivery, and neonatal outcomes, the proportion of GDM cases observed in the study cohort should not be interpreted as reflecting the prevalence of GDM in the general obstetric population. The study was conducted in a tertiary referral

hospital serving a large regional population. Therefore, women with increased metabolic or obstetric risk may have been overrepresented in the study cohort compared with the general obstetric population. All procedures performed in this study were consistent with the ethical standards of the institutional research committee and with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to inclusion in the study.

The study included women aged 18-45 with singleton pregnancies who underwent the necessary tests at the relevant gestational weeks; women with multiple pregnancies, diagnosed fetal structural or chromosomal anomalies, a pre-existing diagnosis of diabetes, chronic hypertension, renal failure, or other systemic diseases, and pregnant women under 18 or over 45 years of age were excluded. Additionally, patients with acute or chronic infections that could affect maternal inflammatory status, with hematological diseases, with immunological disorders, or with regular medication use (particularly steroid and immunosuppressive therapies), as well as those with incomplete laboratory or clinical data, were excluded from the analysis.

GDM screening was performed using a 75 g oral glucose tolerance test during gestational weeks 24-28. The fasting, 1 hour, and 2 hours plasma glucose levels measured during the test were evaluated using the following cutoff values: ≥92 mg/dL, ≥180 mg/dL, and ≥153 mg/dL, respectively. Patients exceeding at least one threshold value were classified as having GDM according to International Association of Diabetes and Pregnancy Study Groups recommendations[11].

As part of the study, demographic, clinical, biochemical, and hematological data for the cases were analyzed. Demographic and obstetric characteristics, including maternal age, body mass index (BMI), gravidity, parity, and parameters related to pregnancy outcomes (mode of delivery, birth weight, Apgar scores, presence of meconium, need for neonatal intensive care unit admission, and neonatal intubation status) were evaluated. Biochemical analyses included AFP, hCG, and uE3 levels obtained from second-trimester triple-screening tests; the predictive performance of these markers was evaluated both individually and in combination. As part of the hematological evaluation, composite indices reflecting systemic inflammation were calculated using white blood cell count, hemoglobin, hematocrit, platelet count, neutrophil count, lymphocyte count, and monocyte count obtained from a complete blood count. Systemic inflammatory indices, including SII, SIRI, and AISI, were derived from routinely collected complete blood count parameters. SII was calculated as platelet × neutrophil / lymphocyte, SIRI as neutrophil × monocyte / lymphocyte, and AISI as neutrophil × monocyte × platelet / lymphocyte. Hematological measurements were obtained using automated analyzers, and inflammatory indices were exploratorily assessed for their potential association with GDM.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Distribution characteristics of continuous variables were evaluated visually and statistically before analysis. Normality testing was performed using the Kolmogorov-Smirnov method. Because several variables exhibited non-normal distributions, nonparametric statistical methods were applied where appropriate. Continuous data were summarized using medians and ranges. For comparisons between two independent groups, the Mann-Whitney U test was used. A two-sided p-value below 0.05 was considered statistically significant. An receiver operating characteristic (ROC) analysis was performed to evaluate the predictive performance of second-trimester biochemical markers (AFP, hCG, and E3) for GDM. The area under the curve (AUC) was calculated for each parameter and model, along with the 95% confidence interval (CI). Optimal cutoff points were determined using the Youden index, and the corresponding sensitivity and specificity values were reported. Additionally, logistic regression models were constructed to evaluate the combined predictive effects of biochemical markers. In the first stage, a basic model was constructed that included the variables AFP, hCG, and E3; this model was then expanded by adding maternal BMI to obtain an extended model. The discriminative performance of the models was compared using ROC curves. Additionally, indices reflecting inflammation and immune response (SII, SIRI, and AISI) were evaluated using ROC analysis for exploratory purposes. Because significant right-skewness was observed in some variables, a logarithmic transformation was applied and sensitivity analyses were conducted. Similar findings obtained from transformation analyses supported the stability of the statistical results.

Results

Among the 405 pregnancies evaluated, 272 women (67.2%) were diagnosed with GDM, whereas 133 women (32.8%) had normal glucose tolerance. A comparison of maternal and neonatal characteristics revealed no statistically significant difference in maternal age between the groups (p=0.928). Gestational age, gravidity, and parity were likewise comparable between GDM and control patients (p=0.958, p=0.895, and p=0.312, respectively). Women who developed GDM had significantly higher maternal BMI values than normoglycemic controls (p<0.001). Neonatal birth weight was significantly increased in pregnancies complicated by GDM (p<0.001). Fasting glucose levels were significantly higher in the GDM group (p<0.001). Similarly, both first- and second-hour OGTT glucose measurements were significantly higher in women with GDM than in controls (p<0.001 for each comparison). No meaningful between-group differences were identified for second-trimester triple screening markers, including hCG, AFP, and E3 levels (p=0.717, p=0.500, and

Table 1. Comparison of maternal and neonatal characteristics according to the presence of gestational diabetes

	Presence of gestational diabetes				p-value
	GDM (n=272)		Control (n=133)		
	Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
Maternal age (years)	31.15±5.15	31.00 (20.00-44.00)	31.05±5.59	31.00 (20.00-43.00)	0.928
Neonatal birth weight (g)	3,814.00±464.52	3,800.00 (2570-5200)	3,474.14±411.664	3,450.00 (2670-4500)	<0.001
Maternal BMI	31.43±5.36	30.00 (21.00-44.00)	25.72±5.56	24.00 (19.00-42.00)	<0.001
Gestational age (weeks)	38.11±1.37	38.00 (35.00-40.00)	38.11±1.36	38.00 (35.00-40.00)	0.958
Gravida	2.34±1.31	2.00 (1.00-7.00)	2.38±1.44	2.00 (1.00-7.00)	0.895
Parity	1.08±0.98	1.00 (0.00-4.00)	1.00±1.03	1.00 (0.00-4.00)	0.312
Fasting blood glucose (mg/dL)	105.45±19.72	100.90 (74.30-181.00)	86.40±5.15	89.90 (70.30-91.00)	<0.001
Hcg	44,045.56±33,629.73	36,297.32 (7,831-264,400)	43,005.84±26,981.58	33,574.00 (2,789-171,555)	0.717
AFP	41.52±17.96	38.20 (0.99-125.10)	41.00±19.38	37.05 (2.00-124.30)	0.500
Estriol	1.49±1.02	1.36 (0.01-14.20)	1.46±0.57	1.35 (0.50-4.00)	0.565
OGTT 1 (1 hour glucose tolerance test) (mg/dL)	194.51±39.54	194.90 (75.10-339.40)	156.62±24.82	164.80 (82.30-189.30)	<0.001
OGTT 2 (2 hours glucose tolerance test) (mg/dL)	157.93±56.41	153.75 (57.70-764.70)	125.85±29.17	122.00 (56.90-188.70)	<0.001
Apgar Score (1st minute)	8.40±1.31	9.00 (4.00-9.00)	8.89±0.59	9.00 (5.00-9.00)	<0.001
Apgar Score (5th minute)	8.70±1.44	9.00 (0.00-10.00)	9.71±0.88	10.00 (5.00-10.00)	<0.001

Mann-Whitney U test
BMI: Body mass index, OGTT: Oral glucose tolerance test, SD: Standard deviation, GDM: Gestational diabetes mellitus

p=0.565, respectively). With respect to neonatal outcomes, both 1 minute and 5 minutes Apgar scores were significantly lower in the GDM cohort (p<0.001 for both) (Table 1).

ROC curve analysis demonstrated that second-trimester triple-screening markers (AFP, hCG, and uE3) had limited independent predictive ability for GDM. The calculated AUC values were 0.521 for AFP (95% CI: 0.460-0.582; p=0.500), 0.489 for hCG (95% CI: 0.430-0.548; p=0.717), and 0.482 for uE3 (95% CI: 0.421-0.543; p=0.565), indicating weak discriminatory performance for all three markers. Combining these biochemical parameters into a single model did not improve predictive accuracy, with the combined model yielding an AUC of 0.507 (95% CI: 0.446-0.568; p=0.830). However, the incorporation of maternal BMI into the model resulted in a substantial increase in discriminatory capacity. The BMI-adjusted model achieved an AUC of 0.809 (95% CI: 0.757-0.860; p<0.001), reflecting significantly improved predictive performance. Using an optimal cutoff value of 0.55, the model demonstrated 88% sensitivity and 68% specificity (Table 2).

Figure 1 shows the ROC curves for the model based on the three screening parameters and for the model to which BMI was added. The curve for the triple-marker model lies closer to the diagonal, while the curve for the model incorporating BMI lies closer to the upper-left corner. Correspondingly, discriminatory performance improves with the inclusion of BMI (Figure 1).

To further clarify the independent contribution of maternal BMI to the combined model's predictive performance, an additional ROC analysis was performed using maternal BMI alone. Maternal BMI demonstrated good discriminatory performance in predicting GDM, with an AUC of 0.806 (95% CI: 0.755-60.858; p<0.001). The combined model, including AFP, hCG, E3, and BMI, showed a slightly higher AUC of 0.809 (Figure 2).

When the role of inflammation and immune response indices in predicting GDM was evaluated in an exploratory analysis, no significant discriminatory performance was observed for SII, SIRI and AISI. The AUC value for SII was 0.516 (95% CI: 0.453-0.578; p=0.609), 0.546 for SIRI (95% CI: 0.488-0.605; p=0.129), and 0.540 for AISI (95% CI: 0.481-0.599; p=0.189) (Table 3).

Logistic regression analysis identified maternal BMI as an independent predictor of GDM development. Multivariate analysis demonstrated that increasing BMI was significantly associated with a higher likelihood of GDM (odds ratio: 1.17; 95% CI: 1.100-1.257; p<0.001). No significant relationship was detected between GDM and AFP, hCG, uE3, or the evaluated hematological variables in regression analyses (p>0.05) (Table 4).

Discussion

The present study evaluated whether second-trimester triple-screening markers could predict GDM and found that AFP, hCG, and uE3 had limited ability to distinguish pregnancies complicated by GDM. Predictive performance improved substantially after maternal BMI was incorporated into the model.

Table 2. Second-trimester biochemical parameters and the predictive performance of combined models for gestational diabetes (ROC analysis)

	AUC (95% CI)	p-value	Cut-off (Youden)	Sensitivity	Specificity
AFP	0.521 (0.460-0.582)	0.500	30.75	0.73	0.32
hCG	0.489 (0.430-0.548)	0.717	7503	1.00	0.02
Estriol	0.482 (0.421-0.543)	0.565	0.70	0.94	0.08
AFP + hCG + estriol	0.507 (0.446-0.568)	0.830	0.66	0.72	0.32
BMI	0.806 (0.755-0.858)	<0.001	22.5	0.91	0.68
AFP + hCG + estriol + BMI	0.809 (0.757-0.860)	<0.001	0.55	0.88	0.68

BMI: Body mass index, AUC: Area under the curve, ROC: Receiver operating characteristic, hCG: Human chorionic gonadotropin, AFP: Alpha-fetoprotein, CI: Confidence interval

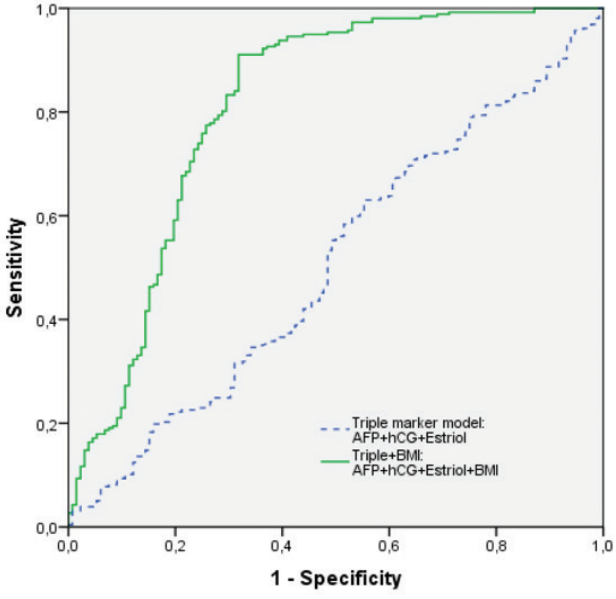

Figure 1. Receiver operating characteristic curves for the triple marker model and the model including BMI

BMI: Body mass index, AFP: Alpha-fetoprotein, hCG: Human chorionic gonadotropin

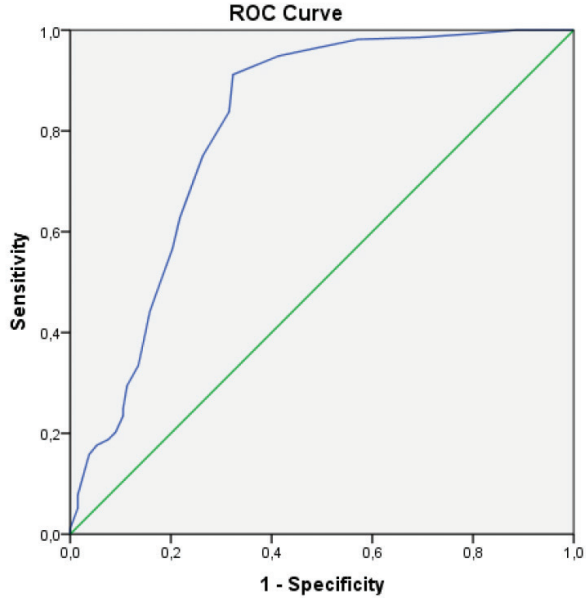

Figure 2. ROC curve of maternal BMI for predicting gestational diabetes mellitus

BMI: Body mass index, ROC: Receiver operating characteristic

Table 3. Results of an exploratory ROC analysis of inflammation and immune combination indices for gestational diabetes

	AUC (95% CI)	p-value	Cut-off (Youden)	Sensitivity	Specificity
SII	0.516 (0.453-0.578)	0.609	545	0.88	0.68
SIRI	0.546 (0.488-0.605)	0.129	1.26	0.88	0.12
AISI	0.540 (0.481-0.599)	0.189	330	0.85	0.19

SII: Systemic immune-inflammation index, SIRI: Systemic inflammatory response index, AISI: Aggregate systemic inflammation index, AUC: Area under the curve, CI: Confidence interval, ROC: Receiver operating characteristic

Table 4. An analysis of the effects of ındependent variables on the probability of gestational diabetes using binary logistic regression

	GDM		Univariate		Multiple	
	No	Yes	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	31.05±5.59	31.15±5.15	1.00 (0.965-1.044)	0.855	0.98 (0.918-1.065)	0.765
BMI	25.72±5.56	31.43±5.36	1.24 (1.181-1.319)	<0.000	1.17 (1.100-12.57)	<0.000
White blood cells	9.85±2.35	10.08±2.27	1.04 (0.955-1.147)	0.333	1.04 (0.819-1.329)	0.729
Hemoglobin	11.54±1.32	11.43±1.31	0.93 (0.797-1.096)	0.405	1.18 (0.530-2.634)	0.683
Hematocrit	35.43±3.40	34.86±3.46	0.95 (0.896-1.012)	0.116	0.88 (0.642-1.206)	0.427
Platelets	246.82±68.34	247.17±64.65	1.00 (0.997-1.003)	0.960	1.00 (0.993-1.006)	0.902
Lymphocytes	1.98±0.71	2.65±12.14	1.01 (0.958-1.074)	0.633	1.15 (0.568-2.350)	0.691
Monocytes	0.63±0.19	0.69±0.53	2.21 (0.828-5.930)	0.113	1.79 (0.177-18.094)	0.622
Neutrophils	8.08±3.17	8.05±2.81	0.99 (0.929-1.070)	0.928	0.96 (0.848-1.104)	0.621
hCG	43,574.20±27,666.82	47,414.25±55,720.46	1.00 (1.00-1.056)	0.462	1.00 (1.00-1.00)	0.118
AFP	41.00±19.38	41.52±17.96	1.00 (0.990-1.013)	0.793	1.00 (0.983-1.021)	0.864
Estriol	1.46±0.57	1.49±1.020	1.04 (0.811-1.333)	0.757	0.72 (0.492-1.071)	0.106

OR: Odds ratio, CI: Confidence interval, BMI: Body mass index, hCG: Human chorionic gonadotropin, GDM: Gestational diabetes mellitus

Furthermore, inflammatory indices derived from complete blood count parameters did not meaningfully contribute to GDM prediction.

Maternal obesity has consistently been identified as one of the strongest clinical risk factors associated with GDM development because of its close relationship with insulin resistance and altered metabolic regulation[12]. Similarly, BMI remained independently associated with GDM in both univariate and multivariate analyses in our cohort. The marked improvement in AUC observed after adding BMI indicates that clinical variables may provide stronger predictive information than isolated biochemical markers during the second trimester. These findings suggest that combined risk assessment models may offer greater clinical utility than single-marker approaches[13].

The association between maternal age and the development of gestational diabetes has been extensively studied in the literature; it has been reported that advanced maternal age is a significant risk factor for GDM, as it is associated with increased insulin resistance and a decrease in pancreatic β-cell reserve[14]. Numerous epidemiological studies have shown that the incidence of GDM increases significantly, particularly among pregnant women aged 30-35[15]. However, it is also emphasized that this association is not consistent across all populations and that the effect of age may vary depending on factors such as obesity, lifestyle, and genetic predisposition[16]. In the present study, however, no statistically significant association was found between maternal age and the development of GDM. This may be due to the relatively homogeneous age distribution in the study group, the predominant influence of stronger metabolic markers such as BMI, or the effect of potential confounding factors. Indeed, the fact that BMI emerged as an independent risk factor in the multivariate analysis suggests that age alone may not be a decisive parameter. This finding appears consistent with the literature indicating that maternal age is a risk marker that contributes to the development of GDM but is not sufficient on its own[17].

Previous investigations examining the relationship between triple screening markers and GDM have produced inconsistent findings. AFP has been proposed as a potential marker because of its relationship with placental physiology; however, reported associations with GDM remain controversial[18]. In this study, the low AUC value for AFP and the statistically non-significant results indicate that AFP plays a limited role in predicting GDM. Similarly, although hCG is a placental hormone and might be expected to be associated with metabolic processes, the current study found that hCG levels did not significantly contribute to the prediction of GDM. The presence of studies in the literature supporting an association between hCG and GDM, as well as studies that failed to find a significant association, highlights the uncertainty surrounding this issue[19,20]. Although the association between uE3 levels and GDM has been less extensively studied, some studies have suggested that high uE3 levels may be associated with GDM[21]. However, the results obtained for uE3 in the current study indicate that this parameter is not a significant biomarker for predicting GDM. This suggests that these biochemical parameters measured in the second trimester may not adequately reflect maternal metabolic changes.

The failure to achieve significant predictive performance, despite combining triple-screening test parameters, suggests that these biomarkers may not be directly pathophysiologically

linked to GDM or that this association is weak. However, the significant increase observed with the addition of BMI underscores the importance of evaluating clinical parameters in conjunction with these biochemical markers. This finding suggests that approaches relying solely on laboratory data may be insufficient for predicting GDM, highlighting the need to develop multidisciplinary and multiparameter models[22].

Although the role of inflammation in the pathogenesis of GDM is increasingly recognized, it is noteworthy that the inflammatory indices evaluated in this study (SII, SIRI, AISI) did not demonstrate significant predictive performance for GDM. Some studies in the literature have reported that these indices are associated with insulin resistance and metabolic syndrome[23]. However, physiological changes during pregnancy may affect hematological parameters and, as a result, reduce the specificity of inflammatory markers. The findings of this study suggest that these markers may not be sufficiently sensitive or specific for predicting GDM in the second trimester.

A review of studies investigating various biomarkers in GDM suggests that, in recent years, GAS6, periostin, and various adipokines have been evaluated as potential biomarkers[24,25]. Although some studies have reported that these biomarkers show promising results, there is not yet sufficient evidence to support their use in clinical practice. This situation highlights that, due to the multifactorial nature of GDM, predicting the condition with a single biomarker is difficult and more comprehensive biomarker panels are needed.

Among the strengths of our study are that the data were collected at a single center using standardized protocols and that the study included a large patient cohort. Furthermore, the evaluation of biochemical parameters obtained during routine clinical practice in the second trimester enhances the study's clinical applicability. Furthermore, combined analysis of biochemical markers, clinical (BMI), and hematological parameters has enabled a multidimensional perspective and strengthened the interpretability of the findings.

Study Limitations

Several limitations should be acknowledged when interpreting the findings of this study. Because the data were obtained from a single institution, the applicability of the results to broader populations may be restricted. In addition, the relatively high proportion of women with GDM in the study cohort likely reflects several methodological and population-related factors. First, the retrospective hospital-based design and the inclusion of only women with complete triple-screening, OGTT, delivery, and neonatal outcome records may have introduced selection bias. Second, our institution serves as a tertiary referral center, resulting in a higher proportion of pregnancies at increased metabolic and obstetric risk. Finally, the relatively high maternal BMI may also have contributed to the increased frequency of GDM observed in this cohort. Therefore, the GDM rate reported in the present study should not be interpreted as representative of the prevalence in the general obstetric population. The analysis of inflammatory indices was exploratory; further multicenter studies involving larger patient populations are required to clarify the clinical relevance of these markers for predicting GDM. Biomarkers were measured at a single time point, which limits the assessment of dynamic changes and time-dependent variations during pregnancy. In addition, the failure to account for potential confounding factors (such as nutritional status, physical activity, and genetic predisposition) may limit the interpretation of the results. Future studies should focus on predictive models that incorporate repeated measurements at different gestational stages and integrate biochemical and clinical parameters, and these studies should be conducted in multicenter settings with larger populations. This approach will contribute to the development of more reliable and clinically applicable methods for the early prediction of GDM.

Conclusion

This study demonstrates that second-trimester triple screening test parameters have limited value in predicting GDM. However, adding BMI to these models significantly improved predictive performance. These findings highlight the value of incorporating simple and readily available clinical variables into GDM risk assessment models. Inflammatory indices, however, were found to make no significant contribution to the prediction of GDM. Future studies focusing on multivariate models that evaluate different biomarkers and clinical parameters together may contribute to the development of more effective strategies for the early prediction of GDM.

Ethics

Ethics Committee Approval: Approval for the study was obtained from the Samsun University Clinical Research Ethics Committee (approval number: 2023/15/8, date: 23.08.2023).

Informed Consent: Written informed consent was obtained from all participants prior to inclusion in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.G., S.M.A., Concept: N.G., K.G.Ş., Ne.G., Design: N.G., M.A., Ne.G., Data Collection or Processing: S.M.A., M.A., K.G.Ş., Analysis or Interpretation: S.M.A., M.A., K.G.Ş., Ne.G, Literature Search: Ne.G., Writing: Ne.G.

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manuscript, ChatGPT (OpenAI) was used to assist with English-language editing and improving scientific wording. All generated content was critically reviewed, revised, and approved by the authors, who accept full responsibility for the final version of the manuscript.

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Microplastics in the umbilical vein of fetal growth restricted and healthy fetuses: A preliminary Turkish selected case series

Fetal büyüme kısıtlılığı olan ve sağlıklı fetüslerin umbilikal veninde mikroplastikler: Ön seçilmiş Türk olgu serisi

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Abstract

Objective: Microplastics are ubiquitous environmental pollutants, yet their potential association with fetal growth restriction (FGR) remains unclear. This observational, analytical case-control study aimed to evaluate the presence and size characteristics of microplastics in umbilical venous blood samples from fetuses with FGR, compared with healthy controls.

Materials and Methods: Fourteen pregnant women with singleton pregnancies, aged 20-36 years, who delivered between 36+0 and 39+6 weeks' gestation were included. Pregnancies were classified as FGR (n=8) or healthy controls (n=6). Maternal and neonatal characteristics, including birth weight, Apgar scores, delivery mode, ultrasound and Doppler findings, and neonatal intensive care unit admission, were recorded. Umbilical vein blood samples collected at delivery were analyzed for the presence and particle size of microplastics using micro-Raman spectroscopy.

Results: Microplastics were detected in 83.3% of control cases and 50% of FGR cases, with no statistically significant difference between groups (p=0.198). Mean microplastic particle size was larger in the FGR group than in controls (54.41±20.15 µm vs. 36.28±16.33 µm), although the difference was not statistically significant (p=0.133). Birth weight was significantly lower in the FGR group (p=0.003).

Conclusion: Microplastics were detected in umbilical vein blood samples of both FGR and healthy fetuses. No significant association between the presence of microplastics and FGR was observed; however, further studies with larger sample sizes are required.

Keywords: Microplastics, fetal growth restriction, umbilical cord, IUGR

Öz

Amaç: Mikroplastikler çevrede yaygın olarak bulunan kirleticiler olup, fetal gelişim üzerindeki olası etkileri henüz net değildir. Bu analitik gözlemsel olgu-kontrol çalışmasının amacı, fetal büyüme kısıtlılığı (FBK) olan fetüsler ile sağlıklı kontrollerin umbilikal ven kan örneklerinde mikroplastik varlığını ve partikül boyut özelliklerini karşılaştırmalı olarak değerlendirmektir.

Gereç ve Yöntemler: Otuz altı+0 ile 39+6 gebelik haftaları arasında doğum yapan, 20-36 yaş aralığındaki 14 tekil gebelik çalışmaya dahil edildi. Olgular FBK tanısı alan gebelikler (n=8) ve sağlıklı kontrol grubu (n=6) olarak iki gruba ayrıldı. Maternal ve neonatal demografik özellikler, doğum ağırlığı, Apgar skorları, doğum şekli, ultrason ve Doppler bulguları ile yenidoğan yoğun bakım ihtiyacı kaydedildi. Doğum sırasında umbilikal venden elde edilen kan örneklerinde mikroplastik varlığı ve partikül boyutu mikro-Raman spektroskopisi ile analiz edildi.

PRECIS: Microplastics were detected in the umbilical vein of both fetal growth-restricted and healthy fetuses, with no statistically significant association observed between microplastic presence and fetal growth restriction in this preliminary cohort.

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Bulgular: Kontrol grubundaki olguların %83,3'ünde, FBK grubundaki olguların ise %50'sinde mikroplastik saptandı; gruplar arasında istatistiksel olarak anlamlı fark bulunmadı (p=0,198). Ortalama mikroplastik partikül boyutu FBK grubunda kontrol grubuna kıyasla daha büyük olmakla birlikte (54,41±20,15 µm'ye karşı 36,28±16,33 µm), bu fark istatistiksel olarak anlamlı değildi (p=0,133). Doğum ağırlığı FBK grubunda anlamlı derecede daha düşüktü (p=0,003).

Sonuç: Mikroplastikler hem FBK olan hem de sağlıklı fetüslerin umbilikal ven kan örneklerinde saptanmıştır. Mikroplastik varlığı ile FBK arasında istatistiksel olarak anlamlı bir ilişki gösterilememiştir. Bununla birlikte, bu ilişkinin daha net ortaya konabilmesi için daha geniş örneklemli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Mikroplastikler, fetal büyüme kısıtlılığı, umbilikal kord, FBK

Introduction

Fetal growth restriction (FGR) affects an estimated 5-10% of pregnancies and represents a major cause of adverse perinatal outcomes, ranking among the leading contributors to perinatal mortality[1]. Clinically, FGR is commonly defined by an estimated fetal weight or abdominal circumference below the 10th percentile for gestational age[2]. Its underlying causes are heterogeneous and include maternal medical conditions, multiple gestations, teratogenic exposures, infections, substance use, genetic abnormalities, fetal structural anomalies, and placental dysfunction[3].

Accumulating evidence suggests that environmental exposures play a meaningful role in the development of FGR[4]. Maternal smoking and alcohol intake have been consistently associated with an increased risk of impaired fetal growth[5]. Moreover, exposure to traffic-related air pollutants during the second and third trimesters has been linked to a higher likelihood of FGR[6]. In line with these findings, recent studies conducted in cold-climate settings have demonstrated that even low-level prenatal air pollution exposure may adversely affect birth weight outcomes[7].

Worldwide plastic production currently exceeds 360 million tons annually and continues to grow at an estimated rate of approximately 4% per year[8]. As plastic manufacturing expands, the environmental burden and potential health consequences associated with plastic-derived pollutants have likewise increased[8]. Plastic waste, which is translocated from its production environment to various other environments through multiple mechanisms, degrades over time due to various factors, resulting in the formation of microplastics (MP), defined as particles smaller than 5 mm, and nanoplastics, characterized as particles smaller than 1 micrometer[9]. The pervasive occurrence of MPs in environmental settings and consumables has raised significant concerns about human exposure. Contemporary research has identified the presence of MPs within human placental tissue, breast milk, and meconium[10,11]. Additionally, recent investigations involving animal models and in vitro cell cultures have indicated that exposure to MPs may have deleterious effects on placental tissue and fetal development[12-15]. In 2021, Amereh et al.[16] documented a notable correlation between the presence of MPs in human placental tissue and FGR. Utilizing an ex vivo placental perfusion system on rat placentas within a controlled laboratory environment, it has been established that nanopolystyrene particles can traverse from the maternal uterine circulation into the fetal circulation via the placenta[17,18]. Nevertheless, despite the established presence of MPs within human placental tissue, further elucidation is required regarding the translocation of MPs from the placenta into the umbilical vein[10,11]. In addition, recent human evidence from a systematic review and meta-analysis has reported a high prevalence of MPs in reproductive tissues and suggested a potential association between MP accumulation and adverse pregnancy outcomes, including FGR, although the available data remain limited and heterogeneous[19]. In the present investigation, we examined the presence of MPs in blood specimens collected from the umbilical veins of healthy fetuses and fetuses diagnosed with FGR.

Materials and Methods

Study Population

This prospective observational study was conducted at the obstetrics outpatient clinic of Kütahya Health Sciences University, Evliya Çelebi Training and Research Hospital between May 2021 and May 2022. Fourteen women with singleton pregnancies were enrolled. All participants were between 20 and 36 years of age and had a gestational age between 36+0 and 39+6 weeks at the time of delivery.

Participants were categorized into two groups. The FGR group included pregnancies with an estimated fetal weight below the 10th percentile accompanied by abnormal Doppler findings or with an estimated fetal weight below the 3rd percentile for gestational age. The control group consisted of healthy pregnancies without evidence of FGR[20]. Maternal and neonatal variables, including birth weight, Apgar scores, mode of delivery, ultrasonographic and Doppler findings, and neonatal intensive care unit admission, were prospectively recorded.

Ethical approval was obtained from the Kütahya Health Sciences University Non-Interventional Clinical Research Ethics Committee in Türkiye prior to study initiation (approval number: 2021/12-22, date: 08.07.2021). Written informed consent was obtained from all participants. The study was registered at ClinicalTrials.gov (identifier number: NCT05070715).

Exclusion criteria comprised recent use of medications affecting intestinal absorption, alcohol consumption, invasive dental procedures within the preceding two weeks, multiple

gestations, cardiovascular disease, chronic hypertension, autoimmune disorders, inflammatory bowel disease, gastrointestinal motility disturbances, gestational cholestasis, preeclampsia or eclampsia, sepsis, placental adhesion abnormalities, and known fetal chromosomal or congenital anomalies. Gestational age was determined based on first-trimester ultrasonography and the last menstrual period.

Sample Collection

Umbilical cord blood samples were obtained immediately after delivery, following cord clamping. Ten milliliters of blood were drawn from the umbilical vein using a PTFE syringe and transferred into sterile glass tubes. Samples were stored at -80 °C until further analysis. To minimize the risk of plastic contamination, latex-free neoprene sterile gloves were used during all sampling procedures.

Extraction of Samples

Upon arrival at the laboratory, samples were passed through a 45 µm mesh sieve. The retained material was transferred into sterile glass containers and rinsed with Milli-Q ultrapure water. Organic matter was removed using a highly alkaline digestion solution composed of potassium hydroxide and sodium hypochlorite, based on a modified protocol described previously[21].

The digestion mixture was prepared by combining ultrapure water with a saturated potassium hydroxide solution and sodium hypochlorite (containing active chlorine). Depending on the sample volume, approximately 250-500 mL of the solution was added to each container. Samples were then covered with aluminum foil and incubated in a closed environment at 50-60 °C until complete digestion of the organic material was achieved.

Following digestion, density separation was performed by transferring the solution into a separatory funnel and adding a potassium carbonate solution with a density of 1.6 g/cm^3. After a 24-hours settling period, the supernatant was carefully collected and vacuum-filtered through sterile GF/F filters with a pore size of 0.45 µm. Filter membranes were placed in sterile Petri dishes for subsequent microscopic evaluation.

MP Analysis with µ-Raman Spectroscopy

Filtered samples were examined using a confocal µ-Raman spectroscopy system (Renishaw InVia Qontor, Renishaw Plc., UK) equipped with 532 nm and 785 nm laser sources. Suspected particles were analyzed individually, and the obtained spectra were compared with reference polymer spectra in the instrument library to determine their chemical composition.

Quality Assurance (QA) and Contamination Control

All analytical procedures were conducted under strict contamination-control conditions. Sample preparation and analysis were performed in a laminar-flow cabinet, and all chemicals were prefiltered through GF/F filters. Laboratory equipment was rinsed with acetone prior to use and then stored wrapped in aluminum foil. Procedural blanks were processed alongside samples to assess potential contamination; no MP particles were detected in the procedural blanks.

Statistical Analysis

All statistical analyses were carried out using SPSS software (version 21). Continuous variables were summarized using both the mean (standard deviation) and the median (interquartile range, 25th-75th percentiles). Normality of the distribution was assessed using the Kolmogorov-Smirnov or Shapiro-Wilk test, as appropriate.

Group comparisons for continuous variables were performed using the independent samples t-test or the Mann-Whitney U test, depending on data distribution. Categorical variables were evaluated using the chi-square test. A two-sided p-value of less than 0.05 was considered statistically significant.

QA/Quality Control

This investigation strictly adhered to a protocol that minimized the risk of procedural contamination from the surrounding atmosphere, chemicals, surfaces, and equipment. In this setting, all analyses were conducted in a sealed laminar cabinet, and all chemicals utilized were filtered through a GF/F filter before examination. Furthermore, all equipment involved in the procedures was washed with acetone prior to use and stored wrapped in aluminum foil. Despite all precautions, a procedural blank was used to check for contamination, and all procedures applied to the samples were carried out with the blank. No MP particles were detected in the procedural blank. However, contamination likely resulted from the surgical environment and from the clothing worn by the medical staff who collected the samples during surgery. Plastics are pervasive in daily life and are integral to medical equipment. A substantial portion of medical equipment routinely employed in hospitals is manufactured from plastic or packaged in plastic[22]. Field et al.[22] documented a notable presence of MPs within the surgical environment, with polypropylene emerging as the second most prevalent polymer among these MPs. Therefore, it is essential to consider that the outcomes observed in this study could be attributed to the surgical setting and the sampled individuals.

Results

The mean age of the 14 pregnant women included in the study was 27.645.24 ±years. Eight cases were in the FGR group, and six were in the control group. Age, gravida, parity, number of living children, body mass index, gestation period, Apgar scores, education level, mode of delivery, newborn intensive care unit admission rates, and MPs in the umbilical vein were similar in the FGR and control groups. The FGR group had lower infant birth weights than the control group [2383 (2240-2680) g vs. 2983 (2745-3680) g; p=0.003].

The demographic, delivery, and MP data for the groups are presented in Table 1.

MPs were detected in the umbilical cord in 4/8 cases (50%) in the FGR group and in 5/6 cases (83.3%) in the control group (p=0.198). When the characteristics of MPs were analyzed, polypropylene was the most frequently detected MP, found in 4 cases in the FGR group and in 2 cases in the control group. Regarding color, blue was the most common in the FGR group, occurring in 2 cases, whereas all colors were detected at the same rate in the control group. MP size was 54.41±20.15 µm in the FGR group and 36.28±16.33 µm in the control group (p=0.133).

Two different MP types were detected in 2 samples: one in the FGR group and one in the control group. The characteristics of MPs detected in the umbilical vein are presented in Table 2.

Table 1. Demographic, delivery and microplastics values of groups

		FGR	Control	p
Age (years)		27 (26-34)	27 (21-32)	0.573[a]
Gravity		2 (1-3)	2 (1-2)	0.852[a]
Parity		2 (1-3)	2 (1-2)	0.662[a]
Live		2 (1-3)	2 (1-2)	0.662[a]
BMI (kg/m^2)		26.8 (23.5-29.4)	29 (24.6-30.86)	0.414[a]
GA (days)		265 (258-268)	270 (261-278)	0.228[a]
Birth weight (g)		2383 (2240-2680)	2983 (2745-3680)	**0.003**[a]
1′ Apgar score		9 (8-9)	9 (7-9)	0.950[a]
5′ Apgar score		10 (9-10)	10 (9-10)	0.950[a]
Education level	Less than graduate	5 (62.5%)	5 (83.3%)	0.393[b]
	Graduate	3 (37.5%)	1 (16.7%)	
Delivery mode	Vaginal delivery	3 (%37.5)	2 (33.3%)	0.872[b]
	Cesarean	5 (62.5%)	4 (66.7%)	
NICU admission	Yes	5 (62.5%)	4 (66.7%)	0.280[b]
	No	3 (37.5%)	2 (33.3%)	
Microplastics in umbilical vein	Yes	4 (50%)	5 (83.3%)	0.198[b]
	No	4 (50%)	1 (16.7%)	
Microplastic size (µm)		54.41±20.15	36.28±16.33	0.133[a]

[a]: Mann Whitney U test, [b]: Square test, FGR: Fetal growth restriction, NICU: Newborn ıntensive care unit, BMI: Body mass index, GA: Gestational age

Table 2. Microplastic characterics of patients

Patient number	Patient group	Particle type	Colour	Length (micron)	Polymer type
1	Control	Fragment	Blue	14.8	Polypropylene
2	FGR	Fragment	Brown	74.69	Polypropylene
3	Control	Fragment	Gray	61.59	Cellulose
4	FGR	Fragment	Blue	69.52	Polyethylene
		Fragment	Blue	23.16	Polypropylene
5	Control	Fragment	Yellow	29.59	Polyacrylamide
		Fragment	Black	47.84	Polypropylene
6	Control	Fragment	Yellow	29.13	Polystyrene
7	FGR	Fragment	Transparent	52.38	Polypropylene
8	Control	Fragment	Transparent	34.71	Polyamide
9	FGR	Fragment	Red	52.29	Polypropylene

FGR: Fetal growth restriction

μ-Raman spectroscopy images of MPs detected in FGR and control groups are shown in Figures 1 and 2, respectively.

Discussion

One of the main causes of neonatal mortality and morbidity is fetal growth retardation[23]. The impact of environmental pollutants on the etiology of FGR is becoming increasingly clear. Preterm birth and FGR are two negative pregnancy outcomes that have been linked in recent years to exposure to ambient air pollution during pregnancy[24,25]. MPs are widely utilized in a variety of industries that are closely tied to daily living, such as the synthesis of clothing fibers and regular chemical production[26]. According to recent research, MPs can be found in a variety of items, such as milk and honey[27] and tea bag drinks[28]. Additionally, MPs have been found in bodily fluids such as breast milk, newborn excrement, and meconium[29]. MPs have been found in healthy pregnant placenta tissue examined using Raman and laser direct infrared spectroscopy in recent research[10,11]. Similar to our investigation, Ragusa et al.[10] used Raman spectroscopy for their findings. The placentas from four women contained a total of twelve MP pieces (designated #1-#12). More precisely, three MPs were discovered in the chorioamniotic membranes: four on the maternal side and five on the fetal side. According to reports, home coatings, adhesives, plasters, polymers, and cosmetic and personal care goods are likely the source of MPs, with three of the MPs found being polypropylene[30]. Studies in cell cultures have demonstrated that nanoplastics negatively affect human placental cells. Human placental cells exposed to polystyrene nanoplastics (PS-NPs) had more intracellular reactive oxygen species, which caused deoxyribonucleic acid damage, cell cycle arrest in the G1 or G2 phase, inflammation, and apoptosis[31]. Research on animals produced similar results.

The study of Aghaei et al.[32] on mice showed that maternal MP exposure causes significant changes in placental metabolism. The study of Chen et al.[12] on mice revealed that PS-NPs caused fetal growth retardation and significantly impaired cholesterol metabolism in both the placenta and fetus. Zhang et al.[33] showed in a rat study that maternal exposure to PS-NPs damages the placental barrier and causes neurotoxicity. In the study published by Amereh et al.[16] in 2022, they reported that placental MPs may be associated with FGR. They examined placental tissue from 30 healthy pregnant women and 13 women diagnosed with FGR. Their study found MPs in the placentas of all 13 FGR cases but in only 3 of 30 control cases. When the properties of MPs were analyzed, polyethylene was the most common, accounting for 67.7%, followed by polystyrene at 33.3%. Raman spectroscopy was used in the investigations, as in our study. The study also reported negative correlations between MP load and birth weight, infant height, head circumference, and 1st-minute Apgar score. However, the relationship between MPs in the placenta and FGR could not be demonstrated at

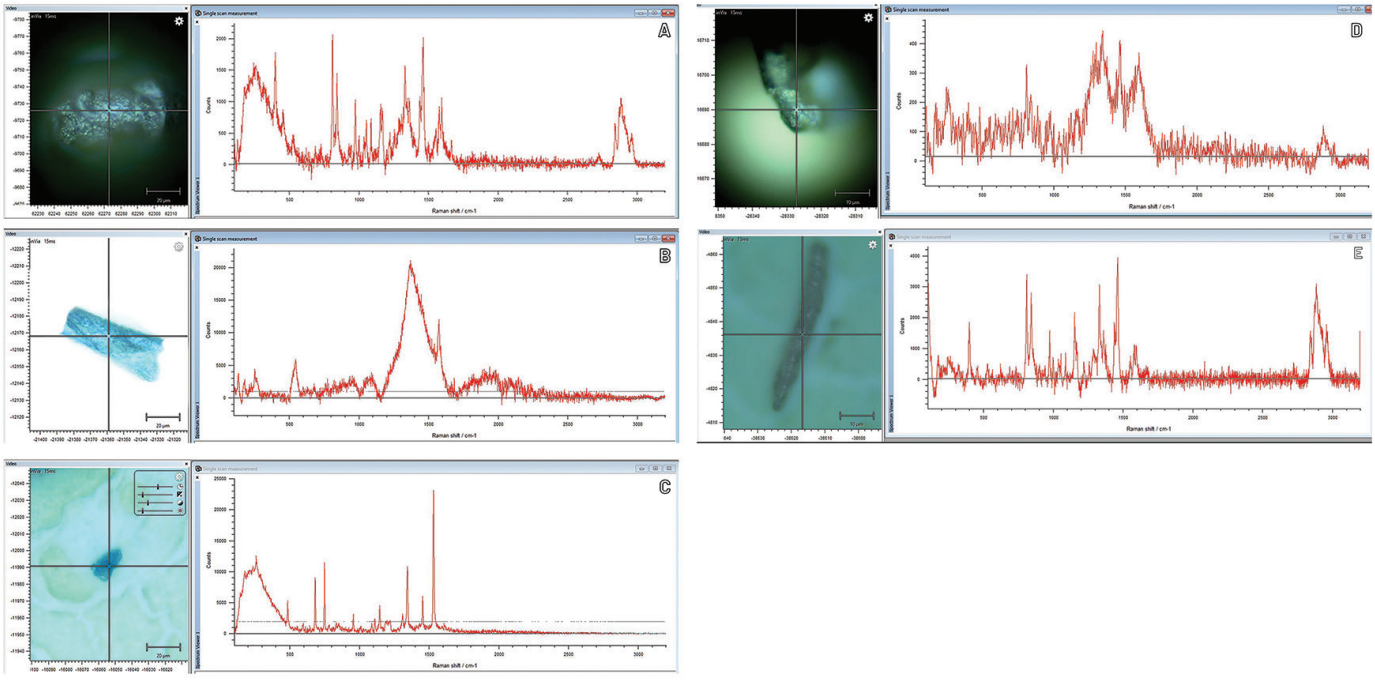

Figure 1. Representative μ-Raman spectroscopy images and spectra of microplastics detected in umbilical vein samples from the FGR group. Panels A-E demonstrate representative polypropylene and polyethylene microplastic fragments identified in the FGR group

FGR: Fetal growth restriction

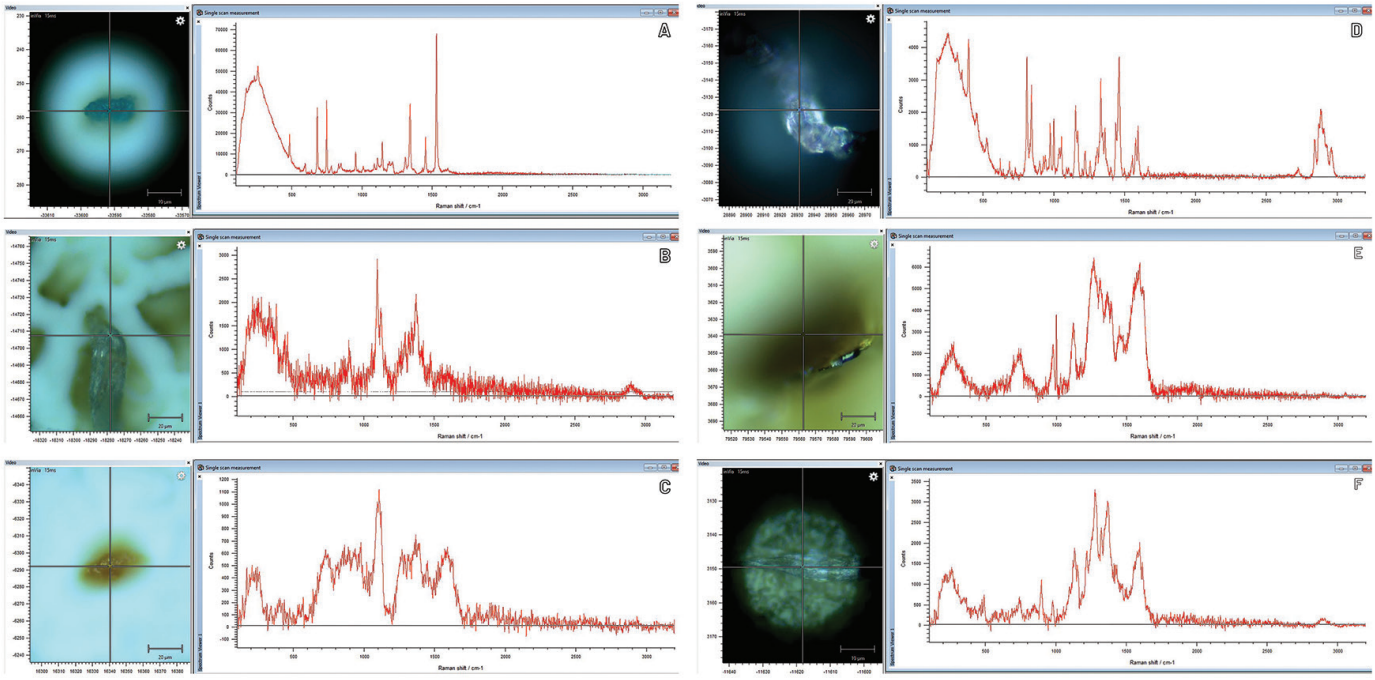

Figure 2. Representative µ-Raman spectroscopy images and spectra of microplastics detected in umbilical vein samples from the control group. Panels A-F demonstrate representative polypropylene, cellulose, polyacrylamide, polystyrene, and polyamide microplastic fragments identified in the control group

the molecular level in human studies. Recent evidence from a systematic review and meta-analysis including human studies has demonstrated a high prevalence of MPs in reproductive tissues and suggested a potential association with adverse pregnancy outcomes, including FGR[19]. However, these findings are based on a limited number of heterogeneous studies, each with relatively small sample sizes.

In contrast to these pooled findings, our study did not demonstrate a statistically significant association between MPs detected in the umbilical vein and FGR. This discrepancy may be explained by differences in biological compartments (placental tissue versus umbilical circulation), methodological variability, and the limited sample size of our study. This highlights the current gap between experimental and clinical evidence and underscores the need for translational studies that bridge experimental findings and real-world human data on the impact of MPs on fetal development.

Furthermore, there is currently limited evidence regarding the transfer of MPs from the placenta to the umbilical cord. In the current study, MP in the umbilical vein was detected in 5/6 patients (83.3%) in the control group and 4/8 patients (50%) in the FGR group (p=0.198). Therefore, our study did not observe a statistically significant association between FGR and MPs. When MP properties were examined, polypropylene was the most common MP, detected in four cases in the FGR group and two cases in the control group. Polypropylene was found to be the most common form of MP in the study by Ragusa et al.[10] that demonstrated the presence of MPs in the human placenta. Materials like food packaging and hospital intravenous cannulas contain polypropylene[34]. Identifying the source of MPs detected in human tissues and fluids is extremely challenging. Given that polypropylene was the most frequently found form of MP in the umbilical cord in our investigation and that hospitalized patients' intravenous cannulas were composed of the same material, intravenous cannulas may be a source of MPs.

Study Limitations

The small number of cases and the fact that patients' plastic consumption was not assessed may be considered limitations of our study. A major strength of our study is that, unlike most previous human studies focusing on placental tissue, we directly evaluated MPs in the umbilical vein, thereby providing novel and clinically relevant insight into potential fetal exposure. Taking all precautions to prevent plastic contamination during sample collection and subsequent processing is also a strength of our study.

Conclusion

Levels of MPs in the umbilical vein in the FGR and control groups were statistically comparable in this preliminary study of this Turkish cohort. Consequently, our results indicate that there was no statistically significant association between FGR and MPs in this small Turkish population. These findings should be interpreted in the context of emerging but still

limited human evidence regarding the clinical impact of MPs on pregnancy outcomes. Further large-scale, well-designed prospective studies are required to confirm these findings and better elucidate the clinical implications of MP exposure during pregnancy.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Kütahya Health Sciences University Non-Interventional Clinical Research Ethics Committee in Türkiye prior to study initiation (approval number: 2021/12-22, date: 08.07.2021).
Informed Consent: Written informed consent was obtained from all participants.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: İ.B., Concept: İ.B., H.Ş., A.T., Design: İ.B., H.Ş., Data Collection or Processing: İ.B., Analysis or Interpretation: İ.B., H.Ş., Literature Search: İ.B., H.Ş., A.T., Writing: İ.B., H.Ş., A.T.
Conflict of Interest: No conflict of interest was declared by the authors.
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Postoperative pain after vNOTES versus laparoscopic hysterectomy: A procedure-specific comparative study

vNOTES ile laparoskopik histerektomi sonrası postoperatif ağrının karşılaştırılması: Prosedüre özgü karşılaştırmalı bir çalışma

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Abstract

Objective: Vaginal natural orifice transluminal endoscopic surgery (vNOTES) is associated with lower postoperative pain, reduced analgesic requirements, and faster early postoperative recovery compared with laparoscopy in patients undergoing hysterectomy for benign indications. To compare postoperative pain and early recovery outcomes between vNOTES and conventional laparoscopy in patients undergoing hysterectomy for benign gynecologic indications.

Materials and Methods: This single-center retrospective cohort study included 192 patients who underwent hysterectomy between March 2021 and September 2025. Patients were grouped according to surgical approach (vNOTES vs. laparoscopy). Postoperative pain was assessed using the visual analog scale at 6 and 24 hours. Primary outcomes were pain intensity and the requirement for additional analgesia. Secondary outcomes included operative time, change in hemoglobin, time to first flatus, and length of hospital stay. Multivariable logistic regression analysis was performed to identify predictors of analgesic requirement.

Results: Of 192 patients, 75 (39.1%) underwent laparoscopy and 117 (60.9%) underwent vNOTES. Baseline characteristics were similar between groups. The vNOTES group had significantly lower pain scores at 6 and 24 hours (p=0.001) and a reduced need for additional analgesia (23.9% vs. 60.0%, p=0.001). Operative time was shorter and recovery outcomes, including time to first flatus and length of hospital stay, were significantly improved in the vNOTES group (p=0.001). Surgical approach, uterine weight, and operative time were independent predictors of analgesic requirement.

Conclusion: In this retrospective cohort study, vNOTES was associated with lower postoperative pain scores, reduced analgesic requirement, and earlier recovery than conventional laparoscopy. These findings should be confirmed in prospective randomized studies.

Keywords: vNOTES, laparoscopic hysterectomy, postoperative pain, minimally invasive surgery, gynecology

Öz

Amaç: Vajinal doğal orifis transluminal endoskopik cerrahi (vNOTES), benign endikasyonlarla histerektomi uygulanan hastalarda laparoskopiye kıyasla daha düşük postoperatif ağrı, daha az analjezik gereksinimi ve daha hızlı erken iyileşme sağlar. Benign jinekolojik endikasyonlarla histerektomi uygulanan hastalarda vNOTES ile konvansiyonel laparoskopinin postoperatif ağrı ve erken iyileşme sonuçlarının karşılaştırılması amaçlandı.

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Gereç ve Yöntemler: Bu tek merkezli retrospektif kohort çalışmasına Mart 2021-Eylül 2025 tarihleri arasında histerektomi uygulanan toplam 192 hasta dahil edildi. Hastalar cerrahi yaklaşıma göre vNOTES ve konvansiyonel laparoskopi gruplarına ayrıldı. Postoperatif ağrı, standart kurumsal protokol kapsamında 6. ve 24. saatlerde görsel analog skala kullanılarak değerlendirildi. Primer sonuçlar ağrı şiddeti ve ek analjezik gereksinimi olarak belirlendi. Sekonder sonuçlar ameliyat süresi, hemoglobin değişimi, ilk gaz çıkarma zamanı ve hastanede kalış süresini içermekteydi. Ek analjezik gereksiniminin bağımsız belirleyicilerini saptamak amacıyla çok değişkenli lojistik regresyon analizi gerçekleştirildi.

Bulgular: Toplam 192 hastanın 75'i (%39,1) laparoskopi, 117'si (%60,9) vNOTES yöntemiyle opere edildi. Gruplar arasında demografik ve klinik başlangıç özellikleri açısından anlamlı fark saptanmadı. vNOTES grubunda hem 6. hem de 24. saatlerde postoperatif ağrı skorları anlamlı derecede daha düşük bulundu (p=0,001). Ek analjezik gereksinimi vNOTES grubunda belirgin olarak daha azdı (%23,9'a karşı %60,0; p=0,001). Ayrıca ameliyat süresi daha kısa olup, ilk gaz çıkarma zamanı ve hastanede kalış süresi anlamlı şekilde daha iyiydi (p=0,001). Cerrahi yaklaşım, uterus boyutu ve ameliyat süresi analjezik gereksiniminin bağımsız belirleyicileri olarak saptandı.

Sonuç: Bu retrospektif kohort çalışmasında, vNOTES'nin geleneksel laparoskopiye kıyasla daha düşük postoperatif ağrı skorları, daha az analjezik ihtiyacı ve daha hızlı erken iyileşme ile ilişkili olduğu gözlemlenmiştir. Bu bulguların prospektif randomize çalışmalarla doğrulanması gerekmektedir.

Anahtar Kelimeler: vNOTES, laparoskopik histerektomi, postoperatif ağrı, minimal invaziv cerrahi, jinekoloji

Introduction

Minimally invasive surgery is widely used to manage benign gynecologic conditions because it is associated with reduced surgical trauma, faster recovery, and improved patient comfort. Conventional laparoscopy remains the standard approach; however, abdominal trocar placement may contribute to postoperative pain through parietal tissue injury, potentially increasing analgesic requirements and limiting patient-centered recovery outcomes[1].

To overcome these limitations, vaginal natural orifice transluminal endoscopic surgery (vNOTES) has emerged as an alternative minimally invasive technique that utilizes a transvaginal approach, thereby eliminating abdominal wall incisions. This scarless approach combines the advantages of endoscopic surgery with reduced parietal trauma and has gained increasing acceptance, particularly in hysterectomy and adnexal procedures[2].

By avoiding abdominal wall disruption, vNOTES has the potential to reduce postoperative pain and analgesic requirements while promoting faster functional recovery. Recent studies have reported favorable early postoperative outcomes with vNOTES, including lower pain scores and reduced analgesic consumption compared with conventional laparoscopy[3].

Postoperative pain remains a key determinant of patient-centered outcomes, influencing early mobilization, recovery trajectory, and overall patient satisfaction. Therefore, accurate assessment of pain outcomes across surgical approaches is essential for optimizing clinical decision-making.

However, a major limitation of the current literature is procedural heterogeneity. Many comparative studies evaluating vNOTES and conventional laparoscopy include mixed surgical procedures, such as hysterectomy, salpingectomy, and sterilization, which differ substantially in operative complexity and postoperative pain profiles. This heterogeneity introduces significant confounding and limits the interpretability of pain-related outcomes[4].

To address this limitation, the present study was designed as a procedure-specific analysis restricted to hysterectomy cases to minimize procedural confounding and to provide a more internally valid comparison. The primary objective was to evaluate postoperative pain intensity and analgesic requirements following vNOTES versus conventional laparoscopic hysterectomy. Secondary outcomes included operative time and indicators of early postoperative recovery.

Materials and Methods

This study was designed as a single-center, retrospective comparative cohort study. Clinical and surgical data for patients who underwent hysterectomy for benign gynecologic indications between March 2021 and September 2025 were retrospectively retrieved from institutional electronic medical records.

To address procedural heterogeneity observed in prior comparative studies, the present analysis was restricted to patients undergoing hysterectomy, thereby minimizing confounding due to variations in surgical complexity and postoperative pain profiles.

Eligible patients were aged 18 years or older and underwent hysterectomy for benign indications. Exclusion criteria included suspected or confirmed malignancy, emergency surgery, intraoperative conversion to laparotomy, and incomplete clinical data. All eligible patients within the study period were included. Given the retrospective design and inclusion of the entire available cohort, no a priori sample size calculation was performed. The study was approved by the University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital Scientific Research Ethics Committee (approval number: 122, date: 25.02.2026) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The patient selection process is summarized in Figure 1.

All procedures were performed at a single institution by the same surgical team under standardized perioperative and postoperative care protocols. The choice of surgical approach was not randomized and was determined according to real-world clinical decision-making. vNOTES was generally preferred in patients with adequate vaginal access

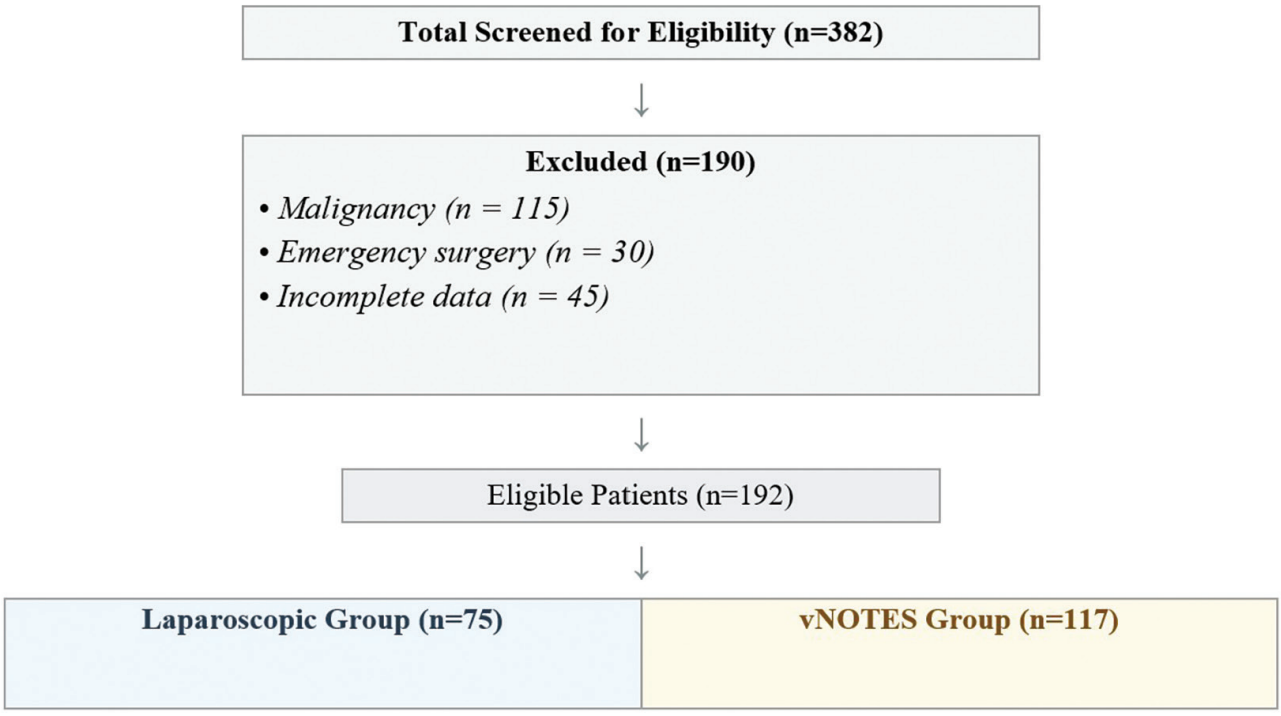

Figure 1. Flow diagram of patient selection and study inclusion
vNOTES: Vaginal natural orifice transluminal endoscopic surgery

and favorable pelvic accessibility. Preoperative assessment included gynecologic examination and ultrasonographic evaluation, including assessment of uterine size, pelvic mobility, and the sliding sign when an adhesion risk was suspected. Importantly, no intentional exclusion of larger uteri from the vNOTES group was performed.

Conventional laparoscopic hysterectomy was performed using a standardized multiport technique with carbon dioxide pneumoperitoneum. In the vNOTES group, transvaginal access was achieved using a dedicated vaginal access platform (GelPoint V-Path, Applied Medical, Rancho Santa Margarita, CA, USA), allowing endoscopic visualization and instrumentation. Vaginal cuff closure was completed according to standard surgical protocols.

A standardized postoperative analgesia protocol was applied to all patients. All patients received intramuscular diclofenac sodium (75 mg twice daily) as routine postoperative analgesia during the first 24 hours after surgery. Postoperative pain was assessed using the visual analog scale (VAS) at 6 and 24 hours after surgery as part of the institutional postoperative care protocol. Rescue analgesia was administered only after pain assessment in patients with a VAS score >4 or in those with inadequate pain control despite the standard analgesic regimen. Therefore, the VAS scores analyzed in this study represent pain intensity measured before administration of rescue analgesia when additional analgesia was required.

Pain was assessed using the VAS at 6 and 24 hours postoperatively (0=no pain, 10=worst imaginable pain). Postoperative pain assessment was an integral component of the institutional routine protocol, and all patients were systematically evaluated at predefined time points (6 and 24 hours postoperatively). Pain scores were recorded in the electronic medical record system. Pain assessments were performed by physicians blinded to the study hypothesis. These assessments were conducted by ward physicians who were not involved in the study design. However, because the study was retrospective, formal blinding of outcome assessment cannot be guaranteed.

The primary outcomes were postoperative pain intensity and the need for rescue analgesia. To complement subjective pain assessment, the requirement for additional analgesia was included as an objective outcome measure. Secondary outcomes included operative time, hemoglobin change, time to first flatus, length of hospital stay, and perioperative complications. Uterine weight was included as a clinically relevant variable to account for potential differences in surgical complexity.

Statistical Analysis

Statistical analyses were performed using SPSS version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro-Wilk test and presented as mean ± standard deviation or median (interquartile range), as appropriate. Between-group comparisons were conducted using the Student's t-test or Mann-Whitney U test. Categorical variables were compared using the chi-square test or Fisher's exact test.

To partially account for the non-randomized study design and potential confounding, univariable and multivariable logistic regression analyses were performed to identify independent predictors of the need for additional analgesia. Variables with p<0.05 in univariable analysis were included in the multivariable model. A two-sided p-value <0.05 was considered statistically significant.

Results

A total of 192 patients who underwent hysterectomy for benign gynecologic indications were included in the analysis, comprising 75 (39.1%) patients in the conventional laparoscopy group and 117 (60.9%) patients in the vNOTES group. The annual distribution of cases remained balanced throughout the study period, with both laparoscopic hysterectomy and vNOTES being performed each year. The numbers of patients undergoing laparoscopy and vNOTES were 8 and 9 in 2021; 13 and 18 in 2022; 17 and 28 in 2023; 20 and 31 in 2024; and 17 and 31 in 2025, indicating that both surgical approaches were consistently utilized throughout the study period.

Baseline demographic and preoperative clinical characteristics are presented in Table 1. There were no statistically significant differences between the groups in terms of age (50.3±7.9 vs. 48.1±8.9 years, p=0.074), body mass index (p=0.963), or preoperative hemoglobin levels (p=0.105). Similarly, parity, menopausal status, history of cesarean section, and surgical history were comparable between the two cohorts. Uterine weight did not differ significantly between groups (p=0.102), suggesting comparable surgical complexity. No significant differences were observed between the two groups in the indications for hysterectomy, including leiomyoma, abnormal uterine bleeding, adenomyosis, cervical intraepithelial neoplasia, and endometrial hyperplasia. Likewise, the rates of concomitant bilateral salpingectomy and bilateral salpingo-oophorectomy were comparable between the groups (all p>0.05).

Table 1. Baseline demographic and clinical characteristics of patients undergoing vNOTES and laparoscopic surgery

	Laparoscopy (n=75)	vNOTES (n=117)	p-value
Age (years)*	50.3±7.9	48.1±8.9	0.074
BMI (kg/m^2)*	29.0±4.7	28.9±6.6	0.963
Parity status**			
Gravidity	3.0 (2.0-5.0)	3.0 (3.0-4.0)	0.855
Parity	3.0 (2.0-4.0)	3.0 (2.0- 4.0)	0.989
Menopausal status, n (%)	44 (58.7%)	54 (46.2%)	0.091
Previous cesarean, n (%)	22 (29.3%)	32 (27.3%)	0.765
Surgical history, n (%)			
None	34 (45.3%)	50 (42.7%)	0.554
Gynecologic surgery	27 (36.0%)	40 (34.2%)	
Non-gynecologic abdominal surgery	7 (9.3%)	8 (6.8%)	
Other	7 (9.3%)	19 (16.2%)	
Indication for hysterectomy			
Leiomyoma	32 (42.7%)	48 (41.0%)	0.814
Abnormal uterine bleeding	24 (32.0%)	41 (35.0%)	0.658
Adenomyosis	11 (14.7%)	16 (13.7%)	0.846
CIN	5 (6.7%)	8 (6.8%)	0.965
Endometrial hyperplasia	3 (4.0%)	4 (3.4%)	0.835
Concomitant procedure			
Bilateral salpingectomy	52 (69.3%)	85 (72.6%)	0.618
Bilateral Salpingo-oophorectomy	18 (24.0%)	26 (22.2%)	0.771
Preoperative Hb*	11.8±1.4	12.2±1.5	0.105

* Mean ± standard deviation, ** median (IQR), BMI: Body mass index, IQR: Interquartile range, vNOTES: Vaginal natural orifice transluminal endoscopic surgery, Hb: Hemoglobin, CIN: Cervical intraepithelial neoplasia

Postoperative outcomes are summarized in Table 2. The vNOTES group demonstrated significantly higher postoperative hemoglobin levels (11.0±1.4 g/dL vs. 10.2±1.7 g/dL, p=0.001) and a significantly lower hemoglobin decrease (1.2±0.9 g/dL vs. 1.6±0.9 g/dL, p=0.001), indicating reduced intraoperative blood loss.

Operative duration was significantly shorter in the vNOTES group than in the laparoscopy group (median 86.0 vs. 105.0 minutes, p=0.001). Additionally, patients undergoing vNOTES experienced an earlier return of bowel function, as reflected by a shorter time to first flatus (10.0 vs. 13.0 hours, p=0.001) and a reduced length of hospital stay (p=0.002).

The requirement for additional analgesia was significantly lower in the vNOTES group than in the laparoscopy group (23.9% vs. 60.0%, p=0.001). Postoperative pain scores were consistently and significantly lower following vNOTES at both 6 hours (median 4 vs. 6, p=0.001) and 24 hours (median 2 vs. 3, p=0.001). No statistically significant differences were observed between the groups in either intraoperative or postoperative complications. All bladder and ureteral injuries were recognized intraoperatively and managed immediately without conversion to laparotomy. Bladder injuries were repaired primarily with layered suturing followed by temporary urinary catheterization, whereas the single ureteral injury was managed intraoperatively by ureteral stent placement in consultation with the urology team. No delayed diagnosis of urinary tract injury occurred during the postoperative period.

In the multivariable logistic regression analysis, laparoscopy (reference category: vNOTES) was independently associated with a higher likelihood of requiring additional analgesia [odds ratio (OR): 4.62, 95% confidence interval (CI): 2.31-9.26, p=0.001]. Patients undergoing laparoscopic hysterectomy had approximately 4.6-fold higher odds of requiring rescue analgesia compared with patients undergoing vNOTES. Additionally, uterine weight (OR: 1.01, 95% CI: 1.01-1.02, p=0.002) and operative time (OR: 1.02, 95% CI: 1.01–1.03, p=0.007) remained independent predictors of the requirement for additional analgesia (Table 3).

Discussion

In the present study, the vNOTES approach in patients undergoing hysterectomy for benign indications was associated with lower postoperative pain scores, reduced need for additional analgesia, and improved early recovery parameters compared with conventional laparoscopy. These

Table 2. Comparison of postoperative results between groups

	Laparoscopy (n=75)	vNOTES (n=117)	p-value
Postoperative hemoglobin*	10.2±1.7	11.0±1.4	0.001
Hemoglobin decrease*	1.6±0.9	1.2±0.9	0.001
Operation time (min)**	105.0 (89.0-125.0)	86.0 (63.0-103.0)	0.001
Uterine weight (g)**	210.0 (140.0-350.0)	255.0 (165.0-375.0)	0.102
Hospitalization (day)**	2.0 (1.0-2.0)	1.0 (1.0-2.0)	0.002
Time to first flatus (hour)**	13.0 (10.0-18.0)	10.0 (8.0-13.0)	0.001
Additional analgesic requirement, n (%)	45 (60.0%)	28 (23.9%)	0.001
VAS pain score (6th hour)**	6.0 (5.0-7.0)	4.0 (3.0-6.0)	0.001
VAS pain score (24th hour)**	3.0 (2.0-4.0)	2.0 (0-3.0)	0.001
Intraoperative complications[a]	3 (4.0%)	2 (1.7%)	NA
Bladder injury	1 (1.3%)	2 (1.7%)	
Ureteral injury	1 (1.3%)	-	
Conversion to laparotomy	1 (1.3%)	-	
Postoperative complications[a]	5 (6.7%)	5 (4.2%)	0.466
Vaginal vault hematoma	1 (1.3%)	2 (1.7%)	
Pelvic infection/abscess	1 (1.3%)	1 (0.8%)	
Urinary tract infection	2 (2.7%)	3 (2.5%)	
Blood transfusion	3 (4.0%)	1 (0.8%)	

* Mean ± standard deviation, ** median (IQR), [a]: Values represent the number of events. Because some patients experienced more than one postoperative complication, the total number of events may exceed the number of affected patients
VAS: Visual analogue scale, vNOTEs: Vaginal natural orifice transluminal endoscopic surgery, IQR: Interquartile range

Table 3. Factors associated with additional analgesic requirement: univariable and multivariable logistic regression

Variable	Univariable OR (95% CI)	p-value (univariable)	Multivariable OR (95% CI)	p-value (multivariable)
Age	1.03 (0.99-1.07)	0.107	-	-
BMI	1.02 (0.97-1.07)	0.366	-	-
Uterine weight	1.01 (1.01-1.02)	0.004	1.01 (1.01-1.02)	0.002
Surgical approach laparoscopy (reference = vNOTES)	4.77 (2.55-8.93)	0.001	4.62 (2.31-9.26)	0.001
Menopausal status	0.60 (0.33-1.08)	0.089	-	-
Operation time	1.02 (1.01-1.03)	0.001	1.02 (1.01-1.03)	0.007

BMI: Body mass index, OR: Odds ratio, CI: Confidence interval, vNOTES: Vaginal natural orifice transluminal endoscopic surgery

findings suggest that, among minimally invasive surgical techniques, the choice of surgical approach plays a key role in determining patient-centered outcomes.

One of the main strengths of this study lies in its procedure-specific design, as the analysis was deliberately restricted to hysterectomy cases. In much of the existing literature, comparisons between vNOTES and laparoscopy often include a heterogeneous mix of procedures, which can introduce variability that is difficult to fully account for[5,6]. Differences in surgical complexity, extent of tissue manipulation, and postoperative pain responses across procedures may act as important confounders and complicate the interpretation of comparative findings[7]. By focusing exclusively on hysterectomy, we aimed to minimize this variability and provide a more internally consistent and clinically meaningful comparison.

In our study, the decrease in postoperative hemoglobin levels was smaller in the vNOTES group, suggesting less intraoperative blood loss. Previous studies have similarly reported that vNOTES may be associated with less blood loss, possibly due to reduced tissue trauma and more limited dissection[8,9]. However, the clinical significance of this difference may still depend on patient selection and surgical expertise.

The significantly shorter operative time observed in the vNOTES group is a notable finding of our study. The literature reports mixed results on this issue. Earlier studies have suggested that vNOTES requires longer operative times during the initial learning curve. In contrast, more recent evidence indicates that operative duration decreases with increasing surgical experience and may even become shorter than that of conventional laparoscopy in experienced centers[10,11].

Our findings are consistent with more recent data, suggesting that when performed by an experienced surgical team, vNOTES does not confer a time disadvantage and may, in fact, be associated with shorter operative times. The shorter operative time may be explained by the more direct transvaginal access and the technical ease of opening and closing the vaginal cuff in experienced hands.

Regarding functional recovery, patients in the vNOTES group experienced an earlier return of bowel function and a shorter hospital stay. These findings are consistent with previous studies reporting faster gastrointestinal recovery and earlier mobilization following vNOTES[12,13]. Reduced peritoneal irritation and lower overall surgical trauma may contribute to these improved outcomes.

The primary finding of this study relates to postoperative pain. Patients undergoing vNOTES reported significantly lower VAS scores at both 6 and 24 hours and a markedly reduced need for additional analgesia. These findings support the hypothesis that minimizing abdominal wall trauma plays a central role in reducing postoperative pain.

Previous studies have consistently demonstrated lower postoperative pain scores and decreased analgesic consumption in patients undergoing vNOTES compared with conventional laparoscopy[14,15]. Previous studies have consistently demonstrated lower postoperative pain scores and reduced analgesic consumption following vNOTES compared with conventional laparoscopy. These findings are generally attributed to the avoidance of abdominal wall incisions, thereby reducing trocar-related somatic pain and overall surgical trauma. Our findings are consistent with this proposed mechanism[16]. From a clinical perspective, these improvements in postoperative pain may facilitate reduced opioid use, earlier mobilization, and enhanced overall patient comfort, all of which are important components of patient-centered recovery.

Multivariable analysis in our study identified the surgical approach and operative time as independent predictors of postoperative analgesic requirement. The association between longer operative duration and increased postoperative pain has been described previously[17]. However, the persistence of an independent effect of the surgical approach suggests that the impact of vNOTES on pain cannot be explained solely by shorter operative time.

In terms of safety, no significant differences were observed between the two groups with respect to perioperative and postoperative complication rates. This finding indicates that the improved recovery profile associated with vNOTES does

not come at the expense of surgical safety and is consistent with the current literature[18].

Study Limitations

Several limitations of this study should be considered. The retrospective design and lack of randomization may increase the risk of selection bias. However, the inclusion of all eligible cases from a single center and the use of standardized perioperative protocols may have partially mitigated this effect.

Although baseline characteristics were largely comparable and multivariable analyses were performed, the retrospective non-randomized design precludes complete adjustment for selection bias and unmeasured confounding. Importantly, no deliberate case selection was performed between groups. On the contrary, larger uteri were preferentially managed using the vNOTES approach, reflecting its application even in potentially more challenging cases. Conventional laparoscopy was primarily favored for patients with a preoperative suspicion of significant abdominal adhesions, which was assessed by ultrasonographic evaluation, including the sliding sign. While this approach reflects real-world clinical decision-making, it may still have influenced case allocation, and this influence should be considered when interpreting the findings.

Postoperative pain assessment using the VAS is inherently subjective and may vary depending on individual pain perception; however, the use of standardized time points for assessment may have contributed to measurement consistency. However, due to the retrospective design, formal blinding of pain assessment was not feasible, and this may have introduced measurement bias. Although pain evaluation was performed as part of a standardized institutional protocol, variability in documentation and subjective pain reporting cannot be entirely excluded.

Importantly, to strengthen the reliability of pain-related outcomes, the requirement for additional analgesia was included as an objective measure, providing supportive evidence alongside subjective VAS scores. Nevertheless, because the study was retrospective and the surgical approach was not randomized, selection bias cannot be completely excluded. Although baseline characteristics were comparable between groups and adjusted analyses were performed to reduce the effect of measured confounders, unmeasured confounding may still have influenced the results. Although the study was conducted over a five-year period, both surgical approaches were performed consistently throughout the study. Nevertheless, because vNOTES is a relatively new technique, the potential influence of increasing surgical experience (the learning curve) cannot be completely excluded and should be considered when interpreting the findings.

The single-center nature of the study may limit the generalizability of the results; further validation in larger multicenter studies would be valuable.

Conclusion

In patients undergoing hysterectomy, the vNOTES approach is associated with lower postoperative pain, reduced analgesic requirements, and faster recovery compared with conventional laparoscopy. By adopting a procedure-specific analytical approach, this study minimizes the impact of procedural heterogeneity and provides more reliable comparative evidence. These findings suggest that vNOTES may be a promising alternative to conventional laparoscopy for hysterectomy. However, given the retrospective non-randomized design, prospective randomized studies are warranted to confirm these observations.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Türkiye, Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital Scientific Research Ethics Committee (approval number: 122, date: 25.02.2026) and conducted in accordance with the Declaration of Helsinki.

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

Footnotes

Authorship Contributions

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

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Vulvar re-antisepsis and urinary tract infection after CO_2 cystoscopy in laparoscopic hysterectomy

Laparoskopik histerektomide CO_2 sistoskopi öncesi vulvar re-antisepsi ve idrar yolu enfeksiyonu

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Abstract

Objective: To evaluate whether vulvar re-antisepsis before carbon dioxide (CO_2) cystoscopy after laparoscopic hysterectomy is associated with a lower incidence of postoperative urinary tract infection (UTI).

Materials and Methods: This prospective observational study included 98 women who underwent laparoscopic hysterectomy between November 25, 2025, and March 1, 2026. All patients received routine preoperative abdominal and vulvovaginal preparation with 10% povidone-iodine. According to routine intraoperative practice and surgeon preference, 53 patients underwent standard antisepsis alone, and 45 received additional vulvar and periurethral re-antisepsis immediately before CO_2 cystoscopy. The primary outcome was a postoperative UTI, defined according to Centers for Disease Control and Prevention/National Healthcare Safety Network criteria. Logistic regression was used to explore factors associated with postoperative UTI. The study was registered at clinicaltrials.gov (NCT07232446).

Results: Postoperative UTI occurred in 8 patients (15.1%) in the standard antisepsis group and in 4 patients (8.9%) in the re-antisepsis group (p=0.538). Vulvar re-antisepsis was not associated with postoperative UTI in the exploratory multivariable model [adjusted odds ratio (OR)=0.89, 95% confidence interval (CI): 0.19-4.23; p=0.881]. Delayed catheter removal (>6 hours) was associated with postoperative UTI (adjusted OR=12.87, 95% CI: 2.13-77.62; p=0.005).

Conclusion: Vulvar re-antisepsis before CO_2 cystoscopy did not result in a statistically significant reduction in postoperative UTIs. Given the limited number of events and the non-randomized design, these findings should be interpreted as exploratory.

Keywords: Antisepsis, carbon dioxide, cystoscopy, laparoscopic hysterectomy, urinary tract infection

Öz

Amaç: Laparoskopik histerektomi sonrasında uygulanan karbon dioksit (CO_2) sistoskopi öncesinde vulvar re-antisepsi yapılmasının postoperatif idrar yolu enfeksiyonu (İYE) insidansında azalma ile ilişkili olup olmadığını değerlendirmektir.

Gereç ve Yöntemler: Bu prospektif gözlemsel çalışmaya 25 Kasım 2025 ile 1 Mart 2026 tarihleri arasında laparoskopik histerektomi uygulanan 98 kadın dahil edildi. Tüm hastalara ameliyat öncesinde %10 povidon-iyot ile rutin abdominal ve vulvovajinal antiseptik hazırlık uygulandı. Rutin intraoperatif uygulama ve cerrah tercihine göre 53 hastaya yalnızca standart antisepsi uygulanırken, 45 hastaya CO_2 sistoskopiden hemen önce ek

PRECIS: In this prospective observational study, vulvar re-antisepsis before carbon dioxide cystoscopy was not associated with a significant reduction in postoperative urinary tract infection after laparoscopic hysterectomy.

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vulvar ve periüretral yeniden antisepsi uygulandı. Birincil sonlanım noktası, *Centers for Disease Control and Prevention/National Healthcare Safety Network* kriterlerine göre tanımlanan postoperatif İYE idi. Postoperatif İYE ile ilişkili faktörleri araştırmak amacıyla lojistik regresyon analizi kullanıldı. Çalışma clinicaltrials.gov'a kaydedilmiştir (NCT07232446).

Bulgular: Postoperatif İYE, standart antisepsi grubunda 8 hastada (%15,1), re-antisepsi grubunda ise 4 hastada (%8,9) görüldü (p=0,538). Keşifsel çok değişkenli modelde vulvar re-antisepsi postoperatif İYE ile ilişkili değildi [düzeltilmiş olasılık oranı (OR)=0,89; %95 güven aralığı (GA): 0,19-4,23; p=0,881]. Gecikmiş kateter çıkarılması (>6 saat) postoperatif İYE ile ilişkili bulundu (düzeltilmiş OR=12,87; %95 GA: 2,13-77,62; p=0,005).

Sonuç: CO_2 sistoskopi öncesinde uygulanan vulvar re-antisepsi, postoperatif İYE sıklığında istatistiksel olarak anlamlı bir azalma göstermedi. Olay sayısının sınırlı olması ve randomize olmayan tasarım göz önüne alındığında, bu bulgular keşifsel olarak yorumlanmalıdır.

Anahtar Kelimeler: Antisepsi, karbondioksit, sistoskopi, laparoskopik histerektomi, idrar yolu enfeksiyonu

Introduction

Urinary tract injury is a significant complication of laparoscopic hysterectomy, particularly when pelvic anatomy is distorted by fibroids, adhesions, prior surgery, endometriosis. Intraoperative cystoscopy is therefore commonly used to assess urinary tract integrity and to facilitate early recognition of bladder or ureteral injury[1-4].

Routine or liberal use of cystoscopy after laparoscopic hysterectomy has therefore been advocated by many surgeons as a practical means of identifying unsuspected bladder or ureteral injury before the patient leaves the operating room. Early recognition allows immediate correction and may reduce delayed morbidity, although the broader effect of universal cystoscopy on postoperative injury rates remains debated. Systematic reviews and large cohort studies have shown that cystoscopy clearly improves intraoperative recognition of urinary tract injury, yet its association with delayed postoperative genitourinary complications has been less consistent across studies[2-5].

Another practical issue concerns how cystoscopy is performed. Historically, saline distension with intravenous indigo carmine was widely used to confirm ureteral patency, but dye shortages and concerns regarding availability, cost, workflow, and adverse effects have stimulated interest in alternative techniques. Studies evaluating sodium fluorescein, phenazopyridine, mannitol, dextrose-containing media, and other approaches have shown that visualization of ureteral jets can be achieved in different ways, but these techniques may differ in surgeon satisfaction, ease of interpretation, operative efficiency, and postoperative urinary outcomes[6-8].

In this context, carbon dioxide (CO_2) cystoscopy has emerged as an attractive alternative. CO_2 provides a clear visual field, avoids reliance on dyes or diuretics, and may shorten the time needed to visualize ureteral jets. Prior studies have suggested that CO_2 cystoscopy is feasible, safe, and efficient in gynecologic laparoscopy, with satisfactory visualization of bladder mucosa and ureteral efflux. More recent prospective data have further supported its diagnostic utility during laparoscopic gynecologic surgery, including total laparoscopic hysterectomy[9,10].

Despite the growing acceptance of intraoperative cystoscopy, attention has increasingly shifted from injury detection alone to the broader perioperative consequences of the procedure itself. Postoperative urinary symptoms, transient retention, delayed catheter removal, retained bladder fluid, and urinary tract infection (UTI) have been examined as clinically relevant outcomes after cystoscopy performed during laparoscopic hysterectomy. Although some trials found no clear effect of retained cystoscopy fluid on postoperative voiding outcomes, other studies have suggested that specific cystoscopy media—particularly dextrose-based solutions—may influence postoperative urinary symptoms or UTI risk[7,8,11].

From an infection-control perspective, contamination of the operative field by vaginal flora remains a longstanding concern during hysterectomy. Vaginal and vulvovaginal preparation are, therefore, routine components of gynecologic surgery, but the optimal agent and the ideal timing of antiseptic application remain unknown. Earlier research emphasized that hysterectomy-related infectious morbidity is closely linked to bacterial contamination from the vagina and suggested that additional or better-timed antiseptic measures might be beneficial, although the available evidence was limited and methodologically heterogeneous[12,13]. More recent randomized and observational studies comparing povidone-iodine and chlorhexidine before hysterectomy have shown lower intraoperative bacterial counts with the latter in some settings, yet the effect on postoperative infectious outcomes—including UTI—has been inconsistent[14-17].

Most published studies on gynecologic antisepsis have focused on preoperative vaginal preparation. By contrast, very little attention has been paid to whether a second antiseptic application immediately before intraoperative cystoscopy might reduce contamination during urethral and vulvar instrumentation. This question is clinically relevant because cystoscopy is typically performed after completion of a hysterectomy, at a time when the operation is already underway, tissue handling has occurred, and the initial surgical preparation may no longer fully reflect the microbial conditions in the vulvovaginal field. At the same time, current surveillance definitions make clear that UTI is strongly influenced by urinary tract instrumentation and catheter exposure, underscoring the importance of perioperative measures that may reduce avoidable contamination[18].

To our knowledge, no study has specifically evaluated whether vulvar re-antisepsis immediately before CO_2 cystoscopy after laparoscopic hysterectomy is associated with postoperative UTI rates. The present study was designed to compare

postoperative urinary symptoms and UTI outcomes between patients who underwent standard antiseptic preparation alone and those who received additional vulvar re-antisepsis before intraoperative CO_2 cystoscopy.

Materials and Methods

Study Design

This prospective observational study evaluated the effect of vulvar re-antisepsis, administered before CO_2 cystoscopy performed after laparoscopic hysterectomy, on postoperative UTIs at a high-volume tertiary referral center. Patients who underwent laparoscopic hysterectomy between November 25, 2025, and March 1, 2026, were included in the study. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital Non-Interventional Research Ethics Committee (approval number: 2025/08-24, date: 11.09.2025). The study was prospectively registered at clinicaltrials.gov (NCT07232446), and written informed consent was obtained from all participants before enrollment. Given the observational design and the procedural nature of the intervention, surgeons, patients, and outcome assessors were not blinded to group allocation.

Study Population

Women undergoing laparoscopic hysterectomy for benign gynecologic indications were eligible for inclusion. Patients were enrolled consecutively during the study period. Patients with preoperative urinary symptoms were excluded. Additional exclusion criteria were uncontrolled diabetes mellitus, immunosuppression, a positive preoperative urine culture, a known urinary tract anomaly, conversion to laparotomy, continuation of surgery via the vaginal route, and a postoperative pathological diagnosis of malignancy. A total of 98 patients were included in the final analysis. Of these, 53 patients were managed with standard antisepsis alone, and 45 underwent additional vulvar re-antisepsis before cystoscopy.

Perioperative Management

All procedures were performed laparoscopically. In patients without contraindications, perioperative antibiotic prophylaxis was administered as 2 g of intravenous cefazolin. Surgical field preparation with 10% povidone-iodine included both the abdominal and vulvovaginal operative fields. After sterile draping, laparoscopic hysterectomy was carried out using a standard intraperitoneal approach.

Study Groups

Patients were classified based on whether vulvar antisepsis was repeated immediately before intraoperative cystoscopy. Group allocation was not randomized and reflected surgeon-specific routine practice. Some surgeons routinely performed repeated vulvar and periurethral antisepsis immediately before cystoscopy, whereas others did not. Thus, the operating surgeon's usual practice, rather than predefined patient-level UTI risk criteria or case complexity, primarily determined the decision. In the standard antisepsis group, all patients underwent abdominal and vulvovaginal preparation with 10% povidone-iodine before surgery; however, no repeat vulvar antiseptic application was performed immediately before cystoscopy. In the vulvar re-antisepsis group, immediately before cystoscope insertion, the vulvar and periurethral areas, including the urethral meatus and vaginal introitus, were prepared again by the operating team using sterile gauze and 10% povidone-iodine. The antiseptic solution was allowed to remain in contact with the prepared area for approximately 1 minute before cystoscope insertion. This additional preparation was performed after completion of laparoscopic hysterectomy and immediately before CO_2 cystoscopy.

The same perioperative antibiotic prophylaxis protocol was applied in both groups. All patients included in the study received intravenous cefazolin before skin incision. No patient had a documented beta-lactam allergy; therefore, no alternative prophylactic regimen was required.

CO_2 Cystoscopy Technique

After completion of laparoscopic hysterectomy, intraoperative cystoscopy was performed using CO_2 as the bladder distension medium. Before cystoscopy, the bladder was emptied and the Foley catheter balloon was deflated. Bladder insufflation was achieved using a trocar and Foley catheter setup. A 30 degree, 5 mm optic was then used to evaluate the bladder mucosa and the bilateral ureteral jet efflux. After cystoscopic evaluation, the Foley catheter was reinserted into the bladder and the balloon was reinflated. CO_2 cystoscopy was performed using the technique reported by Inan et al.[10].

Postoperative Management and Follow-up

The routine postoperative practice was to remove the urinary catheter 6 hours after surgery. No predefined criteria were used for catheter retention beyond 6 hours. When catheter removal was delayed, timing was individualized based on the patient's postoperative clinical condition and intraoperative or surgical circumstances. All patients were followed for 6 weeks postoperatively. Patients were routinely scheduled for outpatient follow-up visits on postoperative days 10 and 30 and were assessed for urinary symptoms during these visits. Postoperative urinary symptoms included dysuria, urinary urgency, urinary frequency, suprapubic pain or tenderness, and costovertebral angle tenderness or flank pain/tenderness, consistent with the symptom components of the Centers for Disease Control and Prevention/National Healthcare Safety Network (CDC/NHSN) criteria for symptomatic UTI, and were assessed based on patient-reported complaints during postoperative follow-up. The operating surgical team assessed postoperative urinary symptoms during routine follow-up

visits. When clinically indicated, urinalysis and urine culture were obtained, and treatment was initiated as appropriate. The same follow-up approach and clinical threshold for obtaining urinalysis and urine culture were applied in both groups.

Data Collection

Clinical data were recorded prospectively using a structured case report form. Baseline variables included age, body mass index, gravidity, parity, menopausal status, smoking status, diabetes mellitus, previous abdominal surgery, and hormone replacement therapy use. Operative variables included surgical indication, operation time, and cystoscopy time; postoperative variables included delayed catheter removal, urinary symptoms, and UTI. Operation time was recorded separately from cystoscopy time and did not include the cystoscopy duration. Delayed catheter removal was defined as removal of the catheter later than 6 hours after surgery. Postoperative UTI was defined according to the CDC/NHSN criteria. Diagnosis required compatible urinary symptoms together with a positive urine culture, in accordance with CDC/NHSN criteria[18].

Statistical Analysis

Continuous variables were assessed for distributional characteristics and are presented as mean ± standard deviation or median (interquartile range), as appropriate. Categorical variables are presented as numbers (%). Between-group comparisons of continuous variables were performed using the independent-samples t-test or Mann-Whitney U test, as appropriate. Categorical variables were compared using the χ^2 test or Fisher's exact test. No formal a priori sample size calculation was performed because this was an exploratory, prospective, observational study that included consecutive eligible patients enrolled during the predefined study period. Logistic regression analysis was used to explore factors associated with postoperative UTI. The regression model included the following variables: vulvar re-antisepsis, age, body mass index, diabetes mellitus, operation time, delayed catheter removal (>6 hours), and cystoscopy time. Because of the limited number of postoperative UTI events, multivariable logistic regression was considered exploratory. Covariates were selected based on clinical relevance, and no automated stepwise variable-selection procedure was used. Adjusted estimates were interpreted cautiously because of the risk of model overfitting and imprecision. Crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. All statistical analyses were performed using SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA). All tests were two-sided; a p-value of <0.05 was considered statistically significant. Given the exploratory nature of the analyses, no formal adjustment for multiple comparisons was applied.

Results

A total of 107 patients were assessed for eligibility. Nine patients were excluded because of a positive preoperative urine culture (n=5), uncontrolled diabetes mellitus (n=3), or a known urinary tract anomaly (n=1). No patients met the remaining predefined exclusion criteria during the study period. The final study population consisted of 98 patients, of whom 53 were included in the standard antisepsis group and 45 were included in the vulvar re-antisepsis group (Figure 1). All patients included in the study completed the 6 weeks follow-up and were analyzed.

Baseline demographic and preoperative characteristics were comparable between the groups. The median age was 51 (47-56.5) years in the standard antisepsis group and 48 (46-55.5) years in the vulvar re-antisepsis group (p=0.226). Gravidity, parity, postmenopausal status, body mass index, smoking status, diabetes mellitus, previous abdominal surgery, and hormone replacement therapy use did not differ significantly between the groups (Table 1).

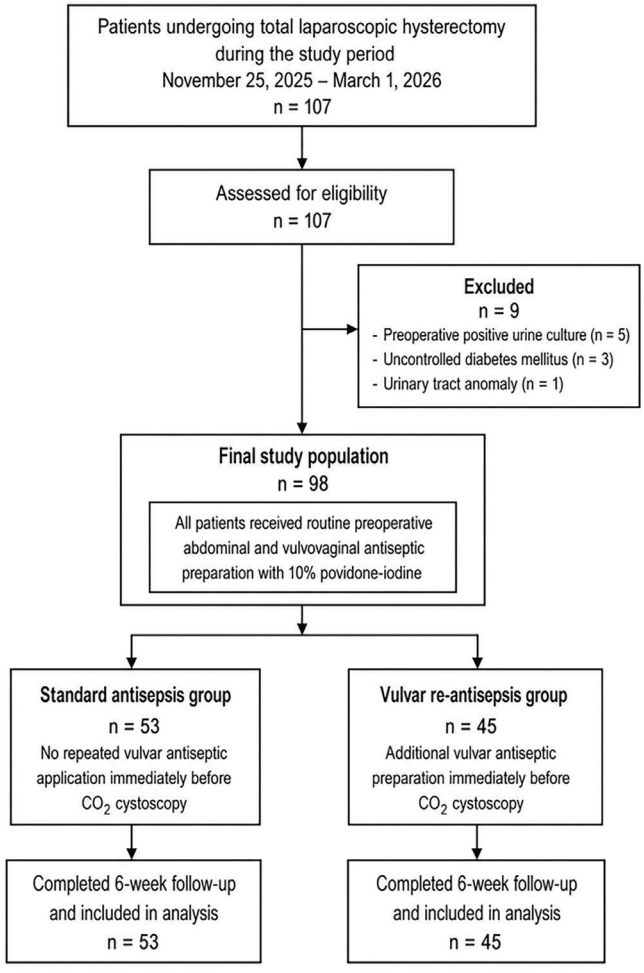

Figure 1. Participant flow diagram

The distribution of surgical indications did not differ significantly between the groups (p=0.140). Myoma was the most common indication in both groups, accounting for 43.4% of cases in the standard antisepsis group and for 40.0% in the vulvar re-antisepsis group. The median operation time was 140 (101-210) minutes in the standard antisepsis group and 124 (89.5-142) minutes in the vulvar re-antisepsis group; this difference was not statistically significant (p=0.070). The median cystoscopy time was 3 (2-5) minutes in the standard group and 3 (2-4) minutes in the vulvar re-antisepsis group (p=0.730). Delayed catheter removal (>6 hours) occurred in 10 patients (18.9%) in the standard antisepsis group and in 4 patients (8.9%) in the vulvar re-antisepsis group (p=0.247) (Table 2).

Postoperative urinary symptoms occurred in 17 patients (32.1%) in the standard antisepsis group and in 8 patients (17.8%) in the vulvar re-antisepsis group, respectively, corresponding to an absolute risk difference of -14.3 percentage points (95% CI: -31.1 to 2.5). Although the frequency was lower in the vulvar re-antisepsis group, the difference was not statistically significant (p=0.162). Similarly, postoperative UTI diagnosed according to CDC/NHSN criteria was observed in 8 patients (15.1%) in the standard antisepsis group and in 4 patients (8.9%) in the vulvar re-antisepsis group, corresponding to an absolute risk difference of -6.2 percentage points (95% CI: -18.9 to 6.5), although this difference was not statistically significant (p=0.538) (Table 2). No adverse events or unintended effects related to vulvar re-antisepsis or CO_2 cystoscopy were observed in either group.

Univariable logistic regression analysis showed no significant association between vulvar re-antisepsis and postoperative UTI (crude OR: 0.55, 95% CI: 0.15-1.96, p=0.355). Age, body mass index, diabetes mellitus, and cystoscopy time were

Table 1. Baseline demographic and preoperative characteristics

	Standard antisepsis (n=53)	Vulvar re-antisepsis (n=45)	p-value
Age, years	51 (47-56.5)	48 (46-55.5)	0.226
Gravidity	2 (2-3)	2 (2-3)	0.402
Parity	2 (2-3)	2 (1-2)	0.179
Postmenopausal, n (%)	30 (56.6%)	25 (55.6%)	0.917
BMI, kg/m^2	27.19±4.07	26.46±4.30	0.391
Smoking, n (%)	13 (24.5%)	13 (28.9%)	0.653
Diabetes mellitus, n (%)	10 (18.9%)	9 (20.0%)	0.888
Previous abdominal surgery, n (%)	33 (62.3%)	30 (66.7%)	0.678
HRT use, n (%)	2 (3.8%)	2 (4.4%)	1.000

Continuous variables are presented as median (IQR) or mean ± SD, as appropriate; categorical variables are presented as n (%)
BMI: Body mass index, HRT: Hormone replacement therapy, IQR: Interquartile range, SD: Standard deviation

Table 2. Operative characteristics and postoperative urinary outcomes

	Standard antisepsis (n=53)	Vulvar re-antisepsis (n=45)	p-value
Operation indication, n (%)			
Abnormal uterine bleeding	8 (15.1%)	11 (24.4%)	0.140
Myoma	23 (43.4%)	18 (40.0%)	
Pelvic organ prolapse	13 (24.5%)	4 (8.9%)	
Endometrial hyperplasia	6 (11.3%)	5 (11.1%)	
Other	3 (5.7%)	7 (15.6%)	
Operation time, min	140 (101-210)	124 (89.5-142)	0.070
Cystoscopy time, min	3 (2-5)	3 (2-4)	0.730
Delayed catheter removal (>6 hours), n (%)	10 (18.9%)	4 (8.9%)	0.247
Postoperative urinary symptoms, n (%)	17 (32.1%)	8 (17.8%)	0.162
Postoperative UTI, n (%)	8 (15.1%)	4 (8.9%)	0.538

Continuous variables are presented as median (IQR); categorical variables are presented as n (%)
UTI: Urinary tract infection, IQR: Interquartile range

not significantly associated with postoperative UTI in this analysis. Operation time was associated with postoperative UTI in the crude model (crude OR: 1.01, 95% CI: 1.00-1.02, p=0.013), and delayed catheter removal (>6 hours) was strongly associated (crude OR: 9.75, 95% CI: 2.54-37.43, p=0.001) (Table 3).

In the exploratory multivariable model, vulvar re-antisepsis was not associated with postoperative UTI (adjusted OR: 0.89, 95% CI: 0.19-4.23, p=0.881). Among the variables entered into the model, delayed catheter removal (>6 hours) was the only factor that remained statistically associated with postoperative UTI (adjusted OR: 12.87, 95% CI: 2.13-77.62, p=0.005). Operation time was no longer statistically significant after adjustment (adjusted OR: 1.01, 95% CI: 1.00-1.02, p=0.101) (Table 3).

Discussion

In this prospective observational study, we evaluated whether vulvar re-antisepsis, performed immediately before intraoperative CO_2 cystoscopy after laparoscopic hysterectomy, reduced postoperative UTIs. Although the rate of postoperative UTI was numerically lower in the vulvar re-antisepsis group than in the standard antisepsis group, this difference did not reach statistical significance. In contrast, delayed catheter removal was the only factor that remained statistically associated with postoperative UTI in the exploratory multivariable model.

Urinary tract injury and its early intraoperative detection remain central concerns in laparoscopic hysterectomy. Routine cystoscopy has been widely adopted to improve detection of ureteral and bladder injuries, and several studies have demonstrated that intraoperative cystoscopy increases the rate of immediate recognition of urinary tract injuries, thereby reducing delayed morbidity[2,4]. Although cystoscopy is beneficial for detecting injuries, its impact on postoperative urinary outcomes—particularly UTI—remains unclear.

Previous studies investigating cystoscopy-related factors have yielded heterogeneous results regarding postoperative urinary morbidity. For example, the type of distension medium has been suggested as a potential contributor to infection risk. Dextrose-containing media have been associated with increased rates of postoperative UTI, possibly due to transient glucosuria promoting bacterial growth[7]. In contrast, Misal et al.[8] compared postoperative urinary symptoms after benign laparoscopic hysterectomy between patients undergoing cystoscopy with 50% dextrose and those undergoing cystoscopy with saline and intravenous indigo carmine. They reported no significant difference in postoperative urinary symptoms or empiric treatment for UTI between the groups, suggesting that the relationship between the cystoscopy medium and postoperative urinary morbidity may depend on patient characteristics, perioperative context, and the specific distension protocol used. These findings suggest that while cystoscopy itself is an important diagnostic tool, modifiable perioperative factors may influence infection risk.

CO_2 cystoscopy represents an alternative approach that avoids the use of liquid distension media and dyes. Previous reports have demonstrated that CO_2 cystoscopy is feasible, safe, and efficient for assessing ureteral patency during laparoscopic gynecologic surgery[9,10]. In the present study, CO_2 cystoscopy provided a standardized intraoperative assessment for all patients, thereby minimizing variability attributable to the cystoscopic technique. This is important when interpreting the effects of additional interventions, such as vulvar re-antisepsis.

The rationale for vulvar re-antisepsis before cystoscopy is the potential for contamination during the procedure. Although preoperative vaginal and vulvovaginal antisepsis is a standard component of gynecologic surgery, bacterial recolonization of the operative field during the procedure is well recognized. Earlier studies have shown that vaginal antiseptic preparation reduces bacterial load and may decrease postoperative infectious complications[12,13]. More recent analyses comparing antiseptic agents have demonstrated differences in microbial colonization, although their impact on clinical infection outcomes, including UTI, has been inconsistent[14-17]. All patients in the present study received routine preoperative abdominal and vulvovaginal antisepsis. Therefore, the comparison was not between antisepsis and no antisepsis, but rather between routine preoperative antisepsis

Table 3. Exploratory logistic regression analysis of factors associated with postoperative UTI

	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Vulvar re-antisepsis	0.55 (0.15-1.96)	0.355	0.89 (0.19-4.23)	0.881
Age	1.02 (0.94-1.09)	0.697	0.99 (0.90-1.09)	0.819
BMI	1.00 (0.87-1.16)	0.968	1.01 (0.84-1.21)	0.928
Diabetes mellitus	0.81 (0.16-4.05)	0.799	0.84 (0.11-6.15)	0.863
Operation time	1.01 (1.00-1.02)	0.013	1.01 (1.00-1.02)	0.101
Delayed catheter removal (>6 hours)	9.75 (2.54-37.43)	0.001	12.87 (2.13-77.62)	0.005
Cystoscopy time	1.02 (0.75-1.39)	0.906	0.59 (0.32-1.09)	0.093

BMI: Body mass index, OR: Odds ratio, CI: Confidence interval, UTI: Urinary tract infection

alone and routine antisepsis followed by repeated vulvar preparation performed immediately before cystoscopy.

Despite this theoretical rationale, our findings indicate that a single additional vulvar antiseptic application immediately before cystoscopy did not result in a statistically significant reduction in postoperative UTI rates. This may be explained by several factors. First, UTI after gynecologic surgery is multifactorial and is influenced by host characteristics, perioperative management, and urinary tract instrumentation. Second, the effect of transient contamination during cystoscopy may be limited compared with that of more dominant factors, such as catheterization duration. Finally, the relatively low number of events in our cohort may have limited the statistical power to detect a modest effect. Given the limited number of postoperative UTI events and the exploratory nature of this study, the absence of statistical significance should not be interpreted as definitive evidence of no effect. The findings indicate that vulvar re-antisepsis before CO_2 cystoscopy did not result in a statistically significant reduction in postoperative UTI in this cohort.

From a practical perspective, vulvar re-antisepsis requires only a brief additional preparation step and uses an antiseptic already routinely available in the operating room, but it nonetheless adds a procedural step and may modestly affect operative workflow. Formal cost and workflow analyses were not performed in this study. In the absence of a demonstrated reduction in postoperative UTI, the value of routinely adopting this practice should therefore be evaluated in adequately powered randomized studies.

Notably, delayed catheter removal (>6 hours) was the only factor that remained statistically associated with postoperative UTI in the exploratory multivariable model. This finding is consistent with the well-established relationship between urinary catheterization and infection risk. According to CDC/NHSN definitions and prior studies, catheter duration is a key determinant of catheter-associated UTI, with longer exposure increasing the likelihood of bacterial colonization and infection[18]. Delayed catheter removal occurred more frequently in the standard antisepsis group, although the difference was not statistically significant. This imbalance may have contributed to the numerically higher postoperative UTI rate in that group. Because the reasons for delayed catheter removal were not systematically recorded, and retention beyond 6 hours was individualized according to clinical and surgical circumstances, the observed association should not be interpreted as evidence that delayed catheter removal itself caused postoperative UTI. Delayed catheter removal may represent a potentially modifiable postoperative factor, but it may also serve as a marker of more complicated surgery or postoperative recovery. Therefore, catheter duration should be minimized whenever clinically feasible; the present association should be interpreted cautiously.

Operative time was numerically longer in the standard antisepsis group, and this difference approached statistical significance; this may reflect greater surgical complexity in some cases. Although operation time was recorded separately from cystoscopy time, the between-group difference in operation time may have influenced postoperative catheter management and urinary outcomes. In addition, the estimates for cystoscopy time should be interpreted with caution because the direction and magnitude of the association may be unstable in the context of the limited number of postoperative UTI events and possible model overfitting. Therefore, neither operation time nor cystoscopy time should be overinterpreted as a definitive, independent determinant of postoperative UTI in this cohort.

Although the overall distribution of surgical indications did not differ statistically between groups, numerical imbalances were observed for some, particularly pelvic organ prolapse and those categorized as "other." These differences may reflect variations in case mix or be related to surgical complexity or postoperative catheter management. Therefore, residual confounding related to surgical indication cannot be excluded.

The present study has several strengths. The prospective design and standardized surgical approach, including routine use of CO_2 cystoscopy, allowed for a relatively homogeneous cohort. In addition, detailed perioperative data collection enabled the evaluation of multiple potential risk factors for postoperative UTI.

Study Limitations

The observational and non-randomized design introduces the possibility of selection bias; therefore, causality cannot be inferred from these findings. Because group allocation reflected surgeon-specific routine practice, residual confounding related to surgeon-level practice patterns or differences in case mix cannot be excluded. Although re-antisepsis was not assigned according to predefined patient-level UTI risk or case-complexity criteria, the possibility of residual confounding inherent to the non-randomized design remains. The sample size was relatively small, limiting the power to detect differences in low-frequency outcomes such as UTI. In addition, only 12 postoperative UTI events occurred, limiting the stability of the multivariable model and increasing the risk of overfitting. The reasons for delayed catheter removal were not systematically recorded, which limits interpretation of this delay as an independent postoperative risk factor and suggests that it may also reflect more complex surgical or recovery-related circumstances. Therefore, adjusted ORs should be interpreted as exploratory estimates rather than definitive evidence of independent causal relationships. Urine cultures were obtained only when clinically indicated, rather than systematically from all patients. Therefore, asymptomatic bacteriuria or minimally symptomatic infections may have been missed, and ascertainment bias cannot be completely excluded. In addition, postoperative urinary assessment was performed

by the surgical team, which was aware of group allocation; therefore, differential ascertainment between groups cannot be completely excluded. Species-level microbiological data from positive urine cultures were not systematically recorded; therefore, postoperative UTI episodes could not be further characterized according to causative pathogens or antimicrobial resistance patterns. However, the same clinical threshold for urine testing was applied in both groups. The study was conducted at a single center, which may limit generalizability.

Conclusion

This prospective observational study did not demonstrate a statistically significant reduction in postoperative UTI when vulvar re-antisepsis was performed before CO_2 cystoscopy following laparoscopic hysterectomy. Delayed catheter removal was associated with postoperative UTI, suggesting that catheter management may be clinically important in this setting. Given the limited number of events and the non-randomized design, these findings should be interpreted as hypothesis-generating.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital Non-Interventional Research Ethics Committee (approval number: 2025/08-24, date: 11.09.2025).
Informed Consent: Written informed consent was obtained from all participants before enrollment.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.K., S.K., U.D., A.B., Concept: A.K., S.S.K., V.K., A.B., Design: A.K., S.S.K., V.K., A.B., Data Collection or Processing: A.K., N.Ö.K., A.G., Analysis or Interpretation: A.K., S.S.K., S.K., U.D., N.Ö.K., A.G., A.H.İ., Literature Search: A.K., S.S.K., S.K., U.D., A.G., A.H.İ., Writing: A.K., S.S.K., V.K., A.H.İ.
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Prognostic determinants of survival in early-stage endometrial cancer: A retrospective analysis

Erken evre endometriyal kanserde sağkalımın prognostik faktörleri: Retrospektif analiz

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Abstract

Objective: The aim of this study was to investigate prognostic determinants of survival in early-stage endometrial cancer (EC), focusing on clinicopathological parameters including histologic grade, tumor stage, myometrial invasion, lymphovascular space invasion (LVSI), and preoperative CA-125, as well as the role of adjuvant therapies in disease-free survival (DFS) and overall survival (OS).

Materials and Methods: We retrospectively analyzed women treated surgically for International Federation of Gynecology and Obstetrics 2009 stage I-II EC at a tertiary center between January 2011 and January 2023. Demographic and pathological data were collected, including age, body mass index, reproductive history, menopausal status, comorbidities, tumor size, histologic subtype and grade, depth of myometrial invasion, LVSI, serum CA-125 levels, and surgical procedures. Adjuvant therapies—external beam radiotherapy, vaginal brachytherapy, and chemotherapy—were also documented. Associations between these variables and survival outcomes were assessed using Kaplan-Meier and Cox regression analyses.

Results: A total of 241 women with early-stage EC were included. Median age was 57 years (range: 34-86 years). Of these, 181 (75.1%) were stage IA, 47 (19.5%) were stage IB, and 13 (5.4%) were stage II. Histologic grades were grade 1 in 44.8%, grade 2 in 39.0%, and grade 3 in 16.2%. During a median follow-up of 75 months, recurrence occurred in 4.6% of patients and mortality in 7.9% of patients. Univariate analysis showed that elevated CA-125, higher stage, and higher grade were associated with worse OS. Multivariate analysis identified histologic grade as an independent predictor of both OS and DFS. Neither adjuvant radiotherapy nor chemotherapy improved survival outcomes.

Conclusion: Histologic grade was the strongest independent prognostic factor for OS and DFS in early-stage EC, surpassing tumor stage, myometrial invasion, and LVSI. These findings highlight the importance of comprehensive surgical staging, especially when high-grade tumors are detected intraoperatively, to ensure accurate risk stratification and appropriate use of adjuvant therapies.

Keywords: Adjuvant therapy, disease-free survival, endometrial cancer, histological grade, overall survival, prognostic factors

Öz

Amaç: Bu çalışmanın amacı, erken evre endometriyal kanserde (EK) sağkalımın prognostik belirleyicilerini araştırmak olup, histolojik grade, tümör evresi, myometriyal invazyon, lenfovasküler alan invazyonu (LVSI) ve preoperatif CA-125 düzeyine ek olarak adjuvan tedavilerin hastalıksız sağkalım (DFS) ve genel sağkalım (OS) üzerindeki rolünü incelemektir.

Gereç ve Yöntemler: Ocak 2011-Ocak 2023 tarihleri arasında üçüncü basamak bir merkezde Uluslararası Jinekoloji ve Obstetrik Federasyonu 2009 evre I-II EK nedeniyle cerrahi tedavi uygulanan kadınlar retrospektif olarak analiz edildi. Yaş, vücut kitle indeksi, üreme öyküsü, menopoz durumu, ek hastalıklar, tümör boyutu, histolojik alt tip ve grade, miyometriyal invazyon derinliği, LVSI varlığı, serum CA-125 düzeyi ve uygulanan cerrahi

PRECIS: Histological tumor grade is the strongest independent prognostic factor for overall and disease-free survival in early-stage endometrial cancer, while adjuvant therapies confer no significant survival benefit.

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prosedür gibi demografik ve patolojik veriler kaydedildi. Ayrıca eksternal radyoterapi, vajinal brakiterapi ve kemoterapi gibi adjuvan tedaviler de dokümante edildi. Sağkalım sonuçları ile değişkenler arasındaki ilişkiler Kaplan-Meier ve Cox regresyon analizleri ile değerlendirildi.

Bulgular: Analize toplam 241 erken evre EK'li kadın dahil edildi. Medyan yaş 57 idi (34-86). Olguların 181'i (%75,1) evre IA, 47'si (%19,5) evre IB, 13'ü (%5,4) evre II idi. Histolojik grade dağılımı %44,8 grade 1, %39,0 grade 2 ve %16,2 grade 3 olarak bulundu. Medyan 75 aylık takip süresinde nüks %4,6, mortalite %7,9 oranında gerçekleşti. Univariate analizde yüksek CA-125, ileri evre ve yüksek grade'in OS üzerine olumsuz etkisi saptandı. Multivariate analizde histolojik grade hem OS hem de DFS için bağımsız prediktör olarak belirlendi. Adjuvan radyoterapi veya kemoterapinin sağkalım üzerine anlamlı katkısı gösterilmedi.

Sonuç: Erken evre EK'de histolojik grade, OS ve DFS için en güçlü bağımsız prognostik faktör olarak ortaya çıkmıştır. Bu bulgu, özellikle yüksek grade tümörlerin intraoperatif saptandığı olgularda doğru risk sınıflaması ve adjuvan tedavilerin dikkatli uygulanabilmesi için kapsamlı cerrahi evrelemenin önemini vurgulamaktadır.

Anahtar Kelimeler: Adjuvan tedavi, hastalıksız sağkalım, endometriyal kanser, histolojik grade, genel sağkalım, prognostik faktörler

Introduction

Endometrial cancer (EC) represents a growing global health burden and has become the most frequently diagnosed gynecologic malignancy in developed nations. According to GLOBOCAN 2020 data, EC accounts for more than 417,000 new cases annually and is the sixth most common cancer in women[1]. In the United States, approximately 66,200 new cases and 13,030 deaths were estimated for 2023, with mortality rates rising substantially with age and reaching 54.9 per 100,000 among women aged 70 years or older[2]. Although EC generally has a more favourable prognosis than other gynecological cancers such as ovarian or cervical carcinoma, it remains a significant cause of morbidity and mortality, especially in patients harboring high-risk pathological features.

The majority of EC cases are detected at an early stage because abnormal uterine bleeding commonly occurs early and prompts timely clinical evaluation. Early diagnosis contributes substantially to the relatively high survival rates reported for early-stage disease[3]. Nevertheless, not all early-stage patients have uniformly favourable outcomes. Subgroups of women experience disease recurrence or succumb prematurely, reflecting biological heterogeneity and the influence of multiple prognostic variables[4]. This clinical reality highlights the need to refine prognostic assessment and individualize treatment strategies beyond conventional staging.

Several clinicopathological factors have been established as important prognostic indicators in patients with EC. Among these factors, age at diagnosis, tumor histologic subtype, histologic grade, depth of myometrial invasion, lymphovascular space invasion (LVSI), and lymph node metastases are consistently reported to influence survival outcomes[5,6]. While low-grade endometrioid adenocarcinoma limited to the endometrium or inner myometrium is associated with an excellent long-term prognosis, patients with high-grade tumors or evidence of LVSI are at a considerably increased risk of recurrence and cancer-related death[7]. The presence of extrauterine spread, even if microscopic, further portends poor outcomes[8]. Accordingly, international guidelines recommend risk stratification to guide adjuvant therapy decisions, typically by incorporating these pathological features into treatment algorithms[9-11].

Surgical staging remains the cornerstone of EC management[12]. The standard approach involves total hysterectomy with bilateral salpingo-oophorectomy (BSO), with or without pelvic and para-aortic lymphadenectomy and, in selected cases, omentectomy. Intraoperative frozen-section evaluation of tumor grade and depth of myometrial invasion is commonly employed to determine whether more extensive staging procedures are necessary[13]. However, the reproducibility of frozen-section diagnosis varies significantly, and inter-institutional discrepancies in surgical practice persist. Consequently, optimal intraoperative risk stratification continues to be debated.

Adjuvant therapy in early-stage EC is highly individualized. Radiotherapy, administered either as external-beam radiotherapy (EBRT) or as vaginal brachytherapy, effectively reduces local and regional recurrence rates but has not consistently improved overall survival (OS)[14]. Chemotherapy is typically reserved for patients with advanced disease or those with multiple high-risk factors; however, its role in early-stage disease remains controversial, with inconsistent evidence regarding a survival benefit[15]. These considerations emphasize the urgent need for reliable prognosticators to identify which patients genuinely benefit from adjuvant modalities, thereby avoiding overtreatment in low-risk individuals and ensuring adequate therapy for those at high-risk.

Therefore, the present study aimed to analyze a large cohort of early-stage EC patients treated at a tertiary referral center over a 12-year period. By comprehensively evaluating clinicopathological and treatment-related parameters, we sought to identify independent prognostic determinants of OS and disease-free survival (DFS) and to assess the real-world impact of adjuvant therapies. The results of this study are expected to contribute to the refinement of prognostic assessment and therapeutic decision-making in early-stage EC, with the ultimate goal of improving patient outcomes and aligning clinical practice with evidence-based precision oncology.

Materials and Methods

This study was designed as a retrospective cohort analysis and conducted at the Department of Obstetrics and Gynecology, İnönü University Faculty of Medicine, a tertiary academic referral hospital serving a large regional population. The study period spanned twelve years (January 2011-January 2023), allowing inclusion of a substantial number of patients with early-stage EC managed according to contemporary surgical and adjuvant treatment standards. Institutional approval for the study protocol was obtained from the İnönü University Scientific Research and Publication Ethics Committee (decision number: 2022/3087, date: 26.04.2022). All procedures were performed in compliance with the ethical principles outlined in the Declaration of Helsinki. Because of the retrospective design, the requirement for individual informed consent was waived.

Patient eligibility was carefully defined to ensure the homogeneity of the study cohort. Only women with histopathologically confirmed endometrial adenocarcinoma diagnosed at International Federation of Gynecology and Obstetrics (FIGO) 2009 stage I or II were included. These patients had undergone primary surgical staging at our institution and had complete clinical, pathological, and follow-up records available. Patients with advanced disease (stage III–IV), non-endometrioid histologies such as serous carcinoma, clear cell carcinoma, or carcinosarcoma, as well as those with a history of previous or synchronous malignancies were excluded to eliminate confounding survival influences. After applying these criteria, 241 patients were deemed eligible for inclusion from an initial pool of 385 women diagnosed with EC during the study period.

Data extraction was performed through a systematic review of hospital medical records, operative notes, and pathology reports. A comprehensive dataset was assembled, covering demographic characteristics [including age at diagnosis, body mass index (BMI), reproductive history, and menopausal status], personal and family medical history (with particular attention to systemic comorbidities such as hypertension, diabetes, and cardiovascular disease), and lifestyle factors such as smoking. Tumor-related characteristics were meticulously recorded, including histological subtype, histological grade, tumor size, depth of myometrial invasion, LVSI, and preoperative serum CA-125 levels. Detailed surgical data were documented for each patient, including whether the surgical procedure was limited to total abdominal hysterectomy with BSO (TAH+BSO) or whether it was extended to include systematic pelvic and para-aortic lymphadenectomy and infracolic omentectomy. Postoperative treatment records were also reviewed to determine the use of adjuvant therapies, including EBRT, high-dose-rate vaginal brachytherapy, and systemic chemotherapy.

Surgical procedures were performed by a dedicated gynecologic oncology team. The extent of surgery was tailored according to intraoperative frozen-section assessment, which provided immediate evaluation of the histological grade and depth of myometrial invasion. Patients with superficial myometrial invasion (<50%) and low-grade tumors (grade 1-2) were considered low-risk and typically underwent TAH+BSO alone. In contrast, patients with deep myometrial invasion (≥50%) or high-grade tumors (grade 3) were classified as high-risk and underwent comprehensive surgical staging, including pelvic and para-aortic lymphadenectomy, and infracolic omentectomy. Lymph node dissections were systematically performed, with the pelvic dissection encompassing common, external, and internal iliac, obturator, sacral, and parametrial nodal groups, while para-aortic dissection extended cranially from the aortic bifurcation to the level of the renal veins.

Pathological assessment was performed by specialized gynecologic pathologists. The maximum tumor dimension was measured macroscopically, while the percentage of myometrial invasion was calculated as the depth of tumor infiltration divided by the total myometrial thickness. LVSI was defined as the presence of unequivocal tumor emboli within endothelial-lined lymphatic or vascular spaces. Histological classification was carried out according to the World Health Organization criteria, and staging was confirmed using the FIGO 2009 classification system[16]. Final reporting was based on permanent sections, although intraoperative frozen-section data were also recorded to compare operative decision-making with definitive pathological findings.

Adjuvant treatment decisions were made within a multidisciplinary tumor board composed of a gynecologic oncology team, radiation oncologists, medical oncologists, and pathologists. EBRT was delivered with either a linear accelerator or cobalt-60 equipment. The standard treatment fields extended from the L5-S1 interspace superiorly to the obturator foramen inferiorly, including the whole pelvis and bilateral regional lymphatic drainage. The typical total EBRT doses ranged from 45 to 50 Gy and were delivered in fractions over five weeks. Vaginal brachytherapy was administered via a high-dose-rate Nucletron system with a vaginal cylinder applicator, with the prescribed dose delivered at a depth of 5 mm from the cylinder surface. The proximal half of the vagina was routinely treated to prevent local relapse, with a cumulative dose of approximately 18-24 Gy delivered in multiple fractions. Chemotherapy was not routinely offered to all patients with early-stage disease but was reserved for those deemed high-risk by the tumor board. The most commonly used regimen consisted of paclitaxel (175 mg/m^2) and carboplatin (area under the curve 5-6), which was administered every three weeks for three to six cycles. In selected cases, cisplatin- and doxorubicin-based regimens were employed.

Follow-up data were meticulously collected from outpatient clinic records and hospital databases. The primary outcomes of interest were OS and DFS. OS was defined as the time interval between the date of pathological diagnosis and the date of death from any cause or the date of last follow-up.

DFS was defined as the time interval between the date of surgery and the first documentation of local, regional, or distant recurrence, or death, whichever occurred first. Recurrence was confirmed either histologically or radiologically and classified as vaginal, pelvic, abdominal, lymphatic, or distant. Patients were followed at three-month intervals for the first two years after treatment, at six-month intervals for the next three years, and annually thereafter.

Statistical Analysis

Data were summarized as mean ± standard deviation, median (minimum-maximum), and frequency (percentage). The Kolmogorov-Smirnov test was used to assess normality. Depending on the distribution and data structure, independent-samples t-test, Pearson's chi-square test, and Fisher's exact test were applied. DFS was defined as the absence of metastasis or death from any cause. DFS and OS were estimated from the date of surgery. Factors including CA-125 level, myometrial invasion, stage, LVSI, pathological grade, chemotherapy, external radiotherapy, and brachytherapy were evaluated for their effects on DFS, OS, and recurrence. The impact of each factor on DFS, OS, and recurrence was assessed by univariate analysis. A p-value <0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics version 25.0 and the R programming language with appropriate packages.

Results

During the twelve-year study period, a total of 385 women were diagnosed with EC at our institution. After applying the predefined eligibility criteria, 241 patients with FIGO 2009 stage I-II disease and complete clinicopathological data were included in the final analysis. The median follow-up duration was 75 months (range: 2-141 months), allowing robust assessment of both short- and long-term outcomes. At the time of data censoring, 222 patients (92.12%) were alive, 19 (7.88%) had died, and 11 (4.56%) had developed disease recurrence.

The median age of the study population was 57 years (range: 34-86 years), with the majority of patients postmenopausal at the time of diagnosis (70.95%). Comorbid conditions were common; 22.8% of patients had hypertension, 29.5% had diabetes mellitus, and smaller proportions had thyroid disease, cardiovascular disorders, asthma, or hepatic disease. The median BMI was 31.8 kg/m², which is consistent with the high prevalence of obesity reported in this patient population. Pathological evaluation revealed that 181 women (75.10%) had stage IA disease, 47 (19.50%) had stage IB disease, and 13 (5.40%) had stage II disease. With respect to tumor grade, 108 patients (44.82%) had grade 1 tumors, 94 patients (39.00%) had grade 2 tumors, and 39 patients (16.18%) had grade 3 tumors. Myometrial invasion greater than 50% was identified in 60 women (24.90%), whereas LVSI was detected in 41 women (17.01%). The median tumor diameter was 3 cm (range: 0.1-10 cm). Preoperative CA-125 levels were available for all patients, with a median of 12.8 U/mL (range: 2.7-273 U/mL). Peritoneal cytology was obtained in 154 patients and was positive in only two cases (0.83%). Because of the very low number of positive samples, it was not included in survival analyses.

Regarding primary surgical management, all patients underwent TAH+BSO. Of 114 women (47.3%), surgery was limited to hysterectomy and oophorectomy; whereas 74 women (30.7%) also underwent pelvic and para-aortic lymphadenectomy, and 53 women (22.0%) underwent omentectomy in addition to hysterectomy and lymphadenectomy. Intraoperative frozen-section analysis plays a key role in guiding the extent of staging, with patients classified as high-risk (deep myometrial invasion or high-grade histology) more frequently undergoing extended procedures.

Adjuvant therapies were administered according to postoperative risk stratification and multidisciplinary tumor board recommendations. Eighty women (33.2%) received adjuvant radiotherapy; of these, 40 underwent external-beam pelvic radiotherapy (mean dose, 4,860 cGy) and 40 received vaginal brachytherapy (mean dose, 1,800 cGy). Chemotherapy was given to 19 patients (7.88%), with 17 treated with a paclitaxel-carboplatin regimen and 2 treated with cisplatin-doxorubicin. The median number of chemotherapy cycles was 4 (range: 2-6). Importantly, receipt of adjuvant therapy did not significantly correlate with survival outcomes in univariate and multivariate analyses. The baseline demographic and clinical characteristics of the cohort are summarized in Table 1.

When patients were stratified according to survival status, several significant differences emerged between patients who died and those who survived. Women who died had significantly higher gravidity and parity, higher preoperative CA-125 levels, and higher histological grade. Specifically, patients who died had a mean CA-125 level of 47.03 U/mL, whereas survivors had a mean level of 21.95 U/mL (p<0.001). In addition, 26.3% of the deceased patients had grade 3 tumors, compared with 15.3% of survivors (p=0.029). Body weight was also significantly higher in patients who died (92.4 kg vs. 84.3 kg; p=0.007). No significant associations were observed between survival and menopausal status, family history of cancer, smoking status, comorbid conditions, tumor size, or extent of surgery. Recurrence occurred in 11 of 241 patients (4.6%).

Compared with non-recurrent cases, those with recurrence more often had grade II-III histology (grade II: 54.5% vs. 38.3%; grade III: 27.3% vs. 15.7%) and deeper myometrial invasion (≥50%: 54.5% vs. 23.5%; p=0.062). The median tumor diameter was larger in the recurrence group [4.2 cm (1-7.5) vs. 3.0 cm (0.1-10); p=0.06], while the preoperative CA-125 levels were similar (median 10.7 U/mL vs. 12.9 U/mL; p=1.00).

Table 1. Demographic and clinical data of the study cohort

Variable		Early stage endometrial cancer (n=241)
Age (year)*		57 (34-86)
Gravidity**		4.12±2.76
Parity*		3 (0-12)
History of dilatation curettage*		0 (0-3)
Abortion*		0 (0-8)
Weight (kg)**		84.9±13.58
Height (cm)**		163.8±6.99
BMI (kg/m^2)**		31.85±6.07
Menopause status***	Premenopause	70 (29.05)
	Postmenopause	171 (70.95)
Family history of cancer***	Absent	166 (68.87)
	Present	75 (31.13)
Smoking***	Non-smoker	227 (94.21)
	Smoker	10 (4.14)
	Quit smoking	4 (1.65)
Medical disease***	No	90 (37.34)
	Hypertension	55 (22.82)
	Diabetes mellitus	71 (29.46)
	Thyroid disease	9 (3.73)
	Cardiac disease	5 (2.07)
	Asthma	8 (3.32)
	Hepatic disease	3 (1.24)
CA-125 (U/mL)*		12.8 (2.7-273)
Tumor diameter (cm)*		3 (0.1-10)
Stage***	1a	181 (75.10)
	1b	47 (19.50)
	2	13 (5.40)
Grade***	I	108 (44.82)
	II	94 (39.00)
	III	39 (16.18)
Myometrial invasion***	<1/2	181 (75.1)
	>1/2	60 (24.9)
LVSI***	Absent	200 (82.99)
	Present	41 (17.01)
Peritoneal cytology***	Negative	152 (63.07)
	Positive	2 (0.83)
	Not available	87 (36.10)

Table 1. Continued

Variable		Early stage endometrial cancer (n=241)
Surgical procedure***	TAH+BSO	114 (47.30)
	TAH+BSO+PPLND	74 (30.71)
	TAH+BSO+PPLND+ omentectomy	53 (21.99)
Adjuvant chemotherapy***		19 (7.88)
Adjuvant chemotherapy regimen***		
	Paclitaxel+carboplatin	17 (7.05)
	Cisplatin+doxorubicin	2 (0.83)
Number of chemotherapy courses**		4.55±1.96
Adjuvant radiotherapy*		80 (33.20)
Adjuvant radiotherapy type***	External radiotherapy	40 (16.60)
	Brachytherapy	40 (16.60)
Total external pelvic radiotherapy dose (cGy)*		4860 (3430-14020)
Total brachytherapy dose (cGy)*		1800 (1200-3600)
External pelvic radiotherapy duration (days)*		25 (19-77)
Brachytherapy duration (day)*		3 (2-6)
Overall survival (months)**		139.609±2.464
Disease-free survival (months)**		144.093±2.025
Follow-up period (month)*		75 (2-141)
Recurrence***	No recurrence	230 (95.44)
	Recurrence is present	11 (4.56)
Location of recurrence***	Absent	230 (95.44)
	Abdomen	4 (1.66)
	Pelvic	6 (2.07)
	Lymph node	1 (0.41)
Mortality***	Absent	222 (92.12)
	Present	19 (7.88)

BMI: Body mass index, LVSI: Lymphovascular space invasion, TAH: Total abdominal hysterectomy, BSO: Bilateral salpingo-oophorectomy, PPLND: Pelvic para-aortic lymph node dissection, *: Median (minimum-maximum), **: Mean ± standard deviation, ***: n (%)

Adjuvant therapy was more frequent among recurrent cases: chemotherapy (27.3% vs. 7.0%; p=0.046), particularly paclitaxel-carboplatin (27.3% vs. 6.1%; p=0.027), and there was a trend toward higher use of EBRT (36.4% vs. 15.7%; p=0.090). Group-wise comparisons by OS status are shown in Table 2, and recurrence-related comparisons are summarized in Table 3.

Table 2. Comparison of demographic and clinical data according to survival in patients with early stage endometrial cancer

		Mortality				p-value
		Survivor		Non-survivor		
		Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
Age (year)		57.2±10.09	57 (34-86)	61.42±12.03	62 (39-82)	0.092
Gravidity		3.97±2.66	4 (0-14)	5.79±3.39	6 (0-12)	0.011
Parity		3.08±2.08	3 (0-11)	4.53±3.22	4 (0-12)	**0.047**
Dilation curettage		0.36±0.75	0 (0-3)	0.53±0.7	0 (0-2)	0.109
Abortion		0.48±1.05	0 (0-8)	0.74±1.05	0 (0-4)	0.062
Weight (kg)		84.26±13.56	83 (54-150)	92.37±11.84	88 (73-113)	**0.007**
Height (cm)		163.58±6.98	164.5 (145-179)	166.37±6.78	167 (149-174)	0.076
BMI (kg/m^2)		31.69±6.05	31.21 (19.36-57.16)	33.67±6.16	31.05 (26.06-46.84)	0.156
CA-125 (U/mL)		21.95±29.79	12.45 (2.7-230)	47.03±59.01	30.7 (7.94-273)	**<0.001**
Tumor diameter (cm)		3.36±1.92	3 (0.1-10)	4.24±2.23	4.2 (0.7-9.5)	0.064
		Count	**Percent**	**Count**	**Percent**	
Menopause status	Premenopause	67	95.7%	3	4.3%	0.288
	Postmenopause	155	90.6%	16	9.4%	
Family history of cancer	Absent	152	91.6%	14	8.4%	0.853
	Present	69	92.0%	6	8.0%	
Medical disease	No	84	93.3%	6	6.7%	0.669
	Hypertension	51	92.7%	4	7.3%	
	Diabetes mellitus	63	88.7%	8	11.3%	
	Thyroid disease	9	100.0%	0	0.0%	
	Cardiac disease	4	80.0%	1	20.0%	
	Asthma	8	100.0%	0	0.0%	
	Hepatic disease	3	100.0%	0	0.0%	
Smoking	Non-smoker	210	92.51%	17	7.49%	0.583
	Smoker	10	100.0%	0	0.0%	
	Quit smoking	4	100.0%	0	0.0%	
Surgical procedure	TAH+BSO	109	95.6%	5	4.4%	0.076
	TAH+BSO+PPLND	64	86.5%	10	13.5%	
	TAH+BSO+PPLND+ omentectomy	49	92.5%	4	7.5%	
Grade	I	105	47.30%	3	15.8%	**0.029**
	II	83	37.40%	11	57.90%	
	III	34	15.30%	5	26.3%	
Myometrial invasion	<1/2	170	93.92%	11	6.08%	0.172
	>1/2	52	86.67%	8	13.39%	
LVI	Absent	184	92.0%	16	8.0%	1.000
	Present	38	92.7%	3	7.3%	

Table 2. Continued

		Mortality				p-value
		Survivor		Non-survivor		
		Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
Stage	1a	170	93.9%	11	6.1%	0.136
	1b	40	85.1%	7	14.9%	
	2	12	92.3%	1	7.7%	
Chemotherapy	None	205	92.3%	17	7.7%	0.651
	Received	17	89.5%	2	10.5%	
Adjuvant chemotherapy regimen	None	205	92.3%	17	7.7%	0.764
	Paclitaxel+carboplatin	15	88.2%	2	11.8%	
	Cisplatin+doxorubicin	2	100.0%	0	0.0%	
Number of chemotherapy courses		4.47±1.37	4 (2-6)	5±4.58	6 (0-9)	0.543
External radiotherapy	None	188	93.5%	13	6.5%	0.100
	Received	34	85.0%	6	15.0%	
Brachytherapy	None	186	92.5%	15	7.5%	0.531
	Received	36	90.0%	4	10.0%	
Total external pelvic radiotherapy dose (cGy)		4853.87±930.6	4600 (3430-9500)	7136.67±3962.31	4950 (4500-14020)	0.226
External pelvic radiotherapy duration (days)		26.61±4.77	25 (19-50)	38.67±21.08	27.5 (25-77)	0.140
Brachytherapy duration (day)		4.03±1.32	3 (2-6)	2.75±0.5	3 (2-3)	0.055
Total brachytherapy dose (cGy)		2416.67±791.92	1800 (1200-3600)	1650±300	1800 (1200-1800)	0.055

BMI: Body mass index, LVI: Lymphovascular invasion, TAH: Total abdominal hysterectomy, BSO: Bilateral salpingo-oophorectomy, PPLND: Pelvic para-aortic lymph node dissection, SD: Standard deviation, min: Minimum, max: Maximum, statistically significant p values are indicated in bold

Univariate Cox regression analyses identified elevated serum CA-125 [hazard ratio (HR): 1.009; 95% confidence interval (CI): 1.003-1.016; p=0.004], higher disease stage (HR: 2.69; 95% CI: 1.009-6.722; p=0.048), and higher histological grade (HR: 4.85; 95% CI: 1.35-17.40; p=0.015) as predictors of poorer OS. Kaplan-Meier survival curves illustrated that patients with grade 3 tumors experienced significantly worse survival compared with those harboring grade 1 or 2 tumors. Multivariate Cox regression analysis confirmed histological grade as the only independent predictor of OS (HR: 5.942, 95% CI: 1.593-22.158; p=0.008). Neither adjuvant chemotherapy nor EBRT nor vaginal brachytherapy demonstrated significant associations with OS after adjustment. The Cox proportional-hazards estimates for OS are reported in Table 4; the corresponding Kaplan-Meier curve is depicted in Figure 1, and Kaplan-Meier curves stratified by stage are shown in Figure 2.

DFS outcomes followed a similar pattern. The overall DFS was excellent, with a mean of 144 months. Eleven women experienced disease recurrence during follow-up, most commonly in the pelvic region (54.5%), followed by the abdominal cavity (36.4%), and in the lymph nodes (9.1%). Comparison of recurrent and non-recurrent cases revealed that a history of prior dilatation and curettage procedures, as well as receipt of adjuvant chemotherapy, was more common among recurrent cases. However, according to the univariate regression analysis, only adjuvant chemotherapy was a significant predictor of shorter DFS (HR: 4.830; 95% CI: 1.487-15.687; p=0.009). In multivariate analysis, histological grade was again retained as an independent prognostic factor for DFS (HR: 0.456; 95% CI: 0.060-3.438; p=0.046), underscoring its consistent impact across survival endpoints. The multivariable results for DFS are presented in Table 5; the Kaplan–Meier curve for DFS is shown in Figure 3 and the stage-stratified analysis is presented in Figure 4.

Table 3. Comparison of demographic and clinical data according to recurrence in patients with early stage endometrial cancer

		Recurrence				p-value
		Non-recurrent		Recurrent		
		Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
Age (year)		57.4±10.4	57 (34-86)	60.36±7.62	61 (47-72)	0.31
Gravidity		4.04±2.74	4 (0-14)	5.73±2.72	6 (2-12)	0.05
Parity		3.13±2.17	3 (0-11)	4.55±2.81	4 (2-12)	0.08
Dilation curettage		0.35±0.72	0 (0-3)	1±1	1 (0-3)	**<0.001**
Abortion		0.51±1.06	0 (0-8)	0.18±0.4	0 (0-1)	0.40
Weight (kg)		84.7±13.63	84.5 (54-150)	89.09±12.35	89 (68-113)	0.21
Height (cm)		163.76±6.91	165 (146-179)	164.55±8.99	167 (145-174)	0.53
BMI (kg/m^2)		31.79±6.09	31.21 (19.36-57.16)	33.13±5.62	31.05 (26.06-43.76)	0.38
CA-125 (U/mL)		23.9±33.89	12.9 (2.7-273)	24.43±26.13	10.7 (2.9-85)	1.00
Tumor diameter (cm)		3.38±1.96	3 (0.1-10)	4.38±1.8	4.2 (1-7.5)	0.06
Menopause status	Premenopause	70	30.4%	0	0.0%	0.067
	Postmenopause	160	69.6%	11	100.0%	
Family history of cancer	Absent	160	69.9%	6	50.0%	
	Present	69	30.1%	6	50.0%	
Medical disease	No	85	37.0%	5	45.5%	0.958
	Hypertension	52	22.6%	3	27.3%	
	Diabetes mellitus	68	29.6%	3	27.3%	
	Thyroid disease	9	3.9%	0	0.0%	
	Cardiac disease	5	2.2%	0	0.0%	
	Asthma	8	3.5%	0	0.0%	
	Hepatic disease	3	1.3%	0	0.0%	
Smoking	Non-smoker	218	96.03%	9	3.97%	0.739
	Smoker	10	100%	0	0.0%	
	Quit smoking	4	100%	0	0.0%	
Surgical procedure	TAH+BSO	109	95.61%	5	4.39%	0.905
	TAH+BSO+PPLND	71	95.95%	3	4.05%	
	TAH+BSO+PPLND+ Omentectomy	50	94.34%	3	5.66%	
Grade	I	106	46.1%	2	18.2%	0.181
	II	88	38.3%	6	54.5%	
	III	36	15.7%	3	27.3%	
Myometrial invasion	<1/2	176	67.0%	5	36.4%	0.062
	>1/2	54	23.5%	6	54.5%	
LVI	Absent	192	83.5%	8	72.7%	0.405
	Present	38	16.5%	3	27.3%	

Table 3. Continued

		Recurrence				p-value
		Non-recurrent		Recurrent		
		Mean ± SD	Median (min-max)	Mean ± SD	Median (min-max)	
Stage	1a	175	76.1%	6	54.5%	0.270
	1b	43	18.7%	4	36.4%	
	2	12	5.2%	1	9.1%	
Chemotherapy	None	214	93.0%	8	72.7%	**0.046**
	Received	16	7.0%	3	27.3%	
Adjuvant chemotherapy regimen	None	214	93.0%	8	72.7%	0.027
	Paclitaxel+carboplatin	14	6.1%	3	27.3%	
	Cisplatin+doxorubicin	2	0.9%	0	0.0%	
Number of chemotherapy courses			4 (0-6)	6.33±2.52	6 (4-9)	0.170
External radiotherapy	None	194	84.3%	7	63.6%	0.090
	Received	36	15.7%	4	36.4%	
Brachytherapy	None	193	83.9%	8	72.7%	0.398
	Received	37	16.1%	3	27.3%	
Total external pelvic radiotherapy dose (cGy)		5148.18±1829.02	4860 (3430-14020)	5850±2700	4500 (4500-9900)	0.610
External pelvic radiotherapy duration (days)		28.24±9.90	25 (19-77)	31.25±12.50	25 (25-50)	0.810
Brachytherapy duration (day)		3.97±1.30	3 (2-6)	3.0±0.0	3 (3-3)	0.250
Total brachytherapy dose (cGy)		2383.78±806.06	1800 (1200-3600)	1800±0	1800 (1800-1800)	0.250

BMI: Body mass index, LVI: Lymphovascular invasion, TAH: Total abdominal hysterectomy, BSO: Bilateral salpingo-oopherectomy, PPLND: Pelvic para-aortic lymph node dissection, SD: Standard deviation, min: Minimum, max: Maximum, statistically significant p values are indicated in bold

Table 4. Cox regression analysis of factors associated with overall survival

	Univariate analysis				Multivariate analysis			
	HR	95% CI		p-value	HR	95% CI		p-value
		Lower limit	Upper limit			Lower limit	Upper limit	
CA-125	1.009	1.003	1.016	0.004	1.008	0.999	1.017	0.070
Myometrial invasion	0.827	0.178	3.83	0.808	0.538	0.110	2.629	0.444
Stage	2.69	1.009	6.722	0.048	0.759	0.118	4.887	0.772
LVI	1.35	0.39	4.65	0.636	0.595	0.134	2.635	0.494
Grade	4.85	1.35	17.4	0.015	5.942	1.593	22.158	**0.008**
Chemotherapy	2.1	0.498	9.46	0.302	1.912	0.305	11.967	0.489
External radiotherapy	2.68	1.02	7.07	0.046	3.543	0.871	14.411	0.077
Brachytherapy	1.28	0.42	3.85	0.663	0.637	0.171	2.373	0.501

CI: Confidence interval, HR: Hazard ratio, LVI: Lymphovascular invasion, statistically significant p-values are indicated in bold

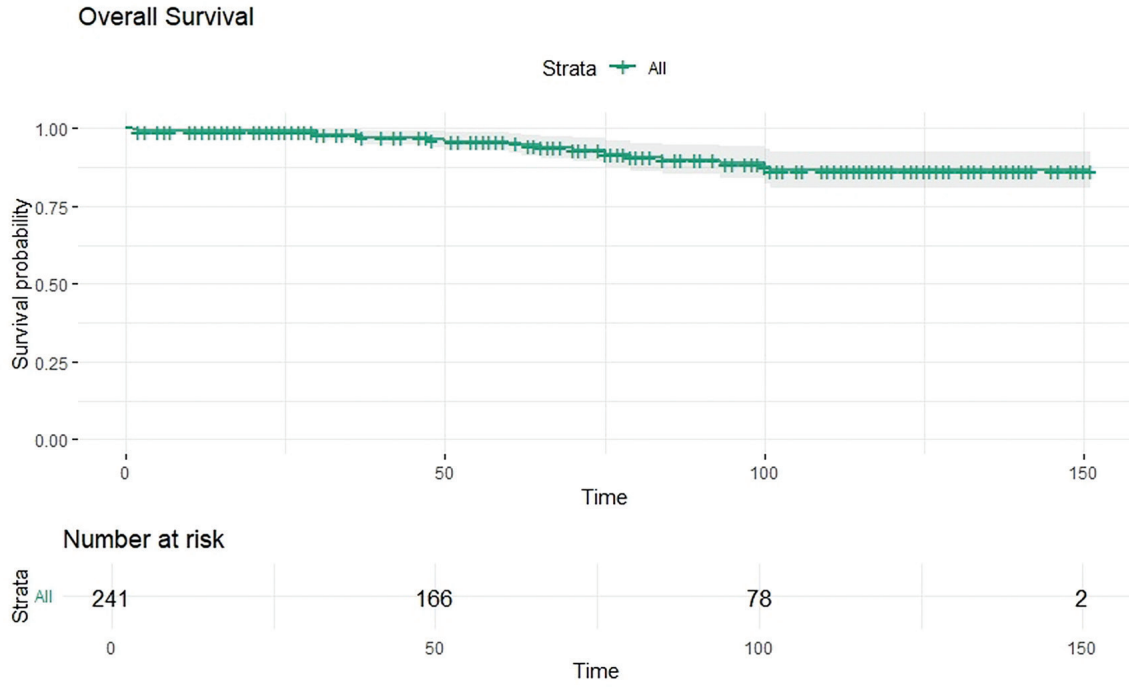

Figure 1. Kaplan-Meier curve for overall survival. The plot illustrates the overall survival probability of the study cohort (n=241) over time. The shaded area represents the 95% confidence interval, and the risk table below shows the number of patients at risk at different time points

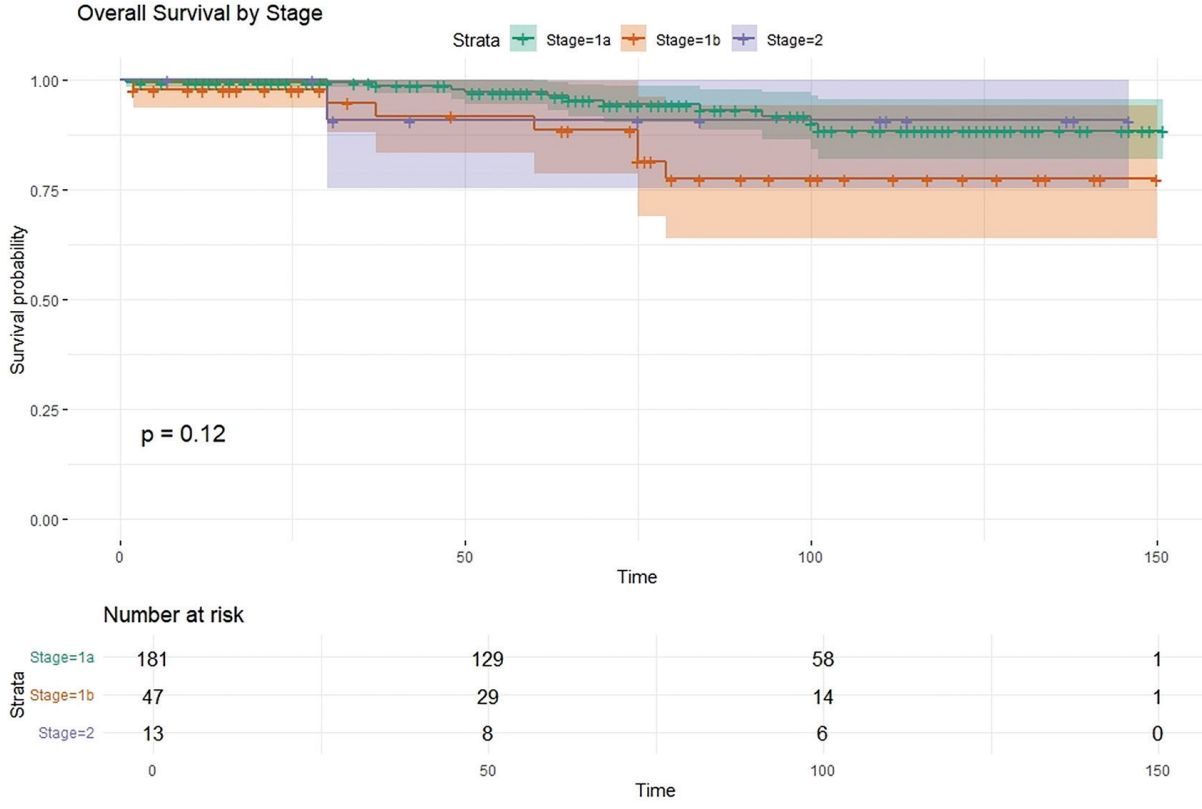

Figure 2. Kaplan-Meier curves for overall survival by endometrial cancer stage. Overall survival probabilities are shown according to disease stage (stage 1a, stage 1b, stage 2). The shaded areas represent 95% confidence intervals. The risk table below indicates the number of patients at risk at different time points. Comparison across groups revealed no statistically significant difference (log-rank test, p=0.12)

Table 5. Cox regression analysis of factors associated with disease-free survival

	Univariate analysis				Multivariate analysis			
	HR	95% CI		p-value	HR	95% CI		p-value
		Lower limit	Upper limit			Lower limit	Upper limit	
CA-125	1.001	0.985	1.018	0.880	0.998	0.979	1.017	0.824
Myometrial invasion	0.618	0.069	5.549	0.668	0.476	0.050	4.541	0.519
Stage	0.421	0.051	3.499	0.423	1.313	0.217	7.936	0.767
LVI	0.422	0.111	1.6	0.205	1.906	0.167	21.710	0.603
Grade	0.227	0.038	1.359	0.104	0.456	0.060	3.438	**0.046**
Chemotherapy	0.16	0.043	0.619	**0.008**	0.245	0.038	1.567	0.137
External radiotherapy	0.3	0.09	1.01	0.052	0.426	0.051	3.548	0.430
Brachytherapy	0.538	0.143	2.031	0.361	0.778	0.160	3.793	0.756

CI: Confidence interval, HR: Hazard ratio, LVI: Lymphovascular invasion, statistically significant p values are indicated in bold

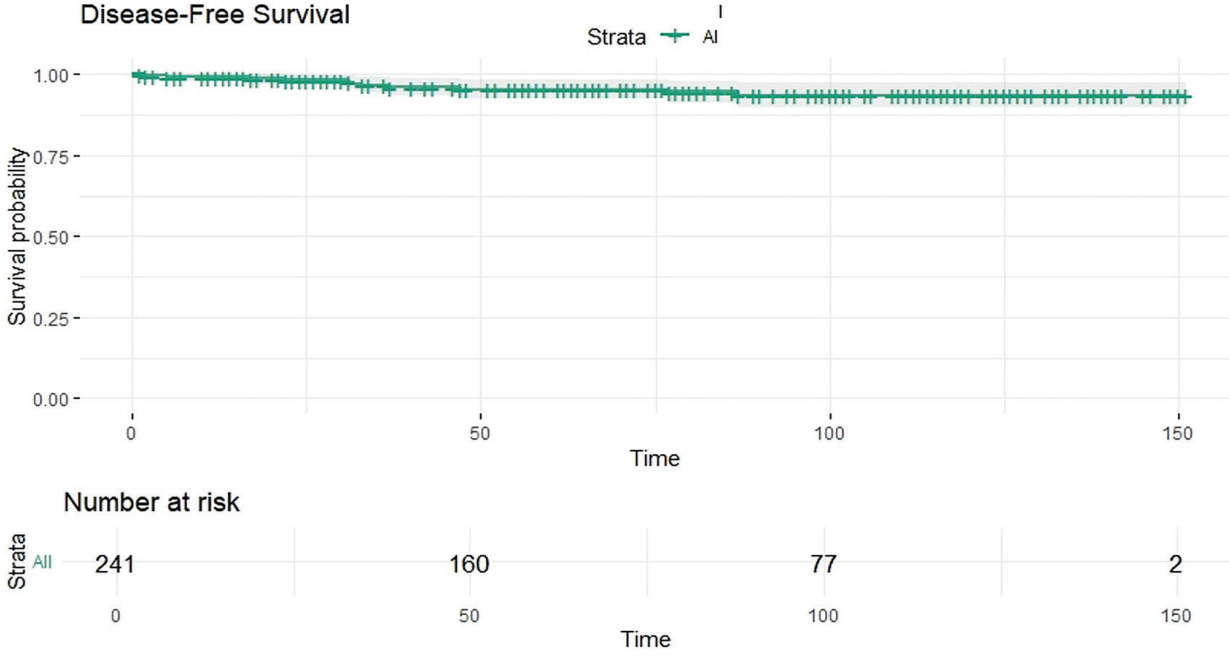

Figure 3. Kaplan-Meier curve for disease-free survival. The plot demonstrates the disease-free survival probability of the study cohort (n=241) over time. The shaded area represents the 95% confidence interval, and the risk table indicates the number of patients at risk at corresponding time points

Discussion

The present retrospective cohort study evaluated the prognostic determinants of survival in 241 women with early-stage EC managed at a tertiary referral center over a twelve-year period. With a median follow-up of more than six years, our data confirm the overall favourable prognosis of patients with FIGO stage I-II disease and highlighted the persistent prognostic importance of tumour grade as the most consistent independent predictor of both OS and DFS. While factors such as elevated serum CA-125 levels, increased body weight, and higher stage were associated with poorer outcomes in univariate analyses, only histological grade remained independently significant in multivariate models. These findings reinforce the notion that histological aggressiveness is the principal determinant of clinical behavior in early-stage EC, and they align with the body of international evidence underscoring grade as a key prognostic marker.

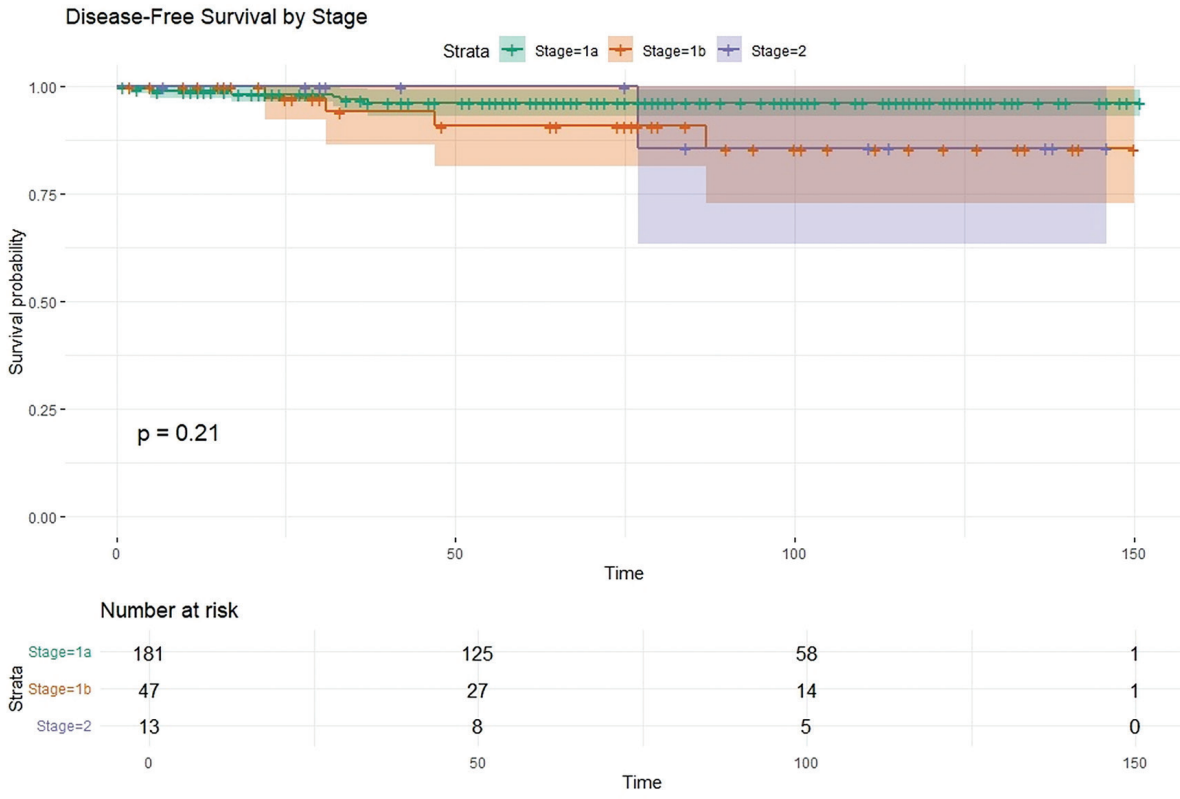

Figure 4. Kaplan-Meier curves of disease-free survival according to endometrial cancer stage. Patients with stage 1a, stage 1b, and stage 2 disease were compared. Shaded areas represent 95% confidence intervals. The number of patients at risk at each time point is shown below the graph. Differences between groups were assessed using the log-rank test and were not statistically significant (p=0.21)

Our results are concordant with those of large multicenter studies and population-based registries. Analyses by the Gynecologic Oncology Group (GOG) and the SEER database have consistently reported five-year survival rates of 85-90% or higher for stage I disease and have demonstrated that grade 3 histology carries a substantially higher risk of recurrence and mortality than grades 1 and 2[17]. Similarly, the PORTEC-1 and PORTEC-2 trials established that high-grade tumors, even when confined to the uterus, are associated with an increased risk of locoregional relapse, thereby justifying the use of adjuvant radiotherapy in selected high-risk patients[18,19]. Our findings support these observations, with grade 3 histology conferring nearly a sixfold higher hazard of death compared with low- or intermediate-grade disease. Importantly, this effect persisted after adjusting for other recognized prognostic variables, underscoring the dominant influence of tumor grade on outcomes.

Beyond histological grade, several additional pathological factors have been implicated in prognosis, including LVSI. LVSI is increasingly recognized as a key adverse prognostic factor in EC and has been incorporated into contemporary risk stratification systems and the updated FIGO 2023 staging framework. Recent large-scale studies and meta-analyses have demonstrated that LVSI is strongly associated with lymph node metastasis, disease recurrence, and cancer-related mortality, particularly in early-stage, high-grade tumors and in advanced-stage disease[20,21]. In the present cohort, although LVSI was identified in 17.0% of patients, it did not emerge as an independent predictor of overall or DFS in multivariate analyses. This finding should be interpreted in the context of the study population, which consisted exclusively of FIGO 2009 stage I-II endometrioid carcinomas with a low overall event rate, as well as the strong collinearity observed between LVSI and established adverse pathological features such as high histological grade and deep myometrial invasion. When these interrelated variables were analyzed simultaneously, histological grade remained the dominant independent determinant of prognosis. Moreover, the absence of molecular classification in this retrospective cohort represents an important limitation, as emerging evidence indicates that the prognostic impact of LVSI varies across molecular subgroups, with greater relevance in p53-abnormal and no specific molecular profile tumors, and limited significance in POLE-mutated cancers[22,23]. Therefore, the lack of independent prognostic significance of LVSI in our analysis should not be interpreted as contradictory to current

staging paradigms but rather as reflective of cohort-specific characteristics and methodological constraints.

In recent years, the prognostic assessment of EC has undergone a paradigm shift from reliance on traditional clinicopathological parameters toward an integrated molecular framework. While histological grade, depth of myometrial invasion, and LVSI have long constituted the cornerstone of risk stratification, contemporary classification systems increasingly incorporate molecular and immunohistochemical markers to capture tumor biology more precisely. However, this transition has occurred gradually in real-world practice, and a substantial proportion of patients treated during the past decade were managed in the absence of systematic molecular profiling. In this context, the present study reflects a transitional, pre-molecular clinical setting and provides robust long-term outcome data based on well-established pathological prognostic factors. Importantly, these conventional parameters remain clinically relevant, as they continue to guide management decisions in settings where molecular testing is unavailable or incomplete, and serve as the foundation upon which modern molecular risk stratification has been built. Future studies integrating LVSI with molecular classification are warranted to refine individualized risk assessment and optimize adjuvant treatment strategies in early-stage EC.

The prognostic significance of the serum CA-125 level has been extensively debated in the literature. Some investigators have proposed that elevated preoperative CA-125 may reflect occult extrauterine disease or aggressive tumor biology, and several studies have correlated higher CA-125 levels with poorer survival[24,25]. In our cohort, patients who died had significantly higher CA-125 levels compared with survivors, and univariate analysis confirmed CA-125 as a predictor of OS. However, its prognostic value was attenuated in multivariate analysis, suggesting that CA-125 may function more as a surrogate marker of high-grade disease or advanced stage rather than as an independent determinant, particularly once grade and stage are accounted for. This interpretation is consistent with the observations of Todo et al.[26], who noted that while CA-125 elevation was associated with worse outcomes, its predictive capacity diminished once grade and stage were included in multivariable models.

Another important observation from our study is the lack of significant survival benefit from adjuvant radiotherapy or chemotherapy in early-stage disease. Approximately one-third of our patients received adjuvant radiotherapy, and less than 10% received chemotherapy; however, neither modality independently influenced OS or DFS. This finding resonates with results from randomized trials such as ASTEC/EN.5 and GOG-99, which demonstrated that adjuvant radiotherapy improves locoregional control but does not confer an OS advantage in early-stage, low-to-intermediate-risk EC[27,28]. Similarly, the addition of chemotherapy in early-stage, high-intermediate-risk patients has yielded inconsistent results, with no clear survival benefit demonstrated in most studies. Our findings therefore support the growing consensus that adjuvant therapy should be individualized and primarily reserved for patients with substantial risk factors such as high-grade histology, deep myometrial invasion, or LVSI.

In contrast to grade, variables such as age, BMI, menopausal status, tumor size, and depth of myometrial invasion did not emerge as independent predictors of survival in our multivariate analysis. While obesity and comorbid metabolic disorders are well-established risk factors for the development of EC, their impact on prognosis after diagnosis remains less certain. Several studies have reported that extreme obesity may be associated with poorer survival, possibly due to technical challenges in surgical staging or increased perioperative morbidity[29]. Our analysis revealed greater body weight among deceased patients, but this association did not remain significant after adjustment, likely reflecting the overwhelming influence of tumor biology rather than host factors in determining outcomes once the disease is established.

The role of the extent of surgery, particularly lymphadenectomy, in early-stage EC has been controversial. While systematic pelvic and para-aortic lymphadenectomy provides valuable staging information, multiple randomized controlled trials, including ASTEC[30] and Benedetti Panici et al.[31], have shown no survival benefit in terms of OS or DFS. In our cohort, nearly half of the patients underwent lymphadenectomy, but the extent of surgery was not associated with survival outcomes. This finding reinforces the position of major guidelines, including the European Society for Medical Oncology and National Comprehensive Cancer Network, which recommend selective rather than routine lymphadenectomy, particularly when intraoperative assessment and preoperative imaging suggest low-risk disease.

The pattern of recurrence in our series is also noteworthy. Despite excellent overall outcomes, 11 women experienced disease relapse, which most frequently occurred in the pelvis and abdomen. This distribution aligns with published data, in which locoregional recurrence predominates in early-stage EC, whereas distant failures are more common in high-grade tumors[32]. Interestingly, in our analysis, adjuvant chemotherapy was associated with higher recurrence rates according to univariate testing. This likely reflects treatment selection bias, as chemotherapy was preferentially administered to patients with high-risk features who inherently carried a greater risk of relapse.

Our study has several strengths. It is based on a relatively large, single-institution cohort with long-term follow-up, comprehensive clinicopathological documentation, and standardized treatment protocols implemented by a dedicated gynecologic oncology team. These features allow

reliable assessment of prognostic variables in a relatively homogeneous patient population. Moreover, the inclusion of multiple survival endpoints (OS and DFS) and rigorous multivariate modelling enhanced the robustness of our conclusions.

Study Limitations

Nevertheless, limitations must be acknowledged. The retrospective design carries inherent risks of selection bias and missing data, although our exclusion of incomplete records helped minimize this issue. The study was conducted in a single tertiary referral center, which may limit its generalizability to broader populations with different demographic and healthcare characteristics. Molecular data, including assessments of recently recognized prognostic classifiers such as POLE mutations, p53 status, and mismatch repair deficiency (as introduced by The Cancer Genome Atlas, TCGA), were not available, which represents an important limitation given their emerging role in FIGO 2023 risk group stratification and their potential to modify the prognostic impact of conventional pathological factors, including LVSI[33]. Furthermore, the lack of systematic availability of immunohistochemical markers, such as p53 and mismatch repair proteins, which are increasingly incorporated into routine diagnostic practice, reflects evolving standards during the long study period and underscores that the findings should be interpreted in the context of pre-molecular, transitional real-world clinical settings. In addition, peritoneal cytology data was not uniformly available throughout the study period, which limited its inclusion in prognostic modeling. Future prospective studies integrating molecular classification with traditional clinicopathological parameters are essential to refine risk-adapted treatment strategies and improve individualized patient care.

Conclusion

In conclusion, our findings confirm that early-stage EC generally has an excellent prognosis, with long-term survival rates exceeding 90%. Among the clinicopathological variables assessed, histological grade emerged as the most consistent and independent determinant of both OS and DFS. Traditional risk factors, such as age, body weight, stage, and extent of surgical staging, were less predictive once grade was accounted for. These results underscore the need for careful pathological evaluation of tumor grade in all patients and suggest that adjuvant treatment decisions should be tailored primarily according to histological aggressiveness rather than stage alone. Future studies incorporating molecular profiling may further refine prognostic assessment and guide personalized therapy in this common gynecologic malignancy.

Ethics

Ethics Committee Approval: Institutional approval for the study protocol was obtained from the İnönü University Scientific Research and Publication Ethics Committee (decision number: 2022/3087, date: 26.04.2022).

Informed Consent: Because of the retrospective design, the requirement for individual informed consent was waived.

Footnotes

Authorship Contributions

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

Conflict of Interest: Ercan Yılmaz MD is Editor-in-Chief in Turkish Journal of Obstetrics and Gynecology. He had no involvement in the peer-review of this article and had no access to information regarding its peer-review. The other authors declare no conflict of interest.

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Adjuvant human papillomavirus vaccination after excisional treatment for cervical precancer

Servikal prekanser için eksizyonel tedavi sonrası adjuvan insan papillomavirüsü aşılaması

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Abstract

Objective: To evaluate whether adjuvant 9-valent human papillomavirus vaccination improves viral clearance and reduces recurrence after excisional treatment for high-grade cervical intraepithelial neoplasia (CIN2+).

Materials and Methods: This retrospective cohort study included women aged 18 years and older who underwent loop electrosurgical excision procedure for histologically confirmed CIN2+ between December 2023 and January 2026. Women with pre-treatment human papillomavirus positivity, negative surgical margins, and available post-treatment follow-up data were included. Patients were stratified according to post-treatment vaccination status. The primary outcome was human papillomavirus clearance at 24 months. Secondary outcomes included clearance at 6 and 12 months, persistent infection, time to viral clearance, and CIN2+ recurrence. Multivariable logistic and Cox regression analyses were performed.

Results: A total of 666 women were included, of whom 212 (31.8%) received adjuvant vaccination and 454 (68.2%) did not. Clearance of human papillomavirus was higher in vaccinated than in unvaccinated women at 6 months (60.8% vs. 46.3%), 12 months (78.3% vs. 58.8%), and 24 months (87.7% vs. 77.8%). Persistent infection at 24 months was lower in the vaccinated group (12.3% vs. 22.2%). CIN2+ recurrence occurred in 3.8% of vaccinated women and 11.9% of unvaccinated women. Adjuvant vaccination was independently associated with faster viral clearance (adjusted hazard ratio: 2.0, 95% confidence interval: 1.5-2.7, p<0.001).

Conclusion: Adjuvant 9-valent human papillomavirus vaccination after excisional treatment was associated with higher viral clearance and lower CIN2+ recurrence.

Keywords: Human papillomavirus vaccines, cervical intraepithelial neoplasia, uterine cervical dysplasia, recurrence, excision

Öz

Amaç: Yüksek dereceli servikal intraepitelyal neoplazi (CIN2+) nedeniyle eksizyonel tedavi uygulanan kadınlarda adjuvan 9-valan insan papillomavirüs aşısının viral klirens ve nüks üzerine etkisini değerlendirmek.

Gereç ve Yöntemler: Bu retrospektif kohort çalışmasına Aralık 2023-Ocak 2026 tarihleri arasında histolojik olarak doğrulanmış CIN2+ nedeniyle loop elektrokoter eksizyon prosedürü uygulanan 18 yaş ve üzeri kadınlar dahil edildi. Tedavi öncesi insan papillomavirüs pozitifliği, negatif cerrahi sınır ve tedavi sonrası takip verisi bulunan hastalar çalışmaya alındı. Hastalar tedavi sonrası aşılama durumuna göre gruplandırıldı. Birincil sonlanım ölçütü 24. ayda insan papillomavirüs klirensiydi. İkincil sonlanım ölçütleri 6. ve 12. ay klirensi, persistan enfeksiyon, viral klirense kadar geçen süre ve CIN2+ nüksü idi. Çok değişkenli lojistik regresyon ve Cox regresyon analizleri yapıldı.

PRECIS: Adjuvant 9-valent human papillomavirus vaccination after excisional treatment for cervical precancer was associated with higher viral clearance and lower recurrence during 24 months of follow-up.

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Bulgular: Toplam 666 kadın değerlendirildi; bunların 212'si (%31,8) adjuvan aşı aldı, 454'ü (%68,2) almadı. İnsan papillomavirüs klirensi aşılanan grupta 6. ayda %60,8'e karşı %46,3, 12. ayda %78,3'e karşı %58,8 ve 24. ayda %87,7'ye karşı %77,8 olarak daha yüksekti. Yirmi dördüncü ayda persistan enfeksiyon aşılanan grupta daha düşüktü (%12,3'e karşı %22,2).CIN2+ nüksü aşılanan kadınların %3,8'inde, aşılanmayanların ise %11,9'unda görüldü. Adjuvan aşılama daha hızlı viral klirens ile bağımsız olarak ilişkiliydi (düzeltilmiş tehlike oranı: 2,0; %95 güven aralığı: 1,5-2,7; p<0,001).

Sonuç: Eksizyonel tedavi sonrası uygulanan adjuvan 9-valan insan papillomavirüs aşısı, daha yüksek viral klirens ve daha düşük CIN2+ nüksü ile ilişkili bulundu.

Anahtar Kelimeler: İnsan papillomavirüs aşıları, servikal intraepitelyal neoplazi, servikal displazi, nüks, eksizyon

Introduction

Cervical cancer remains a significant global health burden, with persistent infection by high-risk human papillomavirus (HPV) recognized as the central etiological factor in its development[1,2]. While most HPV infections are transient, persistent infection—particularly with oncogenic genotypes such as HPV16—can lead to the development of high-grade cervical intraepithelial neoplasia (CIN2/3) and subsequent malignant transformation[3].

Excisional treatment, most commonly performed using loop electrosurgical excision procedure (LEEP), is the standard management for high-grade lesions. Although treatment is highly effective, a considerable proportion of women continue to exhibit persistent HPV infection or develop recurrent disease after treatment. Reported recurrence rates of CIN2+ range from approximately 5% to 15%, even among patients with negative surgical margins[4-6].

Persistent HPV infection has been consistently identified as the strongest predictor of recurrence. Women with ongoing HPV positivity after treatment are at significantly increased risk of developing recurrent high-grade lesions, highlighting the importance of post-treatment viral dynamics in clinical outcomes[5,7].

In Türkiye, HPV vaccination has not yet been incorporated into the national immunization program. Therefore, HPV vaccination is frequently recommended to women presenting in clinical practice, including those undergoing excisional treatment, such as LEEP, and those referred after such treatment. Although prophylactic HPV vaccines are primarily designed to prevent new infections rather than treat existing ones, accumulating evidence suggests that vaccination administered around the time of treatment may reduce the risk of recurrent CIN2+[8,9].However, data regarding its effect on virological outcomes, particularly HPV clearance and persistence, remain limited and inconsistent[9,10].

Given these uncertainties, further real-world data are needed to clarify the impact of adjuvant HPV vaccination on both viral and clinical outcomes following excisional treatment.

In this context, this study aimed to evaluate the effect of post-excisional administration of the 9-valent HPV vaccine on high-risk HPV clearance and on CIN2+ recurrence in women undergoing LEEP for high-grade CIN.

Materials and Methods

Study Design and Setting

This retrospective cohort study was conducted at the Gynecologic Oncology Surgery Clinic at University of Health Sciences Türkiye, İzmir City Hospital between December 2023 and January 2026. The study protocol was approved by the University of Health Sciences Türkiye, İzmir City Hospital Non-Interventional Clinical Research Ethics Committee (approval number: 2025/235, date: 21.05.2025). The study was conducted in accordance with the principles of the Declaration of Helsinki. Due to the retrospective design and the use of anonymized clinical data, the requirement for informed consent was waived.

Study Population

Eligible participants were women aged ≥18 years who underwent LEEP for histologically confirmed CIN2 or CIN3 and had documented HPV positivity prior to treatment.

To minimize the potential influence of residual disease, only patients with negative surgical margins in the excisional specimen were included. Margin negativity was defined as the absence of dysplastic epithelium at both the ectocervical and endocervical resection margins.

Inclusion also required availability of at least one post-treatment HPV test and follow-up data extending to 24 months after excisional treatment.

Patients were excluded if they had invasive cervical carcinoma at baseline, a prior history of cervical excisional treatment, known immunosuppression (including human immunodeficiency virus infection, organ transplantation, or chronic systemic immunosuppressive therapy), pregnancy at the time of treatment, incomplete clinical or virological records, or a history of prophylactic HPV vaccination prior to the index excisional procedure.

Adjuvant HPV Vaccination

Participants were stratified according to HPV vaccination status following excisional treatment.

The vaccinated cohort consisted of women who initiated the 9-valent HPV vaccine (Gardasil 9®) within 90 days of the LEEP procedure and completed the standard three-dose schedule (0, 2, and 6 months). Vaccination status and timing were verified through institutional electronic medical records

and pharmacy administration records to minimize exposure misclassification.

The unvaccinated cohort included women who did not receive HPV vaccination before or after the excisional procedure.

Surgical Procedure and Histopathological Evaluation

All patients underwent LEEP. Surgical specimens were evaluated by experienced gynecologic pathologists. Histopathological examination confirmed the presence of CIN2 or CIN3 and assessed the surgical margin status according to World Health Organization classification criteria.

Follow-up Protocol

Post-treatment surveillance followed the institutional cervical dysplasia follow-up protocol. Follow-up intervals were calculated from the date of the LEEP procedure.

High-risk HPV testing and liquid-based cytology were routinely performed at 6 months and 12 months after treatment, followed by a final HPV test at 24 months.

Patients with abnormal cytology findings and/or persistent HPV positivity during follow-up underwent colposcopic evaluation. Directed cervical biopsies and endocervical curettage were performed when clinically indicated.

Follow-up completeness was assessed for all eligible participants. Patients without available follow-up data for at least one post-treatment HPV test were excluded from the analysis. For patients with partial follow-up, available data were included in time-to-event analyses until the last documented visit.

HPV Testing

High-risk HPV testing was performed using the Cobas 4800 HPV Test (Roche Diagnostics), a clinically validated real-time polymerase chain reaction-based assay that detects oncogenic HPV genotypes, including HPV16 and HPV18, and a pooled group of other high-risk HPV types. Although the assay reports HPV18 separately, no isolated HPV18-positive case was identified in the baseline dataset. Accordingly, HPV18 could not be evaluated as a separate genotype category; cases in which HPV18 was detected together with other high-risk types were included in the multiple high-risk HPV category.

When genotype-specific data were available, type-specific persistence and new genotype acquisition were distinguished. Viral clearance was defined as a negative HPV test following a previously documented positive result without subsequent detection of the same genotype during follow-up.

Persistent infection was defined as the detection of HPV at two or more consecutive follow-up visits.

Baseline demographic and clinical variables included age at treatment, histological grade (CIN2 vs CIN3), and HPV genotype category when available.

These variables were selected based on their potential association with HPV persistence and risk of recurrence, and were included as potential confounders in multivariable analyses.

Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 28.0 (IBM Corp., Armonk, NY, USA). Continuous variables were assessed for normality using the Shapiro-Wilk test and are presented as mean ± standard deviation or median with interquartile range, as appropriate. Categorical variables are presented as frequencies and percentages.

Between-group comparisons were conducted using Student's t-test or the Mann-Whitney U test for continuous variables and the chi-square test or Fisher's exact test for categorical variables.

The association between HPV vaccination and HPV clearance at 24 months was evaluated using logistic regression analysis. Crude and adjusted odds ratios with 95% confidence intervals (CIs) were calculated.

Time to viral clearance and time to CIN2+ recurrence were analyzed using Kaplan-Meier survival curves, and differences between groups were assessed using the log-rank test. Cox proportional hazards regression models were used to estimate adjusted hazard ratios (aHR) while controlling for potential confounders.

The proportional hazards assumption for Cox regression models was assessed using Schoenfeld residuals. No significant violations of the proportional hazards assumption were detected.

Because the study was retrospective, a formal, a priori sample size calculation was not performed. However, a post hoc power assessment was conducted to determine whether the available sample size provided adequate statistical power to detect clinically meaningful differences between groups.

All statistical tests were two-sided, and a p-value <0.05 was considered statistically significant.

Results

Figure 1 shows the flow chart of the study population. A total of 666 women who underwent LEEP for histologically confirmed CIN2 or CIN3 and met the predefined inclusion criteria were included in the final analysis. Among these patients, 212 women(31.8%)received adjuvant 9-valent HPV vaccination following excisional treatment, whereas 454 women (68.2%) did not receive HPV vaccination and served as the comparison group.

Baseline demographic and clinicopathological characteristics of the study population are summarized in Table 1. The mean age of patients in the vaccinated group was 42.9±11.1 years, compared with 43.8±11.7 years in the unvaccinated group, with no statistically significant difference between groups. Similarly, the distribution of final histological diagnoses was comparable. In the vaccinated cohort, 168 patients (79.2%) had CIN2 and 44 patients (20.8%) had CIN3, whereas 352 patients (77.5%) had CIN2 and 102 patients (22.5%) had CIN3 in the unvaccinated group.

Baseline HPV genotype distribution was also comparable between the groups. Among vaccinated women, 17.0% had HPV16 infection, 15.6% had other high-risk HPV types, and 67.5% had multiple HPV infections, whereas the corresponding proportions in the unvaccinated cohort were 38.8%, 28.9%, and 32.4%, respectively. No patient had isolated HPV18 positivity at baseline; therefore, HPV18 was not classified as a separate group. HPV18 co-infections, when present, were included in the multiple high-risk HPV category.

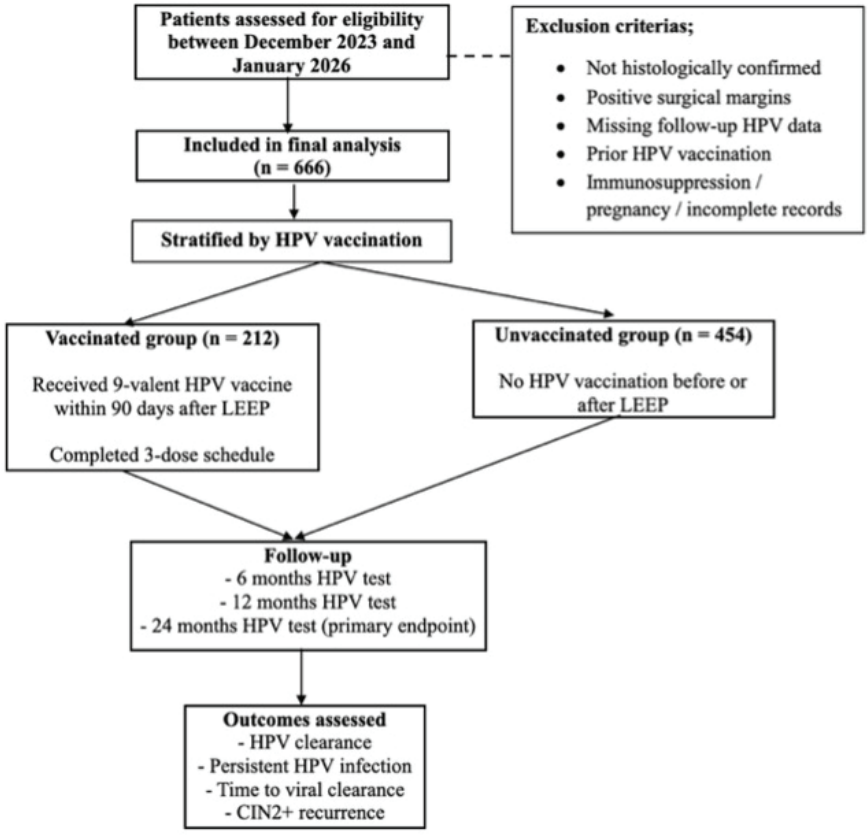

Figure 1. Flow chart of the study population

HPV: Human papillomavirus, LEEP: Loop electrosurgical excision procedure, CIN2+: High-grade cervical intraepithelial neoplasia

Table 1. Baseline demographic and clinical characteristics of women undergoing excisional treatment for high-grade cervical intraepithelial neoplasia according to HPV vaccination status

Characteristic	Vaccinated (n=212)	Unvaccinated (n=454)	p-value
Age (years)			
Mean ± SD	42.9±11.1	43.8±11.7	0.41
Final histology			
CIN2	168 (79.2%)	352 (77.5%)	0.78
CIN3	44 (20.8%)	102 (22.5%)	
Baseline HPV genotype			
HPV16	36 (17.0%)	176 (38.8%)	**0.62**
Other high-risk HPV	33 (15.6%)	131 (28.9%)	
Multiple high-risk HPV	143 (67.5%)	147 (32.4%)	

No isolated HPV18-positive case was identified at baseline. HPV18 co-infections, when present, were included in the multiple high-risk HPV category
HPV: Human papillomavirus, CIN: Cervical intraepithelial neoplasia

Overall, no statistically significant differences were observed between the two groups with respect to baseline demographic or clinicopathological characteristics.

Rates of HPV clearance during follow-up are presented in Table 2. At 6 months after LEEP, HPV clearance was observed in 129 of 212 vaccinated patients (60.8%), compared with 210 of 454 unvaccinated patients (46.3%). Persistent HPV infection at this time point remained in 39.2% of vaccinated women and 53.7% of unvaccinated women.

At the 12 months follow-up, viral clearance increased in both groups but remained higher among vaccinated patients. Specifically, 166 vaccinated patients (78.3%) achieved HPV clearance, whereas 267 unvaccinated patients (58.8%) tested negative for HPV.

At 24 months, which represented the primary endpoint of the study, HPV clearance was documented in 186 vaccinated patients (87.7%), compared with 353 unvaccinated patients (77.8%). Persistent HPV infection at 24 months was therefore observed in 26 vaccinated patients (12.3%) and 101 unvaccinated patients (22.2%).

Persistent HPV infection during follow-up occurred less frequently in the vaccinated group. At the 24 months follow-up, 26 vaccinated patients (12.3%) remained HPV positive compared with 101 patients (22.2%) in the unvaccinated cohort.

In crude comparative analysis, adjuvant HPV vaccination was associated with an approximately 50% reduction in the odds of persistent HPV infection.

During the follow-up period, histologically confirmed CIN2+ recurrence occurred in 62 patients across the entire cohort. Recurrent disease was observed in 8 of 212 vaccinated patients (3.8%), whereas 54 of 454 unvaccinated patients (11.9%) developed CIN2+ recurrence.

Thus, the incidence of recurrent high-grade cervical disease was substantially lower among women who received adjuvant HPV vaccination compared with those who did not.

Figure 2 presents the Kaplan-Meier curves for time to HPV clearance following LEEP according to HPV vaccination status. Kaplan-Meier analysis demonstrated a higher cumulative probability of HPV clearance among vaccinated women compared with unvaccinated patients throughout the 24 months follow-up period. The difference between groups was statistically significant according to the log-rank test (p<0.001).

In multivariable Cox proportional hazards regression analysis adjusting for age, histological grade, and baseline HPV genotype, adjuvant HPV vaccination was independently associated with a significantly increased rate of viral clearance (aHR: 2.0, 95% CI: 1.5-2.7, p<0.001),indicating an approximately twofold higher rate of HPV clearance over time.

Discussion

Principal Findings

In this retrospective cohort study, adjuvant 9-valent HPV vaccination following LEEP for CIN2+ was associated with improved virological and clinical outcomes. Vaccinated women demonstrated higher HPV clearance rates at all follow-up points, reaching 60.8% at 6 months and 87.7% at 24 months, compared with 46.3% and 77.8% in unvaccinated patients, respectively. This was accompanied by a lower rate of persistent infection (12.3% vs. 22.2%) and a reduced

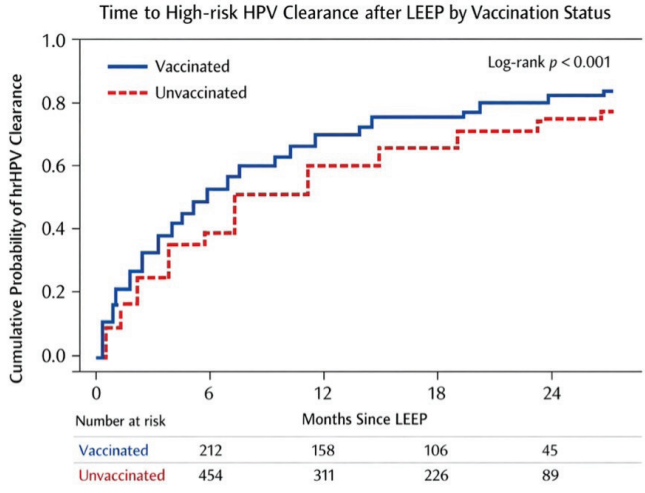

Figure 2. Kaplan-Meier curves for time to hrHPV clearance following LEEP according to HPV vaccination status

hrHPV: High-risk human papillomavirus, LEEP: Loop electrosurgical excision procedure

Table 2. Comparison of HPV clearance and clinical outcomes according to HPV vaccination status

Outcome	Vaccinated (n=212)	Unvaccinated (n=454)	Crude OR (95% CI)	p-value
hrHPV clearance at 6 months	129 (60.8%)	210 (46.3%)	1.81	<0.001
hrHPV clearance at 12 months	166 (78.3%)	267 (58.8%)	2.53	<0.001
hrHPV clearance at 24 months	186 (87.7%)	353 (77.8%)	2.05	0.004
Persistent hrHPV infection at 24 months	26 (12.3%)	101 (22.2%)	0.49	0.004
CIN2+ recurrence	8 (3.8%)	54 (11.9%)	0.29	<0.001

OR: Odds ratio, CI: Confidence interval, CIN2+: High-grade cervical intraepithelial neoplasia, hrHPV: High-risk human papillomavirus

incidence of CIN2+ recurrence (3.8% vs. 11.9%), indicating a clinically relevant benefit in the post-treatment setting.

Biological Plausibility and Interpretation

Persistent HPV infection is a well-established driver of recurrent cervical disease. Previous studies have shown that type-specific persistence, particularly HPV16, significantly increases the risk of CIN2+ recurrence even in margin-negative patients[3,7]. In our cohort, the approximately 10% absolute reduction in persistence among vaccinated women likely contributed to the observed reduction in recurrence. Notably, clearance rates in the unvaccinated group were consistent with expected ranges after excisional treatment (40-55% at 6 months and 65-80% at 24 months) supporting the internal validity of our findings[8,11]. Evidence from observational studies has reported improved viral clearance and reduced persistence following vaccination[12-15].

Comparison with Existing Literature

Our findings are consistent with previous studies that demonstrate a protective effect of HPV vaccination following excisional treatment. A large meta-analysis including over 21,000 women reported a 59% reduction in recurrent CIN2+ (RR 0.41) among vaccinated individuals[16]. Similarly, other meta-analyses and systematic reviews have reported risk reductions ranging between 50% and 80%[14,15]. A large prospective project demonstrated that post-treatment HPV vaccination achieved approximately 80% effectiveness in preventing disease relapse, with sustained benefits observed over long-term follow-up[17]. Similarly, systematic reviews evaluating HPV-positive adult women have reported that vaccination after conization reduces CIN2+ recurrence by up to 87% and improves viral clearance rates to approximately 72.4%, compared with 45.7% in unvaccinated controls[18]. These findings closely parallel the higher clearance rates and reduced persistence observed in our cohort.

In pooled analyses of clinical trials, prior HPV vaccination was associated with a marked reduction in subsequent HPV-related lesions, with decreases exceeding 85-95% for vaccine-type disease endpoints[19]. Likewise, post-hoc analyses of randomized controlled trials have demonstrated approximately 88% efficacy in preventing recurrent CIN2+ after surgical treatment[20].

A recent large retrospective cohort study reported that completion of the 9-valent HPV vaccination schedule was independently associated with significantly higher HPV clearance rates at 12 months, irrespective of excisional treatment status[21]. This supports our observation of consistently higher clearance rates across all follow-up time points (60.8%, 78.3%, and 87.7%), suggesting that vaccination may enhance immune-mediated viral elimination beyond that of surgical excision alone. The natural history of cervical disease after excisional treatment underscores the importance of viral persistence. Longitudinal data indicate that approximately 10-20% of infections persist after treatment, representing a key mechanism underlying recurrence[22]. The reduction in persistent infection observed in our study (12.3% vs. 22.2%) is, therefore, likely a key mechanism underlying the decreased recurrence rates.

Heterogeneity of Evidence

The interpretation of adjuvant HPV vaccination remains challenging because of substantial heterogeneity across study designs and clinical contexts. While our findings demonstrate a clinically meaningful reduction in recurrence (3.8% vs. 11.9%) and a nearly twofold increase in the rate of viral clearance over time (aHR≈2.0), randomized evidence remains limited and less consistent.

Recent evidence syntheses have emphasized this uncertainty. A 2025 Cochrane review concluded that HPV vaccination administered around conisation may reduce CIN2+ risk, but rated much of the evidence as low to very low certainty and noted that randomized evidence was limited to only two trials[23]. A subsequent 2026 systematic review and meta-analysis including 17 studies, four of which were randomized controlled trials, reported an overall reduction in CIN2+ recurrence with adjuvant vaccination, while also underlining between-study heterogeneity and the need for stronger randomized data[24].

Results from randomized or trial-derived data have not been uniformly supportive. In pooled analyses of clinical trials, prior HPV vaccination was associated with a marked reduction in subsequent HPV-related lesions, with decreases exceeding 85-95% for vaccine-type disease endpoints[19]. Likewise, post-hoc analyses of randomized controlled trials demonstrated substantial efficacy in preventing recurrent CIN2+ after surgical treatment[20]. Conversely, a post-hoc analysis of a randomized controlled trial of the HPV16/18 AS04-adjuvanted vaccine did not show a clear effect on viral persistence or lesion recurrence despite modest reductions in new HPV infections[10]. The VIVA randomized placebo-controlled trial in previously treated anal or vulvar high-grade squamous intraepithelial lesion (HSIL) also reported no significant reduction in HSIL recurrence or HPV persistence after nonavalent vaccination[25]. In a related population-based analysis of women with CIN2 managed by active surveillance, vaccination after diagnosis did not reduce progression to CIN3+[26].

In contrast, observational and real-world studies have reported more favorable outcomes, including reductions in recurrence rates approaching 50-60% in pooled analyses[27]. These discrepancies likely reflect differences in baseline risk, margin status, timing of vaccination, HPV genotype distribution, outcome definitions, and residual confounding inherent to non-randomized designs.

Importantly, HPV vaccines are prophylactic rather than therapeutic. Their mechanism is based on the induction of neutralizing antibodies that prevent viral entry[28]. Therefore,

the observed benefits are unlikely to reflect the direct clearance of established infection. Instead, vaccination may reduce reinfection risk[10,29], enhance immune-mediated viral control following excision[30], and provide cross-protection against related HPV genotypes[31].

Clinical Implications

In Türkiye, where HPV vaccination is not included in the national immunization program, the observed reductions in persistence and recurrence support a pragmatic strategy of offering vaccination following excisional treatment. This approach is consistent with European Society of Gynecological Oncology recommendations advocating individualized counseling for HPV vaccination in patients with HPV-related disease[32].

The strengths of this study include a relatively large sample size, a real-world clinical setting, and a standardized follow-up protocol with serial HPV testing at predefined intervals. Importantly, this study offers context-specific evidence from a healthcare setting where HPV vaccination is not part of the national immunization program. In such settings, our findings have direct clinical relevance and support the implementation of opportunistic vaccination strategies following treatment.

Study Limitations

This study has several limitations. Its retrospective design may have introduced selection bias and residual confounding. In addition, vaccination was not randomly assigned, and unmeasured post-LEEP behavioral factors, including smoking status, acquisition of new sexual partners, condom use, and partner HPV status, may have influenced HPV clearance and recurrence. Because these variables were not systematically available in the medical records, their potential effect could not be adjusted for in the multivariable models. The higher proportion of HPV16 infection in the unvaccinated group may also have contributed to the observed between-group differences, given the known association between HPV16 persistence and recurrence risk. Furthermore, baseline differences in HPV genotype distribution may have affected virological outcomes. Accordingly, residual confounding cannot be fully excluded despite multivariable adjustment.

Conclusion

Adjuvant 9-valent HPV vaccination following LEEP was associated with higher HPV clearance rates, reduced persistence of infection, and a significantly lower incidence of CIN2+ recurrence. These findings suggest that vaccination may enhance post-treatment viral control and contribute to a clinically meaningful reduction in recurrence risk. However, given the heterogeneity of existing evidence and the absence of a direct therapeutic effect, these results should be interpreted with caution. In settings where HPV vaccination is not routinely implemented, such as Türkiye, offering vaccination after excisional treatment may represent a pragmatic strategy to improve outcomes. Further prospective, randomized studies are needed to confirm these findings and define optimal patient selection.

Ethics

Ethics Committee Approval: The study protocol was approved by the University of Health Sciences Türkiye, İzmir City Hospital Non-Interventional Clinical Research Ethics Committee (approval number: 2025/235, date: 21.05.2025).
Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: S.Ö., G.Ö.Ş., İ.B.İ., M.S., Concept: S.Ö., Design: S.Ö., Data Collection or Processing: S.Ö., E.G.Ö., G.Ö.Ş., İ.B.İ., Analysis or Interpretation: S.Ö., E.G.Ö., M.S., Literature Search: S.Ö., E.G.Ö., G.Ö.Ş., İ.B.İ., Writing: S.Ö., E.G.Ö., M.S.
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Review / Derleme

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Modulation of oxidative stress and angiogenic pathways by natural compounds in experimental preeclampsia: A narrative review focusing on MDA, sFlt-1, and PlGF

Deneysel preeklampsi modellerinde doğal bileşiklerin oksidatif stres ve anjiyojenik yolları modüle etmesi: MDA, sFlt-1 ve PlGF'ye odaklanan bir anlatımsal derleme

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Abstract

Objective: To synthesize current evidence on the effects of natural compounds on oxidative stress and angiogenic biomarkers, particularly malondialdehyde (MDA), soluble fms-like tyrosine kinase-1 (sFlt-1), and placental growth factor (PlGF), in experimental models of preeclampsia.

Materials and Methods: A narrative review was conducted of studies retrieved from PubMed, Scopus, ScienceDirect, and Web of Science (2016-2026). Experimental animal studies that evaluated natural compounds and reported outcomes related to MDA, sFlt-1, PlGF, or angiogenic pathways were included. Study quality was assessed descriptively using the Joanna Briggs Institute framework.

Results: Nine relevant experimental studies were included in the narrative synthesis. Interventions included *Astragalus* spp., pomegranate juice, young kopyor coconut water, soybean tempeh extract, *Cosmos caudatus* extract, *Nigella sativa*, *Puerariae lobatae* Radix, and olive leaf extract. Most interventions significantly reduced MDA and sFlt-1 levels, indicating reductions in oxidative stress and anti-angiogenic activity. Several compounds increased expression of PlGF and/or vascular endothelial growth factor, suggesting improved placental angiogenesis. Beneficial effects on placental morphology, blood pressure, proteinuria, and fetal outcomes were also reported.

Conclusion: Natural compounds may ameliorate oxidative stress and angiogenic imbalance in experimental models of preeclampsia by reducing MDA and sFlt-1 levels and enhancing PlGF-mediated angiogenesis. These findings support their potential as adjunctive therapies for preeclampsia, although further mechanistic studies and clinical trials are needed to confirm their efficacy and safety. As this narrative review is based exclusively on findings from experimental animal models, these results should be interpreted with caution and not be directly extrapolated to clinical practice until they are confirmed by human studies.

Keywords: Angiogenesis, malondialdehyde, natural compounds, PlGF, preeclampsia, sFlt-1

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Öz

Amaç: Deneysel preeklampsi modellerinde doğal bileşiklerin oksidatif stres ve anjiyojenik biyobelirteçler, özellikle malondialdehit (MDA), çözünür fms benzeri tirozin kinaz-1 (sFlt-1) ve plasental büyüme faktörü (PlGF) üzerindeki etkilerine ilişkin mevcut kanıtları sentezlemektir.

Gereç ve Yöntemler: PubMed, Scopus, ScienceDirect ve Web of Science'tan (2016-2026) elde edilen çalışmaların anlatımsal bir derlemesi yapıldı. Doğal bileşikleri değerlendiren ve MDA, sFlt-1, PlGF veya anjiyojenik yollarla ilgili sonuçları bildiren deneysel hayvan çalışmaları dahil edildi. Çalışma kalitesi, Joanna Briggs Enstitüsü çerçevesi kullanılarak tanımlayıcı olarak değerlendirilmiştir.

Bulgular: Anlatımsal senteze dokuz ilgili deneysel çalışma dahil edilmiştir. Müdahaleler arasında *Astragalus* spp., nar suyu, genç kopyor hindistan cevizi suyu, soya tempeh özü, *Cosmos caudatus* özü, *Nigella sativa*, *Puerariae lobatae* Radix ve zeytin yaprağı özü yer almıştır. Çoğu müdahalenin, MDA ve sFlt-1 seviyelerini önemli ölçüde azaltarak oksidatif stresi ve anti-anjiyojenik aktiviteyi azalttığı gösterilmiştir. Birkaç bileşiğin, PlGF ve/veya vasküler endotelyal büyüme faktörünün ekspresyonunu artırarak plasental anjiyogenezi iyileştirdiği düşünülmüştür. Plasental morfoloji, kan basıncı, proteinüri ve fetal sonuçlar üzerinde de faydalı etkiler bildirilmiştir.

Sonuç: Doğal bileşikler, MDA ve sFlt-1 seviyelerini azaltarak ve PlGF aracılı anjiyogenezi artırarak preeklampsi deneysel modellerinde oksidatif stresi ve anjiyojenik dengesizliği iyileştirebilir. Bu bulgular, preeklampsi için yardımcı tedaviler olarak potansiyellerini desteklemektedir, ancak etkinliklerini ve güvenliklerini doğrulamak için daha fazla mekanistik çalışma ve klinik denemeye ihtiyaç vardır. Bu anlatımsal derleme yalnızca deneysel hayvan modellerinden elde edilen bulgulara dayandığından, bu sonuçlar dikkatli bir şekilde yorumlanmalı ve insan çalışmalarıyla doğrulanana kadar doğrudan klinik uygulamaya aktarılmamalıdır.

Anahtar Kelimeler: Anjiyogenez, malondialdehit, doğal bileşikler, PlGF, preeklampsi, sFlt-1

Introduction

Preeclampsia remains a leading cause of maternal and perinatal morbidity and mortality, affecting approximately 2-8% of pregnancies worldwide and contributing to preterm birth, fetal growth restriction, and long-term cardiovascular complications in both mothers and offspring[1,2]. Because the only definitive treatment remains delivery of the placenta, there is an urgent need for adjunctive strategies that target the underlying pathophysiology while preserving maternal and fetal safety[3]. The pathogenesis of preeclampsia involves abnormal placentation, placental hypoperfusion, oxidative stress, and endothelial dysfunction[4]. Placental ischemia triggers excessive reactive oxygen species (ROS) production and the release of antiangiogenic factors, particularly soluble fms-like tyrosine kinase-1 (sFlt-1), along with alterations in proangiogenic factors such as placental growth factor (PlGF)[5-8]. Elevated sFlt-1 acts as a decoy receptor for vascular endothelial growth factor (VEGF) and PlGF, reducing angiogenic signaling and impairing endothelial integrity, while excessive ROS promotes lipid peroxidation reflected by increased malondialdehyde (MDA), a reliable marker of oxidative cellular damage[9-11].

This angiogenic imbalance—elevated sFlt-1 with reduced PlGF—together with oxidative stress is now regarded as a central, interconnected hallmark of preeclampsia, and the sFlt-1/PlGF ratio has become a valuable biomarker for diagnosis, prognosis, and disease monitoring[12-14]. MDA, sFlt-1, and PlGF were selected as the primary biomarkers for this review because they represent, respectively, the oxidative, antiangiogenic, and proangiogenic arms of this shared pathogenic pathway and are the outcomes most consistently reported across experimental preeclampsia studies, making cross-study comparison feasible. Because oxidative stress and angiogenic imbalance are closely interconnected, natural compounds with antioxidant, anti-inflammatory, and proangiogenic properties—rich in flavonoids, polyphenols, isoflavones, and other phytochemicals—have attracted growing interest as potential adjunctive therapies[15-17]. Recent studies using Nω-nitro-L-arginine methyl ester (L-NAME)-induced preeclamptic rodent models have reported that compounds such as Astragalus spp., pomegranate juice, soybean tempeh extract, olive leaf extract, Cosmos caudatus, Nigella sativa, Puerariae lobatae Radix, and young coconut water can modulate MDA, sFlt-1, and PlGF/VEGF levels and improve placental morphology, blood pressure, and fetal outcomes[5-11,16-19].

Despite this growing body of evidence, previous reviews have generally focused on antioxidant activity or herbal therapy in isolation, without integrating findings on oxidative stress biomarkers, antiangiogenic factors, and proangiogenic factors within a single framework; to date, no narrative review has specifically synthesized evidence on the effects of natural compounds on MDA, sFlt-1, and PlGF together, which represents the specific knowledge gap this review addresses[18,20]. This review therefore aims to synthesize current experimental evidence on how natural compounds influence these three biomarkers and their underlying molecular mechanisms, in order to clarify their therapeutic potential as adjunctive strategies for preeclampsia and to identify priorities for future translational and clinical research.

Materials and Methods

Study Design

This study was conducted as a narrative review to synthesize current evidence regarding the effects of natural compounds on oxidative stress, angiogenic biomarkers, and endothelial dysfunction in experimental preeclampsia models[21]. The review was developed following the principles of the Scale for the Assessment of Narrative Review Articles to

ensure methodological transparency, scientific rigor, and comprehensive reporting[22].

Although a structured and transparent search process, including predefined eligibility criteria and a systematic search strategy, was applied to reduce selection bias, this review does not include meta-analysis, dual independent screening, or a formal risk-of-bias synthesis, and should therefore be understood as a narrative review employing a systematic search component rather than a full systematic review.

Search Strategy

A comprehensive literature search was conducted to identify experimental studies that investigated the effects of natural compounds on oxidative stress, angiogenic biomarkers, and endothelial dysfunction in animal models of preeclampsia. Electronic databases, including PubMed, Scopus, ScienceDirect, and Web of Science, were systematically searched for articles published between January 2016 and March 2026. The search strategy combined medical subject headings (MeSH) and free text terms related to preeclampsia, natural compounds, oxidative stress, angiogenic biomarkers, and animal models. Boolean operators (AND, OR) were applied to maximize search sensitivity and specificity. Manual searches of reference lists from eligible studies were also performed to identify additional relevant articles. The literature search was conducted systematically to improve transparency; however, the review was synthesized narratively rather than following the full methodology of a systematic review. The complete search strategy, including databases, keywords, Boolean operators, and inclusion parameters, is summarized in Table 1.

The complete electronic search strategy applied to each database is provided to ensure transparency. The PubMed search string used was: ("preeclampsia"[MeSH] OR "pre-eclampsia") AND ("natural compounds" OR "phytochemicals" OR "plant extract" OR "antioxidants") AND ("oxidative stress" OR "malondialdehyde" OR "sFlt-1" OR "PlGF" OR "angiogenic") AND ("rat" OR "mice" OR "animal model").Equivalent field-tagged syntax, adapted to each platform's indexing structure, was applied to Scopus (TITLE-ABS-KEY), ScienceDirect, and Web of Science (topic search), using the same three concept blocks (condition AND intervention AND outcome or model) combined with the Boolean operators shown above.

Eligibility Criteria

Studies were included in this review if they fulfilled the following criteria:

1. Original experimental studies involving animal models of preeclampsia;
2. Studies investigating natural compounds, plant-derived bioactive substances, or natural antioxidants;
3. Articles evaluating at least one biomarker associated with oxidative stress or endothelial dysfunction, including sFlt-1, MDA, endothelial nitric oxide synthase (eNOS), VEGF, PlGF, NO, or inflammatory mediators;
4. Studies published in peer-reviewed journals;
5. Articles written in English.

Table 1. Literature search strategy

Component	Description
Study design	Narrative review focusing on the effects of natural compounds as adjuvant therapy for preeclampsia in experimental animal models
Databases used	PubMed, Scopus, ScienceDirect, and Web of Science
Search period	Articles published between 2016 and 2026 were included in the search process
Search approach	Systematic electronic database searching complemented by manual screening of reference lists
Keywords/MeSH terms	"Preeclampsia," "pre-eclampsia," "natural compounds," "natural products," "phytochemicals," "plant extracts," "antioxidants," "oxidative stress," "malondialdehyde," "MDA," "soluble fms-like tyrosine kinase-1," "sFlt-1," "placental growth factor," "PlGF," "angiogenic biomarkers," "endothelial dysfunction," "mice model," "rat model," and "experimental preeclampsia"
Boolean operators	Boolean operators "AND" and "OR" were applied to combine keywords and optimize database searching
Example search syntax	("preeclampsia" AND "natural compounds" AND "oxidative stress") OR ("sFlt-1" AND "MDA" AND "PlGF" AND "experimental preeclampsia")
Additional search method	Manual screening of reference lists from eligible studies was conducted to identify additional relevant articles
Language restriction	Only articles published in English were included
Study type included	Experimental animal studies involving mice or rat models of preeclampsia
Primary outcomes	Biomarker modulation including sFlt-1, MDA, PlGF, VEGF, nitric oxide, and oxidative stress indicators

MeSH: Medical subject headings, sFlt-1: Soluble fms-like tyrosine kinase-1, PlGF: Placental growth factor, MDA: Malondialdehyde, VEGF: Vascular endothelial growth factor

The exclusion criteria included:
1. Clinical studies involving human participants;
2. Review articles, conference abstracts, editorials, and case reports;
3. Studies without biomarker evaluation;
4. Articles with incomplete methodological descriptions or unavailable full text.

Study Selection Process

The study selection process was conducted in several stages. Initially, all retrieved articles were imported into a reference management system to identify and remove duplicate records[23]. Subsequently, titles and abstracts were screened according to the eligibility criteria. A full-text assessment was performed to determine final study inclusion. Studies considered relevant were further evaluated for methodological suitability and biomarker reporting consistency.

Data Extraction

Data extraction was performed using a modified Joanna Briggs Institute (JBI)-based extraction framework developed specifically for experimental preeclampsia studies. The extracted variables included author(s) and publication year; country of study; experimental animal model; method of preeclampsia induction; type of natural compound; dosage and intervention duration; biomarker outcomes (sFlt-1, MDA, PlGF, eNOS, VEGF, NO); laboratory examination methods; mechanistic pathways; and main findings and conclusions. The extraction framework was expanded to emphasize oxidative stress and angiogenic biomarkers in order to strengthen mechanistic interpretation across studies.

Quality Appraisal

Methodological quality assessment was conducted descriptively using the JBI critical appraisal framework for experimental studies. The appraisal considered study randomization, intervention consistency, outcome measurement reliability, biomarker assessment methods, and clarity of statistical analysis[24]. Quality assessment was performed to improve interpretative rigor and minimize potential bias in evidence synthesis.
The detailed results of the JBI appraisal for each included study are summarized in Table 1.1, rather than reported only as a general statement, to allow readers to assess the methodological strength underlying each biomarker finding.

Data Synthesis

Data synthesis was conducted narratively, through thematic grouping based on biomarker mechanisms and therapeutic effects of natural compounds. The findings were categorized into several major themes, including antioxidant activity, angiogenic modulation, endothelial protection, and anti-inflammatory mechanisms. A comparative analysis of studies was performed to identify patterns of biomarker changes associated with various natural compounds.

Ethical Consideration

Since this study was based exclusively on previously published literature and did not involve human participants or experimental animal interventions conducted by the authors, ethical approval was not required.

Table 1.1. Summary of Joanna Briggs Institute (JBI) critical appraisal results (JBI Critical Appraisal Checklist for Randomized Controlled Trials, 13 items)

Author (year)	1	2	3	4	5	6	7	8	9	10	11	12	13	Yes/11	Yes %
Zhu et al.[5]	Y	U	U	NA	U	U	U	NA	Y	Y	Y	Y	Y	6/11	54.5%
Kerdsuknirund et al.[6]	U	U	Y	NA	U	U	U	NA	Y	Y	Y	Y	Y	6/11	54.5%
Yang et al.[8]	Y	U	U	NA	U	U	U	NA	Y	Y	Y	Y	Y	6/11	54.5%
Fitriana et al.[16]	Y	U	Y	NA	U	U	U	NA	Y	Y	Y	Y	Y	7/11	63.6%
Sulistianingsih et al.[11]	U	U	U	NA	U	U	U	NA	Y	Y	Y	Y	Y	5/11	45.5%
Kurniasari et al.[10]	Y	U	Y	NA	U	U	U	NA	Y	Y	Y	Y	Y	7/11	63.6%
Huang et al.[7]	Y	U	Y	NA	U	U	Y	NA	Y	Y	Y	Y	Y	8/11	72.7%
Marpaung et al.[17]	U	U	U	NA	U	U	U	NA	Y	Y	Y	Y	Y	5/11	45.5%
Anggraini et al.[9]	Y	U	U	NA	U	U	U	NA	Y	Y	Y	Y	Y	6/11	54.5%

JBI Checklist items for Randomized Controlled Trials: 1=true randomization used for group assignment; 2=allocation concealment; 3=treatment groups similar at baseline; 4=participants blind to treatment assignment; 5=those delivering treatment blind to assignment; 6=outcome assessors blind to assignment; 7=treatment groups treated identically other than the intervention; 8=follow-up complete/differences adequately described and analyzed; 9=participants analyzed in the groups to which they were randomized; 10=outcomes measured in the same way across groups; 11=outcomes measured in a reliable way; 12=appropriate statistical analysis used; 13=trial design appropriate for the topic, with deviations accounted for in analysis. Y=yes; U=unclear (not explicitly reported in the source article); NA=not applicable to the animal-experimental design of the included studies (items 4 and 8). The "Yes/11" and "Yes %"columns denote the proportion of applicable items (11 of 13; items 4 and 8 excluded) rated "yes" for each study; these values summarize methodological quality for transparency and were not used as an exclusion criterion in this narrative review

Results

Characteristics of Included Studies

A total of 9 experimental studies investigating the effects of natural compounds on preeclampsia models were included in this narrative review. The studies were published between 2016 and 2026 and predominantly used rat and mouse models induced by lipopolysaccharide, reduced uterine perfusion pressure, NO synthase inhibition, and placental hypoxia. Most interventions involved plant-derived bioactive compounds with antioxidant, anti-inflammatory, and endothelial-protective properties.

The majority of the included studies evaluated oxidative stress biomarkers, particularly MDA, eNOS, and sFlt-1. Additional biomarkers included VEGF, PlGF, NO, tumor necrosis factor-alpha (TNF-α), and interleukin-6.

JBI-based Data Extraction of Included Studies

The detailed characteristics, interventions, and biomarker findings of each included study are presented in Table 2.

Across the nine included studies, all natural compounds demonstrated beneficial effects on key biomarkers associated with the pathophysiology of preeclampsia, particularly markers of angiogenic imbalance and oxidative stress. Most studies used L-NAME-induced preeclamptic rat models and evaluated biomarkers, such as sFlt-1, PlGF, MDA, ROS, and inflammatory mediators. Astragalus-based interventions consistently reduced circulating sFlt-1 levels, increased PlGF expression and were accompanied by reductions in oxidative stress markers, including MDA and ROS, as well as improvements in endothelial function and fetal outcomes. Similar findings were reported for pomegranate juice, soybean tempeh extract, Cosmos caudatus extract, Nigella sativa, olive leaf extract, and young kopyor coconut water, all of which significantly attenuated oxidative stress and decreased expression of antiangiogenic factors. Several interventions also improved placental morphology, renal function, blood pressure, and proteinuria. In addition, Puerariae lobatae Radix enhanced VEGF and PlGF expression, suggesting restoration of angiogenic signaling and placental vascular integrity. Overall, the findings indicate that natural compounds may exert protective effects in preeclampsia by reducing oxidative stress, suppressing sFlt-1 overexpression, restoring angiogenic balance, and improving maternal and fetal outcomes.

The included studies consistently demonstrated that natural compounds exert beneficial effects on both oxidative stress and angiogenic dysregulation, two central mechanisms in the pathogenesis of preeclampsia. Most interventions, including Astragalus injection, Astragalus membranaceus, soybean tempeh extract, Cosmos caudatus extract, olive leaf extract, pomegranate juice, and Nigella sativa, significantly reduced circulating sFlt-1 levels, indicating attenuation of anti-angiogenic activity. Several studies also have reported improvements in pro-angiogenic signaling, as evidenced by increased PlGF expression following administration of Astragalus-based therapies, and enhanced PlGF and/or VEGF expression after treatment with Puerariae lobatae Radix. Regarding oxidative stress, reductions in MDA and ROS levels were consistently observed, particularly following treatments with Astragalus injection, Astragalus membranaceus, soybean tempeh extract, Cosmos caudatus extract, and olive leaf extract. Furthermore, Astragalus membranaceus increased the activities of antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), while the young kopyor coconut water reduced placental oxidative injury. Collectively, these findings suggest that natural compounds may ameliorate experimental preeclampsia through dual mechanisms involving suppression of oxidative stress and restoration of angiogenic balance, ultimately contributing to improved placental function and maternal-fetal outcomes. These integrated effects of natural compounds on oxidative stress and angiogenic biomarkers are summarized in Table 3.

Comparative Synthesis Across Studies

When compared across studies, most interventions were tested in L-NAME-induced Wistar or Sprague-Dawley rat models with treatment initiated in mid-to-late gestation (approximately GD 7-20), providing reasonable consistency in the experimental platform used to evaluate biomarker response. However, considerable heterogeneity was observed in dosage (ranging from 200 mg/kg BW to 2000 mg/kg BW), treatment duration, and the specific biomarker panel reported: MDA and sFlt-1 were assessed in the majority of studies, whereas PlGF was assessed in the studies by Zhu et al.[5], Yang et al.[8], and Huang et al.[7], whereas VEGF was assessed only in the study by Huang et al.[7]. Methodological detail also varied, with some studies [e.g., Zhu et al.[5], Sulistianingsih et al.[11]] reporting more comprehensive biomarker panels and statistical detail than others [e.g., Marpaung et al.[17]], which focused narrowly on inflammatory markers. This heterogeneity limits direct pooling of effect sizes across studies, but it does not undermine the qualitative consistency of the overall pattern: interventions that reduced oxidative stress markers also tended to reduce sFlt-1, which supports a shared upstream mechanism that links oxidative and antiangiogenic pathways across diverse natural compounds and experimental models.

Discussion

This narrative review synthesizes evidence from nine experimental studies investigating the effects of natural compounds on oxidative stress and angiogenic biomarkers in preeclampsia models. Rather than restating these findings in detail here, the sections below focus on interpreting their biological plausibility, critically comparing evidence across studies, and outlining the resulting limitations-in line with the Results section presented above.

Oxidative Stress Biomarkers

Oxidative stress is recognized as a central mechanism in the development of preeclampsia[1]. Inadequate trophoblast invasion and impaired spiral artery remodeling result in placental ischemia and hypoxia, leading to excessive production of ROS[25]. This process promotes lipid peroxidation and cellular injury, reflected by increased MDA concentrations[26].

In the present review, most studies reported significant reductions in MDA following administration of *Astragalus* spp., soybean tempeh extract, *Cosmos caudatus* extract, olive leaf extract, and young kopyor coconut water[5,8-11,16]. These findings suggest that natural compounds effectively mitigate oxidative damage in preeclamptic pregnancies.

Table 2. Characteristics and biomarker findings of included experimental studies

Author (year)	Experimental model	Natural compound	Dose and duration	Biomarkers assessed	Main biomarker findings
Zhu et al.[5]	L-NAME-induced preeclamptic Sprague-Dawley rats	Astragalus injection	0.024 mL/g BW/ day during PE induction	sFlt-1, PlGF, MDA, ROS, NO	Decreased sFlt-1, MDA, and ROS; increased PlGF and NO; improved angiogenesis and endothelial function
Kerdsuknirund et al.[6]	L-NAME-induced pregnant rats	Pomegranate juice	Low-, medium-, and high-dose administration (GD 7-20)	sFlt-1, placental histology	Reduced sFlt-1, blood pressure, and proteinuria; improved placental morphology in a dose-dependent manner
Yang et al.[8]	L-NAME-induced preeclamptic rats	*Astragalus membranaceus*	Oral administration during PE induction	sFlt-1, PlGF, MDA, SOD, GSH-Px	Reduced sFlt-1 and MDA; increased PlGF and antioxidant enzyme activity; improved fetal outcomes
Fitriana et al.[16]	Early-onset preeclampsia rat model induced by L-NAME	Young kopyor coconut water	2-3 mL/200 g BW (GD 4-19)	Oxidative stress markers, eNOS	Reduced placental hypoxia and oxidative stress; increased eNOS expression and vascularization
Sulistianingsih et al.[11]	L-NAME-induced Sprague-Dawley rats	Soybean tempeh extract	400 mg/kg BW/day	MDA, sFlt-1, NT-proBNP, Angiotensin II	Significantly reduced MDA and sFlt-1; improved cardiovascular function, placental health, and fetal outcomes
Kurniasari et al.[10]	L-NAME-induced preeclamptic rats	*Cosmos caudatus* (Kenikir) extract	200 mg/kg BW	MDA, IL-6, sFlt-1	Reduced MDA, IL-6, and sFlt-1; improved renal function and reduced glomerulosclerosis
Huang et al.[7]	L-NAME-induced preeclamptic rats	*Puerariae lobatae* Radix	Puerarin-equivalent 72 mg/kg BW	VEGF, PlGF	Increased VEGF and PlGF expression; improved placental barrier integrity and reduced hypertension
Marpaung et al.[17]	Preeclamptic pregnant rat model	*Nigella sativa*	500 and 2000 mg/ kg BW	TNF-α, IL-2, sFlt-1	Significantly reduced inflammatory cytokines and sFlt-1 expression
Anggraini et al.[9]	L-NAME-induced Wistar rat model	OLE	200 mg/kg BW alone or combined with nifedipine (GD 7-20)	MDA, sFlt-1, NGAL, creatinine	Markedly reduced MDA and sFlt-1; improved renal function, proteinuria, and blood pressure

L-NAME: Nω-nitro-L-arginine methyl ester, BW: Body weight, PE: Preeclampsia, GD: Gestational day, sFlt-1: Soluble fms-like tyrosine kinase-1, PlGF: Placental growth factor, MDA: Malondialdehyde, ROS: Reactive oxygen species, NO: Nitric oxide, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, eNOS: Endothelial nitric oxide synthase, NT-proBNP: N-terminal pro-B-type natriuretic peptide, IL: Interleukin, VEGF: Vascular endothelial growth factor, TNF-α: Tumor necrosis factor-alpha, OLE: Olive leaf extract, NGAL: Neutrophil gelatinase-associated lipocalin

Oxidative stress ↓ → MDA ↓

The observed antioxidant effects are consistent with previous experimental and clinical studies demonstrating that phytochemicals rich in flavonoids, polyphenols, and phenolic acids enhance endogenous antioxidant defenses[27]. Polyphenolic compounds can directly scavenge ROS while simultaneously activating antioxidant enzymes such as SOD, catalase, and GSH-Px[28]. Notably, Astragalus membranaceus increased both SOD and GSH-Px activities, supporting the hypothesis that natural compounds exert protective effects through activation of cellular antioxidant systems[8]. These findings align with current theories suggesting that a reduction in oxidative stress may interrupt the progression from placental ischemia to systemic endothelial and vascular injury in preeclampsia.

Angiogenic Biomarkers: sFlt-1

Angiogenic imbalance is considered a hallmark of preeclampsia[29]. Elevated sFlt-1 acts as a circulating anti-angiogenic factor by binding VEGF and PlGF, thereby limiting their biological activity[30]. Excessive sFlt-1 production contributes to placental vascular dysfunction, maternal hypertension, and proteinuria.

sFlt-1 ↓ → VEGF ↑

Most studies included in this review demonstrated significant reductions in sFlt-1 following treatment with Astragalus spp., pomegranate juice, soybean tempeh extract, Cosmos caudatus, olive leaf extract, and Nigella sativa[5,6,8-11,17]. These findings are consistent with previous reports showing that anti-inflammatory and antioxidant interventions may suppress placental sFlt-1 release under hypoxic conditions. Experimental evidence suggests that oxidative stress and inflammatory mediators such as TNF-α stimulate sFlt-1 production; therefore, reduction of oxidative and inflammatory signaling may indirectly restore angiogenic balance. The consistent decrease in sFlt-1 observed across studies supports the potential role of natural compounds in targeting one of the key molecular drivers of preeclampsia.

PlGF Pathway and Placental Angiogenesis

PlGF is a critical regulator of placental angiogenesis and vascular development[31]. Reduced circulating PlGF is frequently observed in women with preeclampsia and is associated with impaired placental perfusion and adverse pregnancy outcomes. In this review, Astragalus spp. and

Table 3. Integrated effects of natural compounds on oxidative stress and angiogenic biomarkers in experimental preeclampsia models

Natural compound	Oxidative stress biomarkers (MDA/ROS)	Anti-angiogenic marker (sFlt-1)	Pro-angiogenic marker (PlGF/VEGF)	Overall biological effect
Astragalus injection	↓ MDA, ↓ ROS	↓ sFlt-1	↑ PlGF	Reduced oxidative stress and restored angiogenic balance
Astragalus membranaceus	↓ MDA, ↑ SOD, ↑ GSH-Px	↓ sFlt-1	↑ PlGF	Enhanced antioxidant capacity and placental angiogenesis
Young kopyor coconut water	Reduced placental oxidative injury	Not assessed	Not assessed	Reduced oxidative damage in placental tissue
Soybean tempeh extract	↓ MDA	↓ sFlt-1	Not assessed	Improved oxidative status and angiogenic profile
Cosmos caudatus extract	↓ MDA, ↓ IL-6	↓ sFlt-1	Not assessed	Antioxidant and anti-inflammatory effects
Olive leaf extract	↓ MDA	↓ sFlt-1	Not assessed	Reduced oxidative stress and anti-angiogenic activity
Pomegranate juice	Beneficial effect on oxidative stress-related parameters	↓ sFlt-1	Not assessed	Improved placental health and angiogenic regulation
Nigella sativa	Reduced inflammatory-oxidative response (↓ TNF-α, ↓ IL-2)	↓ sFlt-1	Not assessed	Modulated inflammation and angiogenic imbalance
Puerariae lobatae Radix	Not directly assessed	Not reported	↑ PlGF, ↑ VEGF	Enhanced placental angiogenesis and vascular development
Overall findings	**Consistent reduction in MDA and oxidative stress-related markers**	**Consistent reduction in sFlt-1 levels**	**Increased PlGF and/or VEGF expression in studies evaluating angiogenic pathways**	**Natural compounds improved oxidative balance and angiogenic regulation in experimental preeclampsia**

MDA: Malondialdehyde, ROS: Reactive oxygen species, sFlt-1: Soluble fms-like tyrosine kinase-1, PlGF: Placental growth factor, VEGF: Vascular endothelial growth factor, SOD: Superoxide dismutase, GSH-Px: Glutathione peroxidase, IL: Interleukin, TNF-α: Tumor necrosis factor-alpha

Puerariae lobatae Radix significantly increased PlGF expression, while Puerariae lobatae Radix also enhanced VEGF levels[5,7]. These findings indicate restoration of pro-angiogenic signaling pathways that are typically suppressed in preeclampsia.

PlGF ↑ → Placental Angiogenesis ↑

The observed increase in PlGF is biologically plausible because several plant-derived bioactive compounds have been shown to improve placental vascular remodeling through modulation of hypoxia-related and angiogenic signaling pathways[32]. Increased PlGF availability may counteract the anti-angiogenic effects of sFlt-1, thereby promoting endothelial cell proliferation, vascular growth, and placental perfusion. Similar findings have been reported in experimental studies demonstrating that restoration of the VEGF-PlGF axis improves placental development and reduces disease severity[29]. Therefore, enhancement of PlGF-mediated angiogenesis represents a promising therapeutic mechanism through which natural compounds may improve maternal and fetal outcomes.

Mechanisms of Natural Compounds in Preeclampsia

The findings of this review, as illustrated in Figure 1, suggest that oxidative stress and angiogenic imbalance are closely interrelated in the pathogenesis of preeclampsia. Placental ischemia stimulates ROS production, which subsequently increases inflammatory signaling and the release of anti-angiogenic factors. Elevated sFlt-1 further suppresses PlGF and/or VEGF activity, resulting in impaired placental vascularization and disease progression. The natural compounds evaluated in this review appear to interrupt this pathogenic cascade at multiple levels by reducing oxidative stress, suppressing sFlt-1 production, and enhancing pro-angiogenic signaling. Such multimodal actions may explain the consistent therapeutic benefits observed across experimental studies.

Critical Synthesis and Translational Considerations

Beyond the overall pattern of biomarker improvement, important differences across studies deserve critical consideration. Compounds evaluated in shorter-duration, later-gestation models (e.g., pomegranate juice, olive leaf extract) predominantly produced reductions in blood pressure and sFlt-1 without directly assessing PlGF or VEGF; by contrast, compounds tested with a broader biomarker panel and earlier intervention (e.g., Astragalus spp., Puerariae lobatae Radix) provided more complete evidence of pro-angiogenic restoration. This suggests that the apparent consistency of benefit across natural compounds may partly

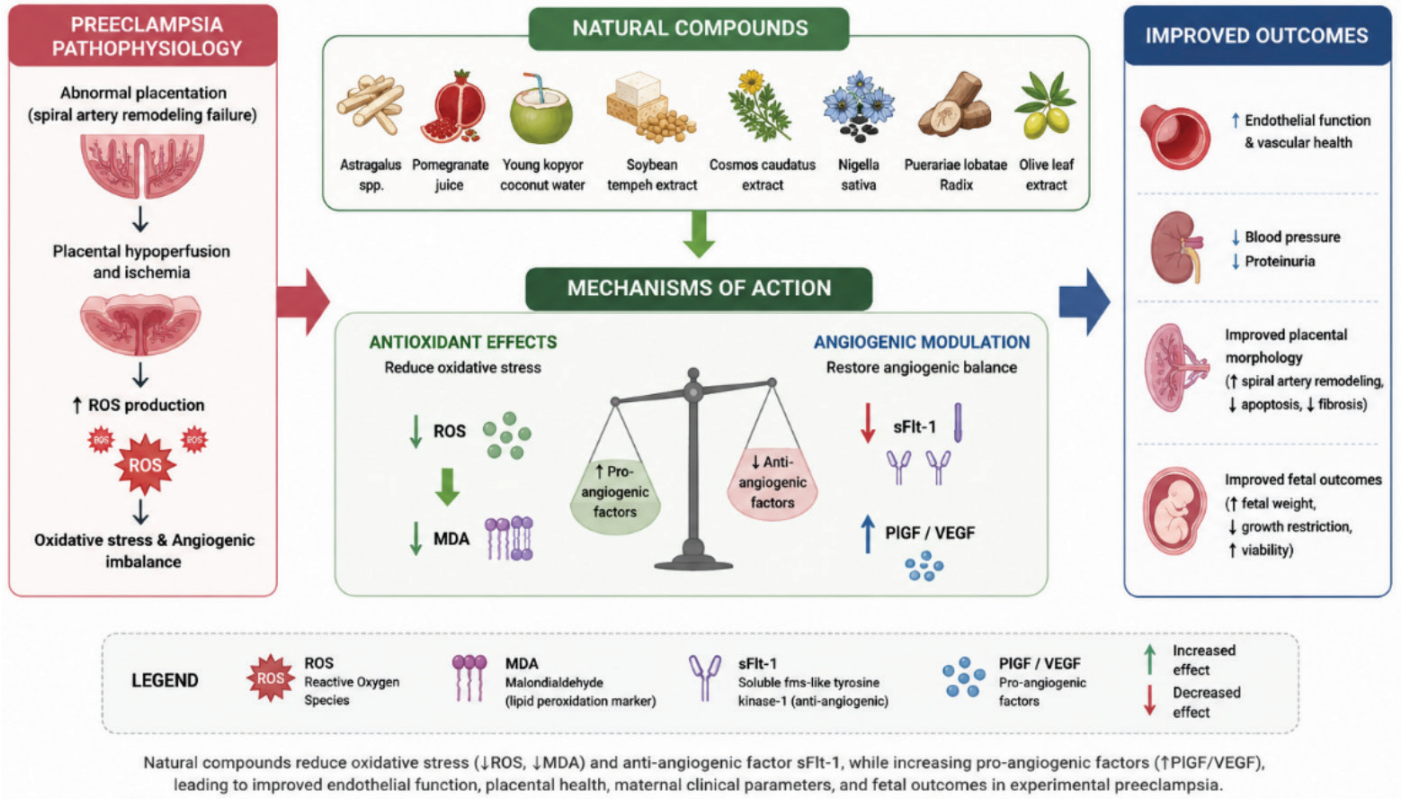

Figure 1. Mechanisms of natural compounds in preeclampsia

MDA: Malondialdehyde, ROS: Reactive oxygen species, sFlt-1: Soluble fms-like tyrosine kinase-1, PlGF: Placental growth factor, VEGF: Vascular endothelial growth factor

reflect differences in the biomarkers measured, rather than uniformly equivalent biological efficacy. Moreover, because L-NAME-induced hypertension models primarily reflect NO deficiency rather than the immunological and trophoblastic abnormalities underlying human preeclampsia, mechanistic conclusions drawn from this model should be extrapolated to human disease with caution.

Future Directions

Future research should focus on standardizing experimental protocols, including animal models, intervention dosages, and biomarker measurements. Additional studies are needed to investigate the effects of natural compounds on PlGF signaling and other angiogenic pathways because evidence in this area remains limited. Furthermore, mechanistic studies exploring molecular targets such as Nrf2/Keap1, HIF-1α, VEGF/PlGF signaling, and inflammatory pathways may provide deeper insights into therapeutic mechanisms. Ultimately, well-designed clinical trials are required to determine whether the promising findings observed in experimental models can be translated into safe and effective adjunctive therapies for women with preeclampsia.

Study Limitations

Several limitations should be considered when interpreting these findings. First, all included studies were conducted in animal models, the majority using L-NAME-induced preeclampsia models, which may not fully replicate the complexity of preeclampsia in humans. Second, substantial heterogeneity existed in intervention types, dosages, treatment durations, and biomarker assessment methods. Third, only a limited number of studies evaluated PlGF directly, whereas MDA and sFlt-1 were assessed more frequently. Finally, differences in experimental design and reporting standards limited direct comparison across studies.

Because only English-language articles were included and no formal assessment of publication bias was performed, language bias and selective reporting of predominantly positive findings cannot be excluded. As with any narrative review, study selection and interpretation may also carry a degree of subjectivity that is not fully mitigated by the structured search strategy employed. Most importantly, none of the included studies evaluated these natural compounds in human pregnancies; the complete absence of clinical-phase data constitutes a major factor limiting the strength and clinical applicability of the conclusions drawn from this review.

Conclusion

This narrative review synthesized evidence from nine experimental studies evaluating the effects of natural compounds on oxidative stress and angiogenic biomarkers in preeclampsia models. The findings consistently demonstrated that natural compounds attenuated oxidative stress by reducing MDA levels and enhancing endogenous antioxidant defenses. In addition, most interventions significantly decreased sFlt-1, a key anti-angiogenic factor implicated in the pathogenesis of preeclampsia. Several compounds, particularly Astragalus spp. and Puerariae lobatae Radix, also increased the expression of PlGF and/or VEGF, indicating restoration of pro-angiogenic signaling pathways and improvement in placental vascular development. Collectively, these findings suggest that natural compounds may target multiple interconnected mechanisms underlying preeclampsia, including oxidative stress, angiogenic imbalance, and placental dysfunction. The simultaneous reduction of MDA and sFlt-1, together with enhancement of PlGF-mediated angiogenesis, highlights their potential as adjuvant therapies for preeclampsia. However, the current evidence is limited to experimental animal studies with considerable methodological heterogeneity. Therefore, further mechanistic investigations and well-designed clinical trials are required to validate the efficacy, safety, optimal dosage, and translational applicability of these natural compounds in human pregnancies complicated by preeclampsia.

Footnotes

Authorship Contributions

Surgical and Medical Practices: N.A.P., N.P., Concept: N.A.P., S.S., D.H.H., Design: N.A.P., N.P., S.S., D.H.H., Data Collection or Processing: N.A.P., Analysis or Interpretation: N.A.P., N.P., S.S., D.H.H., Literature Search: N.A.P., Writing: N.A.P., N.P., S.S., D.H.H.

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Successful placental removal guided by MRI-based mapping in advanced abdominal pregnancy: A case report and literature review

İleri abdominal gebelikte MRG tabanlı haritalama rehberliğinde başarılı plasenta çıkarımı: Bir olgu sunumu ve literatür taraması

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Abstract

Advanced abdominal pregnancy is a rare but life-threatening condition associated with substantial maternal and fetal morbidity and mortality, particularly when diagnosis is delayed beyond the first trimester. Owing to its rarity, there is still no consensus regarding its optimal diagnostic approach or management. We present a case of advanced abdominal pregnancy in a woman who first sought medical care at 24 weeks' gestation. She reported five months of amenorrhea accompanied by progressively worsening abdominal pain, constipation, and dyspareunia. Ultrasonography demonstrated a gestational sac outside the uterine cavity. Magnetic resonance imaging was subsequently performed to define the placental location and its relationship to adjacent structures and to confirm the diagnosis of advanced abdominal pregnancy. Because the patient had persistent severe abdominal pain and clinical findings concerning for an acute abdomen, immediate surgical management was undertaken after counseling and preoperative planning. Laparotomy was performed through a midline abdominal incision. Intraoperatively, the omentum, uterus, adnexa, and bladder were densely adherent to the gestational sac, creating the appearance of a frozen pelvis. The placenta invaded the peritoneum overlying the sigmoid colon and left iliac region, although no major vascular involvement was identified. Organ-sparing surgery was successfully performed, and the placenta was carefully dissected and removed without complications. The postoperative course was uneventful, and the patient was discharged in good condition. Advanced abdominal pregnancy should be considered in the differential diagnosis of pregnant patients presenting with abdominal pain and atypical imaging findings. In symptomatic patients with an acute abdomen, immediate surgery may be preferred over expectant management. Although placental management remains controversial, detailed preoperative imaging is crucial for surgical planning. In selected cases without major vascular invasion, placental removal may be feasible with organ preservation.

Keywords: Advanced abdominal pregnancy, ectopic pregnancy, placenta, magnetic resonance imaging, surgical procedures

Öz

İleri abdominal gebelik, özellikle tanının ilk trimester sonrasına geciktiği durumlarda, anne ve fetüste belirgin morbidite ve mortalite ile ilişkili, nadir ancak yaşamı tehdit eden bir durumdur. Nadir görülmesi nedeniyle, optimal tanısal yaklaşım ve yönetim konusunda halen bir görüş birliği bulunmamaktadır. Bu olgu sunumunda, gebeliğin 24. haftasında sağlık kuruluşuna ilk kez başvuran bir kadında saptanan ileri abdominal gebelik olgusu sunulmaktadır. Hasta, beş aydır amenoreye eşlik eden giderek artan karın ağrısı, konstipasyon ve disparoni yakınmaları ile başvurdu. Ultrasonografide uterin kavite dışında yerleşmiş gestasyonel kese izlendi. Plasentanın yerleşimini ve komşu yapılarla ilişkisini değerlendirmek amacıyla manyetik rezonans görüntüleme yapıldı ve ileri abdominal gebelik tanısı doğrulandı. Hastada persistan şiddetli karın ağrısı ve akut batın ile uyumlu klinik bulgular bulunması nedeniyle danışmanlık verilmesi ve ameliyat öncesi planlamanın ardından acil cerrahi tedaviye karar verildi. Orta hat insizyonu ile laparotomi uygulandı. İntraoperatif olarak omentum, uterus, adneksler ve mesanenin gestasyonel kese ile yoğun şekilde yapışık olduğu ve frozen

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pelvis görünümü oluşturduğu saptandı. Plasentanın sigmoid kolon üzerindeki periton ile sol iliyak bölgeye invaze olduğu ancak majör vasküler tutulumun bulunmadığı görüldü. Organ koruyucu cerrahi başarıyla uygulandı ve plasenta dikkatli diseksiyonla komplikasyonsuz şekilde çıkarıldı. Postoperatif seyir sorunsuzdu ve hasta iyi klinik durumda taburcu edildi. İleri abdominal gebelik, karın ağrısı ve atipik görüntüleme bulguları ile başvuran gebelerde ayırıcı tanıda mutlaka düşünülmelidir. Akut batın bulguları bulunan semptomatik hastalarda bekleyici yaklaşıma kıyasla acil cerrahi tercih edilebilir. Plasenta yönetimi tartışmalı olmaya devam etmekle birlikte cerrahi planlama açısından ayrıntılı preoperatif görüntüleme kritik öneme sahiptir. Majör vasküler invazyonun bulunmadığı seçilmiş olgularda, organ koruyucu yaklaşımla plasentanın çıkarılması mümkün olabilir.

Anahtar Kelimeler: İleri abdominal gebelik, ektopik gebelik, plasenta, manyetik rezonans görüntüleme, cerrahi işlemler

Introduction

Ectopic pregnancy is defined as the placement of the blastocyst outside the endometrium and is seen in approximately 1-2% of cases[1]. Abdominal pregnancy (AP), which constitutes 1% of all ectopic pregnancies or 1/10,000-30,000 of all pregnancies, is the placement of the placenta anywhere in the peritoneal cavity[2-4]. When the gestational age is less than the 20th week of pregnancy, AP has a prognosis and treatment options similar to those for other ectopic pregnancies. However, in cases of advanced AP (AAP), where the gestational age is over 20 weeks, the prognosis is worse than that of other ectopic pregnancies. Two important factors that determine prognosis are gestational age at diagnosis and the location of the placenta. Because AAPs are rare, their treatment is controversial[3]. In particular, there is no consensus on whether the placenta should be removed. In addition, the diagnosis of AAP may be difficult because clinical manifestations are often non-specific and imaging findings may be misinterpreted, especially in late gestation[5,6].

Owing to technological advances and improved diagnostic capabilities, antiphospholipid syndrome is generally diagnosed earlier. Inadequate access to prenatal care and health services may result in a late diagnosis of AAP, negatively affecting maternal prognosis. However, AAP can also be seen in societies where prenatal care is sufficient. This report presents the diagnosis and management of a patient with AAP who had a good socioeconomic status. Published case reports and case series from different settings have shown that delays in diagnosis and variation in placental implantation contribute substantially to maternal and fetal risk[4-6]. This report presents a case of AAP diagnosed at 24 weeks of gestation and managed with magnetic resonance imaging (MRI)-based preoperative placental mapping and organ-sparing surgery. We emphasize that including AP in the differential diagnosis during routine obstetric evaluation may help prevent diagnostic delays and improve surgical planning in advanced cases.

Case Report

The patient, a 39-year-old gravida 0, had oligomenorrheic cycles for more than five years. The patient, who lived in the city center, had a favorable socioeconomic status and did not use contraceptives or receive ovulation induction therapy. She had been experiencing infertility problems for four years. In addition to her delayed menstruation, the patient had experienced progressively worsening abdominal pain, constipation, and dyspareunia for approximately five months. The patient, who had not undergone a gynecological examination in the last year, was in good general condition, conscious, oriented, and cooperative, with an arterial blood pressure of 105/65 mmHg, a heart rate of 86 beats/min, and a hemoglobin level of 12.1 mg/dL. Abdominal examination revealed distension of the suprapubic region. There was abdominal tenderness without rebound or guarding. A pelvic examination could not be performed because of pain. Because of the severity and progression of abdominal pain and the presence of abdominal tenderness, the clinical picture was considered suggestive of acute abdomen.

Ultrasonographic (USG) evaluation revealed no pregnancies in the uterine cavity. The endometrial thickness was 13 mm, and the myometrial parenchyma was homogeneous (Figure 1A-C). The fetus and its appendages, which demonstrated cardiac activity, were located outside the uterus, extending from the Douglas pouch to the left abdominal region. Fetal biometric measurements were compatible with those obtained at 24 weeks of gestation. The placenta extended towards the left pelvic wall. Retroplacental lacunae were also observed. Iliac vascular involvement could not be evaluated using sonography or color Doppler. Pelvic MRI without intravenous contrast administration, performed to delineate the precise placental location, showed an extrauterine pregnancy (Figure 1D-H). The placenta extended to the left pelvic sidewall and sigmoid colon without iliac vascular involvement. MRI was therefore used to delineate placental topography and its relationship to surrounding pelvic structures before surgery.

Written and verbal consent was obtained after all risks were explained to the patient. Although fetal cardiac activity was present, immediate laparotomy was preferred because of persistent severe abdominal pain, clinical concern for acute abdomen, and the possibility of progressive maternal risk with advancing gestation. The decision was made after evaluation of the imaging findings and counseling about the risks of hemorrhage, bowel injury, vascular injury, possible organ loss, and fetal loss. The abdomen was opened through a sub-umbilical median incision (Figure 2). The bladder, omentum, and gestational structures appeared conglomerated and adherent to each other, producing a "frozen pelvis" appearance. The gestational sac was separated from the sigmoid colon, bladder, and omentum by blunt and sharp dissection (Figure 3). Upon inspection, the placenta was adherent to the peritoneum of the pouch of Douglas, the sigmoid colon, and the parietal peritoneum over the left iliac vessels. Invasion of the iliac vessels was not observed.

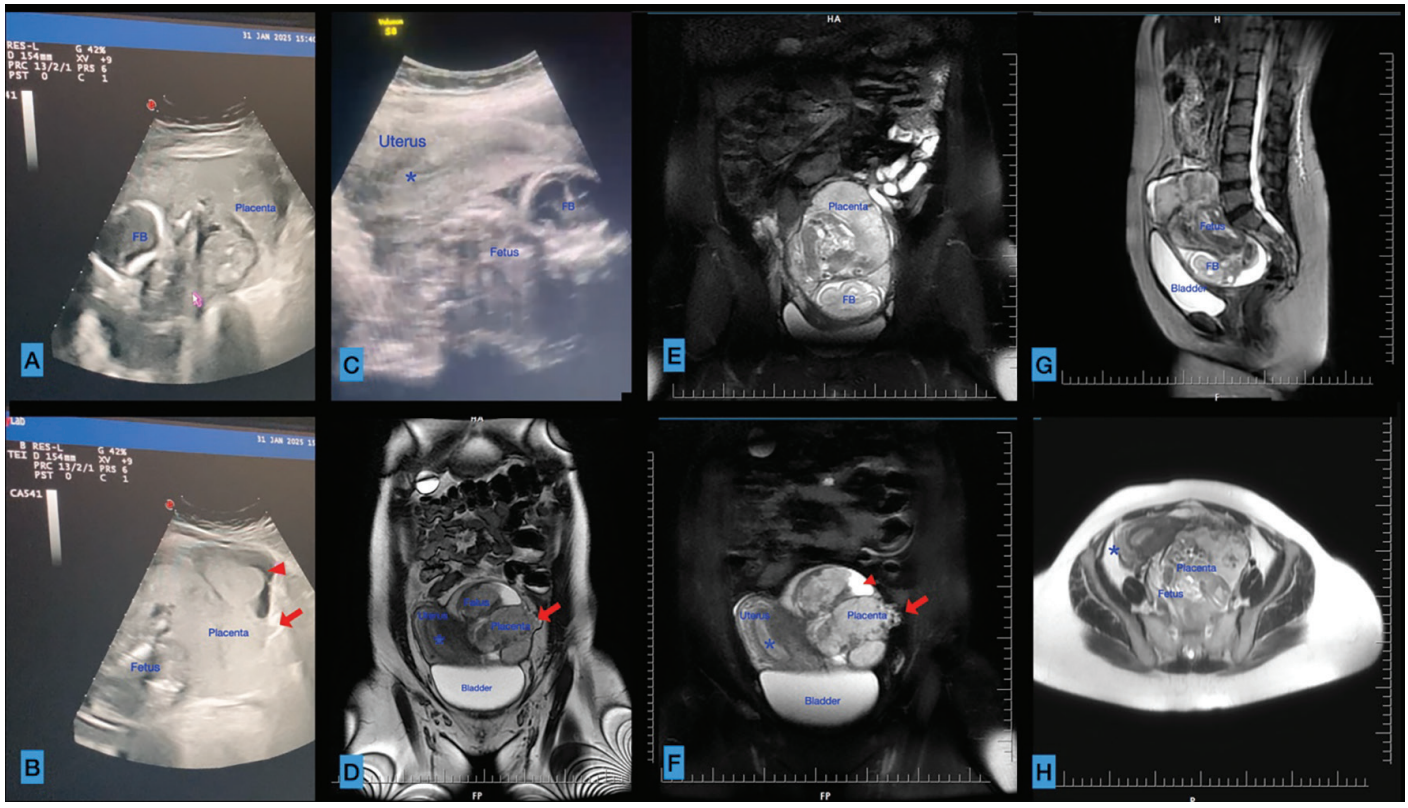

Figure 1. Ultrasonographic (A-C) and magnetic resonance (D-H) images of the patient at the time of diagnosis
Blue *: Endometrium, red arrowhead: placental lacuna. Red arrow: Invasion of the placenta into surrounding tissues, white arrow: fetus
FB: Fetal brain

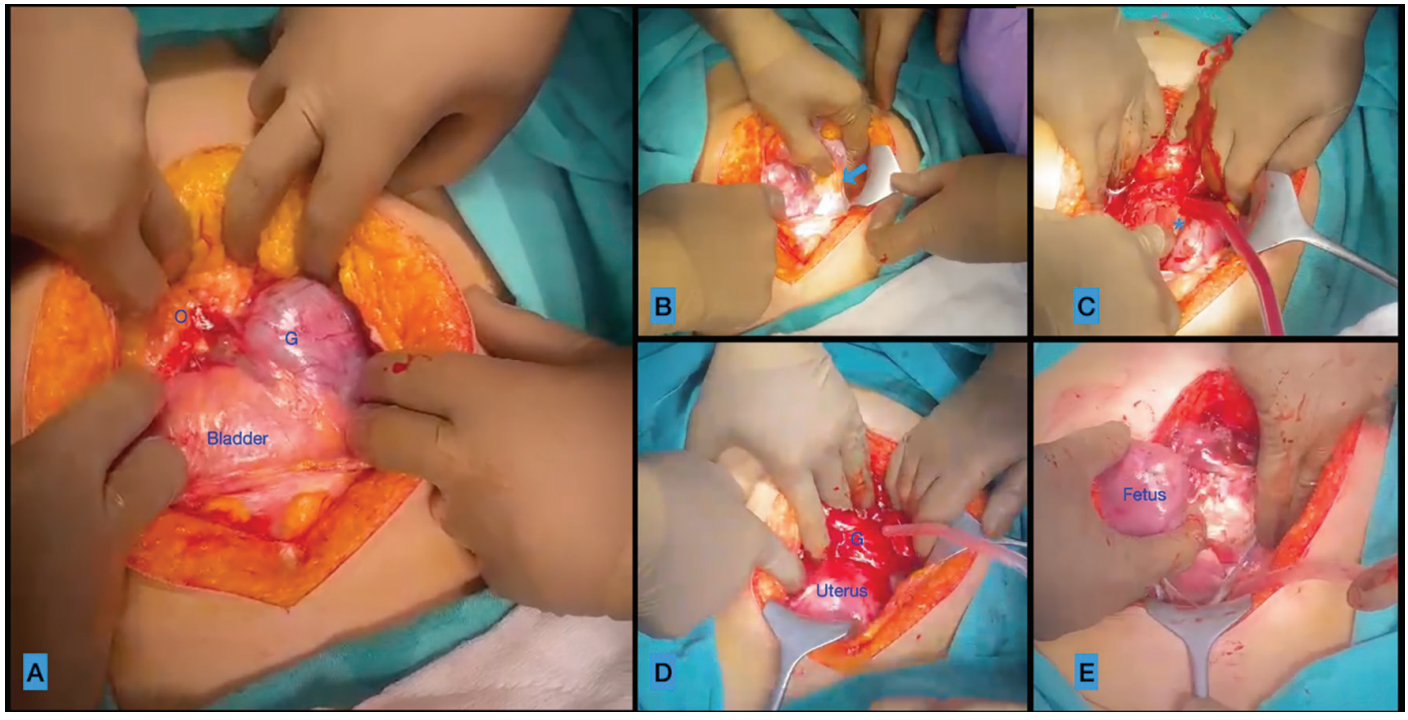

Figure 2. Initial images during surgery
*O: Omentum, G: Gestational sac. Blue arrow: Adhesion between sigmoid colon and gestational sac, blue, *: Fetal head*

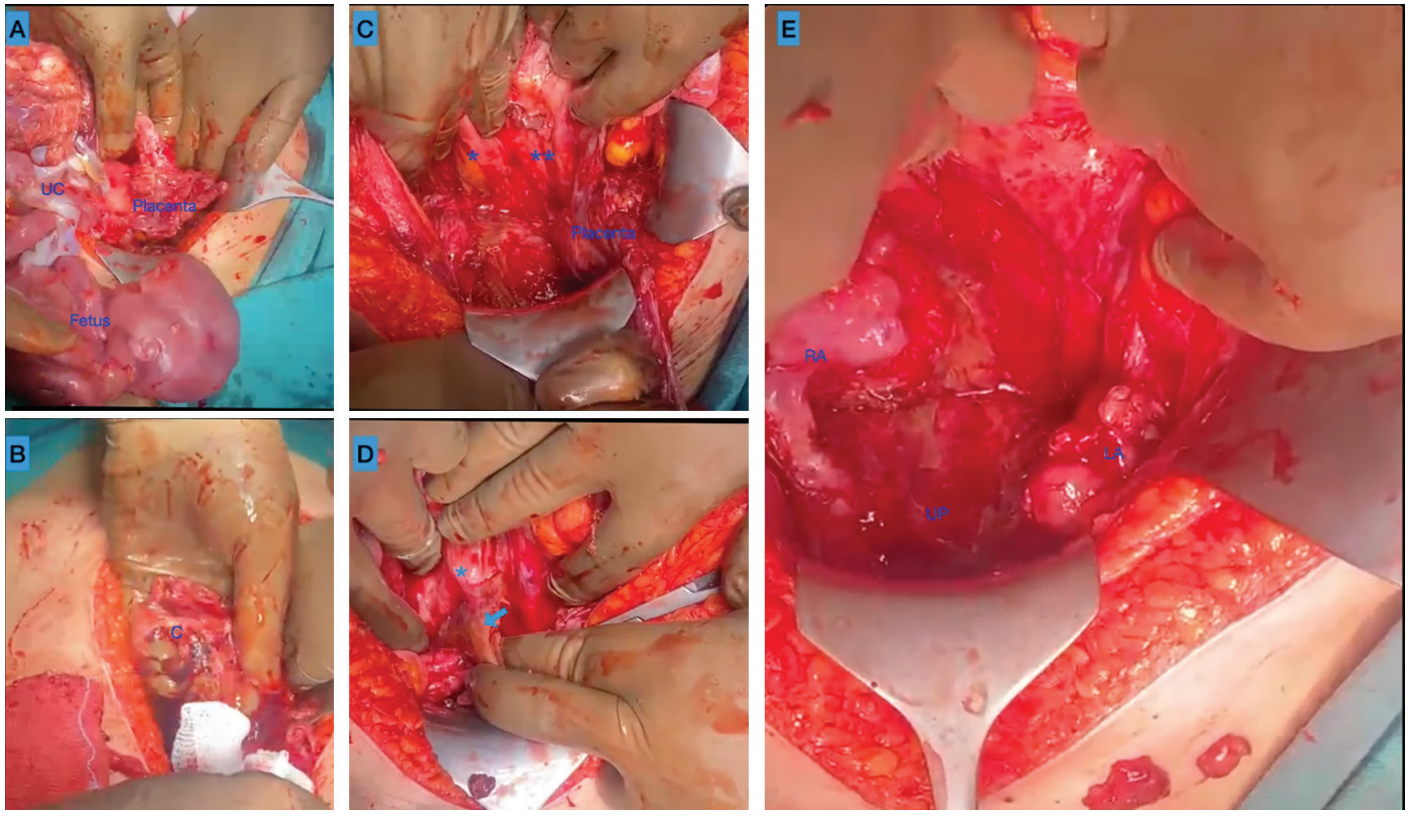

Figure 3. Intraoperative images

*UC: Umbilical cord, blue. *: Placental bed dissected from the sigmoid colon, blue. **: Placental bed dissected from the peritoneum of the left iliac vessels, Blue arrow: Placental remnants adhering to the sigmoid colon, C: Cotyledon, RA: Right adnexa, LA: Left adnexa, UP: Posterior uterus*

A female infant weighing 1100 gr, with an Apgar of 0, was delivered from the intra-abdominal cavity. The placenta was removed by dissection from the peritoneum of Douglas, the serosa of the sigmoid colon, the posterior uterus, and the adnexa. The uterus, bilateral ovaries, and fallopian tubes were also preserved. After bleeding was controlled, the abdomen was closed in accordance with the anatomical layers. No blood transfusion was needed. The patient, who did not develop any complications during postoperative follow-up, was discharged with a full recovery. After the patient's identity was anonymized, written consent was obtained from the patient for the publication of her data and images.

Discussion

Although AAP is rare, its incidence increases in women with risk factors for ectopic pregnancy[2]. It is known that conditions such as fallopian tube-related diseases (such as ectopic pregnancy, tubal surgery, sterilization and pelvic inflammatory disease), contraception, endometriosis, smoking, history of infertility and intra-abdominal surgery increase the risk[7-9]. In addition, low socioeconomic status, inadequate or incomplete antenatal care, and limited medical facilities may cause delays in the diagnosis of AP and increase the frequency of AAP. In our case, no known risk factors for AAP were identified other than infertility and smoking. However, published reports also show that AAP may occur even in women with access to antenatal care, particularly when symptoms are non-specific or imaging findings are misinterpreted[4-6].

There are two types of AAP based on their formation. In secondary AP, the pregnancy initially implants in the fallopian tubes. Subsequently, following tubal abortion or rupture, it attaches to the parietal peritoneum and continues to grow. In the less frequent primary AP, the gestational sac is located above any peritoneal surface in the abdominal cavity[10]. In our case, primary AAP was considered because there were no signs of early-stage pregnancy-related injuries (tuba-utero-peritoneal fistula) in the tubes, ovaries, and uterus. Nevertheless, secondary AP cannot be fully excluded in many advanced cases, because the original implantation site may no longer be clearly identifiable at laparotomy[4,7].

AAP may present with clinical presentations ranging from an asymptomatic state to hemorrhagic/septic shock[11-13]. Depending on the location of the gestational sac in the abdomen, patients may present with non-specific symptoms such as nausea, vomiting, abdominal pain, constipation, and ileus. This creates difficulties in making a correct diagnosis. In our case, the symptoms, abdominal pain, constipation, and dyspareunia, which increased in severity over time following delayed menstruation, could be explained by the placement of the fetus in the pouch of Douglas and of

the placenta in the rectosigmoid colon. The progressively worsening abdominal pain and tenderness in our patient were clinically significant, as they raised concern for an acute abdomen and influenced the decision for immediate surgical intervention.

It may not always be possible to diagnose AAP[11]. The diagnosis may be missed in 50-70% of patients due to the presence of non-specific symptoms, suboptimal anatomical evaluation due to advanced gestational age, and technical/structural limitations in imaging[13,14]. Therefore, the diagnosis of AAP is difficult. The most important parameter in the diagnostic process is the inclusion of AAP in the list of possible diagnoses. Especially in patients who have not undergone any imaging evaluation in the first trimester, as in our case, AAP should be considered in the differential diagnosis. In our case, we suspected an AAP on transabdominal sonography. We established a definitive diagnosis of AAP after transvaginal USG demonstrated empty endocervical and endometrial canals. We then evaluated the relationship between the placental bed and the intestinal and vascular structures using non-contrast-enhanced MRI. MRI was particularly useful in our case because it helped define placental topography and suggested the absence of major iliac vascular invasion, thereby contributing to surgical planning. Similar reports have emphasized that MRI may provide additional value over ultrasonography in delineating placental implantation and adjacent organ relationships in AAP[6,7,15].

Perinatal mortality in AAP is approximately 72%[2]. Perinatal mortality may be related to gestational age and inadequate placental perfusion. In our case, the fetus's gestational age of 24 weeks was considered the main cause of fetal death. The risk of maternal mortality and morbidity also increases with AAP. Although maternal mortality is approximately 12%, abdominal bleeding requiring blood transfusion occurs in 80% of patients[2]. Additionally, the risks of sepsis, relaparotomy, hysterectomy, oophorectomy, and vascular and intestinal injuries increase. The most important factors determining these risks are the gestational week at the time of diagnosis and the location of the placenta. In our case, although the placenta was located in the peritoneum over the sigmoid colon and iliac vessels, we achieved surgical recovery without complications, organ injury, or loss. One possible reason for this finding is that gestational age was relatively low. As pregnancy progresses, invasion may deepen, and treatment may become more difficult. This possibility was one of the reasons we did not favor prolonging pregnancy in the presence of severe pain and acute abdominal findings. Management of AAP is difficult and requires care at an experienced tertiary care hospital. Evidence-based treatment is not available because it is rare. Treatment should be determined by considering the condition of the fetus at the time of diagnosis, the week of pregnancy, and the relationship of the placenta to the intra-abdominal organs. When the fetus is non-viable or has an anomaly at diagnosis, necessary preparations can be made and surgery performed immediately. However, it may not always be possible to make this decision when the fetus is alive or healthy. There are cases in the literature where, after diagnosis, prolonging the pregnancy is offered as an option with patient consent to ensure fetal maturation[16]. At the same time, expectant management has generally been described in carefully selected stable patients under close inpatient surveillance and multidisciplinary monitoring[15-17]. In our patient, despite fetal cardiac activity, immediate laparotomy was preferred because of persistent severe abdominal pain, concern for an acute abdomen, and the possibility of increasing maternal risk with advancing gestation.

Since the MRI examination revealed that the placenta was located away from the main vessels and intestines, no problems may have occurred during the removal of placental tissues in this case. However, in cases in which placental invasion involves the major vascular structures or the bowel, any delay may increase the complication rate. In the present case, the sigmoid colon and iliac vessels may be at risk of deeper involvement during advanced gestational weeks. Therefore, the decision to proceed with immediate surgery in our case was based not only on fetal status but primarily on maternal symptoms and the anticipated progression of local invasion.

In the literature, it has been emphasized that treatment alternatives such as leaving the placenta in place and postoperative arterial embolization or methotrexate administration can be successfully applied in surgical treatment because removing the placenta during surgery may cause excessive bleeding and organ damage/loss in the mother[3,16-20]. When the placenta cannot be removed, close, long-term follow-up is required because resorption of the placenta at the site of invasion may lead to sepsis and secondary bleeding. Recent reports have also shown that even embolization-assisted conservative management may be followed by abscess formation, infection, or delayed reoperation[16,21]. Conversely, attempts at placental removal may result in catastrophic hemorrhage, shock, relaparotomy, or hysterectomy in anatomically unfavorable cases[3,22]. There are also cases in the literature in which the placenta was removed and treated successfully[11,12,23-25]. In the present case, the placenta was removed without any complications. Taken together, these findings suggest that placental management should be individualized according to placental location, expected vascularity, adjacent organ involvement, maternal condition, and local surgical expertise, rather than following a single universal strategy[3,4,7,22].

As summarized in the comparative literature table (Table 1), published reports do not support a single standard approach to AAP. Instead, management appears to depend on the interaction between maternal symptoms, fetal viability, placental anatomy, and institutional preparedness[4,5,7,17,26,27].

Table 1. Comparative summary of published advanced abdominal pregnancy cases and case series relevant to diagnostic and management dilemmas

Study	GA at diagnosis	Maternal/fetal status at diagnosis	Imaging	Management	Placental management	Maternal outcome	Fetal/neonatal outcome
Present case/ Türkiye	24 w	Stable mother; live fetus	US + MRI	Immediate surgery	Removed	Uneventful recovery	1100 g female, Apgar 0
Kim et al.[15], 2013/ Korea	18 w	Stable mother; live fetus	US + MRI	Expectant to 34 w	Removed	Uneventful; uterus preserved	Live female, 2100 g
Marcellin et al.[16], 2014/France	22 w	Stable asymptomatic inpatient; live fetus	US + MRI	Expectant to 32 w	Left *in situ* + embolization/follow-up	Maternal survival	Live birth
Harirah et al.[26], 2016/USA	23 w and 28 w	Stable mothers; live fetuses	US + MRI	Individualized: planned delivery vs. expectant management	Partial removal in one; *in situ* in one	Both survived	Favorable neonatal outcomes
Muroni et al.[3], 2021/Burundi	37 w	Stable mother; live fetus	US	Elective laparotomy	Removed	Massive hemorrhage, shock, relaparotomy; survived	Live birth; later infant death
Owie et al.[27], 2022/Nigeria	27 w	Septic mother; live fetus	US	Immediate surgery	Partially left *in situ*	Survived; transfusion required	Neonatal death within 24 h
Legesse et al.[23], 2023/Ethiopia	37 + 6 w	Stable mother; live fetus	Expert US	Immediate surgery	Removed	No maternal complication	Live female, 2060 g; foot deformity
Alwafai et al.[11], 2024/Germany	Late, missed until IUFD	Deteriorating mother; IUFD	US	Failed induction, then urgent laparotomy	Removed	Good postoperative recovery	Non-viable fetus
Emmanuel et al.[24], 2026/Ghana	29 + 3 w	Stable mother; live fetus	US	Expectant to 32 w	Removed	Hemorrhage, transfusion, postoperative abscess/ relaparotomy; survived	Live male, 1.6 kg, no gross anomaly
Sunday-Adeoye et al.[6], 2011/Nigeria	Mean 32.5 w (range 27-38 w)	Variable maternal status; mixed fetal outcomes	Limited imaging	Mixed surgical management	Removed in 11; *in situ* in others	1 maternal death	Poor perinatal outcomes overall
Harries et al.[5], 2025/South Africa	Median 31 + 4 w	Variable maternal status; 12 live births	US ± MRI	Mostly expedited delivery within 48 hours	Variable	2/17 maternal deaths	12 live births; 10 discharged home
Hoshino et al.[7], 2021/Japan	20-42 w (median 37.5 w)	Variable maternal status; mostly live births in later cases	US + MRI in recent cases	Mixed	Removed at laparotomy in 5/10; delayed removal in others	Maternal survival emphasized	6/7 live births had mainly compression-related deformities
Çetin Arslan[24], 2025/Türkiye	20 w and 29 w	Both diagnosed with live fetuses	US + MRI	Immediate surgery in both	Removed in both	Both survived; high blood loss/ICU care	Both live births

GA: Gestational age, US: Ultrasonography, MRI: Magnetic resonance imaging, ICU: Intensive care unit, w: Weeks, IUFD: Intrauterine fetal death

Our case adds to the limited literature suggesting that, in selected symptomatic patients without major vascular invasion on imaging, MRI-guided immediate organ-sparing surgery with placental removal may be feasible[15,23].

Conclusion

AAP is a preventable yet life-threatening condition associated with high maternal and fetal morbidity and mortality. Early diagnosis—particularly during the first trimester—is critical to improving outcomes. Surgical risk is largely dependent on the location and extent of placental invasion. While placental removal carries significant hemorrhagic risk, leaving the placenta in situ may result in secondary complications. MRI provides valuable information on placental localization and potential vascular involvement and may be particularly helpful for preoperative planning for suspected AAP cases. Management should occur in tertiary centers with multidisciplinary teams, and each case should be approached individually, with informed patient consent and detailed surgical planning. In symptomatic patients with severe pain or acute abdominal findings, immediate surgery may be justified despite the presence of fetal cardiac activity. In selected cases without major vascular invasion, organ-sparing placental removal may be feasible.

Ethics

Informed Consent: After the patient's identity was anonymized, written consent was obtained from the patient for the publication of her data and images.

Footnotes

Authorship Contributions

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

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Letter to the Editor / Editöre Mektup

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Vaginal microbiome metabolite shifts as early predictors of premature ovarian insufficiency in adolescents with novel gene variants

Yeni gen varyantlarına sahip ergenlerde prematür over yetmezliğinin erken belirleyicisi olarak vajinal mikrobiyom metabolit değişimleri

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Keywords: Premature ovarian insufficiency, vaginal microbiome, genetic variants

Anahtar Kelimeler: Prematür over yetmezliği, vajinal mikrobiyom, genetik varyantlar

To the Editor,

Premature ovarian insufficiency (POI) is a condition in which there is a decline in ovarian function prior to the age of 40 years. Elevated follicle-stimulating hormone (FSH) and estrogen deficiency result in sustained systemic complications, predisposing patients to cardiovascular morbidity, pronounced fertility decline, and diminished longevity. Globally, POI continues to pose a significant health burden, occurring in 3.5% of women. Furthermore, its prevalence increased over the past two centuries, although this trend was not statistically significant ($p>0.05$). For individuals with POI, hormone replacement therapy (HRT), coupled with skeletal preservation and reproductive planning, is advised to reduce chronic complications. Additionally, timely access to expert clinical care is crucial for the optimal management and enhanced outcomes for patients with POI[(1)].

Vaginal microbiome metabolite profiling is an integrated omics-based diagnostic framework that combines metabolic profiling with biochemical-functional remodeling to identify biochemical dysregulations associated with POI. This study leverages non-invasive collection of vaginal samples and advanced high-throughput methods, such as 16S ribosomal ribonucleic acid sequencing and liquid chromatography-mass spectrometry, to quantify metabolic fluxes and pathways. In POI, altered *Lactobacillus* predominance and specific metabolic derangements have been demonstrated, reflecting condition-specific microbial profiles. These integrated microbial-metabolite signatures enhance diagnostic accuracy beyond taxonomic profiling alone, suggesting strong predictive potential. Overall, this method is an emerging precision medicine strategy that integrates microbial and genetic data to advance early detection and therapeutic management of POI in at-risk youth[(2)].

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Recent studies suggest that vaginal microbiota plays an integral role in women's reproductive health and that alterations in its composition and diversity have been increasingly associated with ovarian dysfunction in POI. A case-control study showed certain microbial alterations, including an increased abundance of Gardnerella and Atopobium and a reduced abundance of Bifidobacterium ($p=0.017$), in women with POI; these alterations were also correlated with reduced anti-Mullerian hormone levels and increased FSH levels ($p<0.001$). These findings suggest that vaginal microbial metabolites may reflect endocrine and follicular impairment[3]. As HRT is a proposed treatment for POI, a cross-sectional study of 40 women with POI who were receiving oral HRT found that 33.4% (n=11) had a vaginal microbiome dominated by Lactobacillus species, which is typical of a healthy estrogenized vaginal environment. In contrast, 15.2% (n=5) demonstrated predominance of anaerobic bacteria, suggesting that systemic HRT may partially restore a vaginal microbiome similar to that observed in healthy women[4].

Despite intriguing associations, the routine clinical application of microbiome-based diagnostics in POI faces practical barriers to implementation. Most studies are limited by small sample sizes (<100), cross-sectional or case-control designs, or single-center recruitment, which restrict statistical power and generalizability. Vaginal microbiota sequencing requires specialized laboratories and expertise, which are often unavailable in low-resource settings. Moreover, certain microbiome shifts are not disease-specific, as taxa Gardnerella and Atopobium are also found in bacterial vaginitis, which further limits diagnostic specificity[5]. These challenges need to be addressed before vaginal microbiome and metabolite-based tools can be integrated alongside hormonal and genomic tests into routine early detection for women with POI.

Considering these points, emerging literature supports incorporating vaginal microbiome-derived metabolite profiling alongside genetic testing into the early diagnosis of POI, especially in women with genetically unexplained POI. This perspective adds to the existing literature by linking the shift in vaginal microbiome metabolites to genetic susceptibility in ovarian dysfunction. However, larger, population-based prospective studies are required to establish standard reference profiles, and specialized tools are needed to enhance diagnostic specificity and enable early risk stratification in adolescent women before irreversible ovarian decline occurs.

Footnotes

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