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# TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

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## LETTER FROM THE PRESIDENT

Dear TJOD Family,

I am happy to announce the release of the September issue of our media organ, the Turkish Journal of Obstetrics and Gynecology. I would like to extent my appreciation to those who contributed to this issue, that is, our editors, section editors, referees who devotedly evaluated the publications, and the national and international scientists who preferred our journal to publish their scientific studies.

Our journal is prepared by making great efforts, as a result of which it has a significant reputation in the international arena. Our impact factor for 2024 has risen from 1 to 1.3. I also would like to announce here that better developments are on the way.

The Turkish Gynecology and Obstetrics Society represents our country not only through our scientific media organ but also in respected scientific communities. We felt proud to represent our country in the special session allocated to our association in the EBCOG Congress held in Germany from June 5 to June 7, 2025.

We also contributed to the Natural Birth Action Plan 2nd Workshop by participating in the "Evaluation Meeting for the Natural Birth Action Plan" organized by the Ministry of Health in Ankara on July 10, 2025.

While presenting our September issue to the esteemed Gynecology and Obstetrics Society, I would like to express that TJOD is not only a professional association but also the umbrella organization of a large family.

**Best Regards**
**Ismail Mete Itil, Prof. MD.**
**President of TJOD**

TURKISH JOURNAL OF OBSTETRICS AND GYNECOLOGY

## EDITORIAL

Dear Colleagues,

We are proud to present you again with the September issue, the third publication of the year 2025, as the scientific media organ of the Turkish Gynecology and Obstetrics Society In this issue, numerous studies by international scientists have been evaluated.

In the September issue, there are 12 articles in total. One of them is a review study, while 11 articles are original articles. We extend our thanks to section editors, referees, and especially authors.

We would also like to share good news: The impact factor of our journal for 2024 has been determined as 1.3. This rate for 2023 was 1. We can state that our journal is a significant scientific publication organ.

We wish to meet again with the future issues of our scientific journal, whose motto is “together for the better”.

**Ercan Yilmaz, Prof. MD.**
**Fatih Sendag, Prof. MD.**

*Clinical Investigation / Araştırma*

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# Non-invasive diagnosis of endometrioma through cervical swabs using Fourier transform infrared spectroscopy

## *Endometriomanın servikal sürüntü üzerinden Fourier dönüşümlü kızılötesi spektroskopi ile non-invaziv tanısı*

**Aslı Karakaşlı[1], Ümit Görkem[2], Cihan Toğrul[2], Engin Yıldırım[3], Dursun Ali Köse[4], Ömer Yurdakul[4]**

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### Abstract

**Objective:** This study aimed to investigate whether Fourier transform infrared (FTIR) spectroscopy applied to cervical swab samples could detect meaningful biochemical differences between women diagnosed with endometriomas and healthy controls, thereby assessing its potential as a non-invasive diagnostic tool.

**Materials and Methods:** A total of 104 cervical swab samples-52 from women with endometriomas diagnosed via transvaginal ultrasonography and 52 from healthy controls—were initially collected and processed. Following an optimization process and quality control of spectral data, 24 endometrioma and 20 control samples were included in the final analysis. FTIR spectra were obtained in the 4000-600 $cm^{-1}$ range, and the primary outcomes included comparative peak intensities and areas under specific wavenumbers reflecting various bio-organic molecules.

**Results:** Statistically significant differences were observed at 2350 $cm^{-1}$ and 1050 $cm^{-1}$, indicative of alterations in carbon dioxide and carbohydrate metabolism, respectively, in the endometrioma group compared with healthy controls ($p<0.05$). No significant differences were detected in other spectral regions associated with lipids (2950, 1460, 1400 $cm^{-1}$) and proteins (e.g., amid-I and amid-II regions), suggesting that endometrioma may primarily affect carbohydrate metabolism and carbon dioxide balance rather than lipid and protein pathways. Both groups were comparable in demographic and hormonal characteristics, thus bolstering the validity of the findings.

**Conclusion:** FTIR spectroscopy of cervical swab samples revealed distinctive biochemical profiles in women with endometriomas, particularly related to carbon dioxide and carbohydrate metabolism. These data suggest that FTIR analysis, which is rapid and minimally invasive, holds promise for the future development of non-invasive diagnostic strategies for endometrioma. However, larger multicenter studies that include surgical confirmation and disease staging are needed to establish its clinical utility definitively.

**Keywords:** Endometrioma, cervix, spectroscopy, carbohydrates, diagnostic techniques

### Öz

**Amaç:** Bu çalışma, servikal sürüntü örnekleri üzerinde Fourier transform infrared (FTIR) spektroskopisi kullanılarak, endometrioma tanısı almış kadınlarla sağlıklı kadınlar arasında anlamlı biyokimyasal farklılıkların saptanıp saptanamayacağını araştırmayı amaçlamıştır. Böylece FTIR spektroskopisinin non-invaziv bir tanı yöntemi olarak kullanılabilirliği değerlendirilmektedir.

**PRECIS:** Fourier transform infrared spectroscopy of cervical swabs revealed distinct carbohydrate and carbon dioxide metabolism alterations in endometrioma, suggesting its potential use as a non-invasive diagnostic tool for this disease.

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**Gereç ve Yöntemler:** Araştırma kapsamında, transvajinal ultrasonografi ile endometrioma tanısı konulan 52 kadın ve 52 sağlıklı bireyden toplam 104 servikal sürüntü örneği toplanmıştır. Spektral veri optimizasyonu ve kalite kontrol sonrasında, analiz için endometrioma grubundan 24 ve kontrol grubundan 20 örnek seçildi. FTIR spektroskopik ölçümler 4000-600 $cm^{-1}$ dalga sayısı aralığında gerçekleştirilerek, spesifik dalga sayılarında pik yoğunlukları ve alanları karşılaştırıldı.

**Bulgular:** Çalışmanın bulgularına göre endometrioma grubunda sağlıklı kontrollere göre karbonhidrat metabolizmasını yansıtan 1050 $cm^{-1}$ ve karbondioksiti yansıtan 2350 $cm^{-1}$ bölgelerinde istatistiksel olarak anlamlı farklılıklar saptandı ($p<0,05$). Lipit ve proteinlerle ilişkili diğer spektral bölgelerde gruplar arasında anlamlı bir farklılık bulunmadı.

**Sonuç:** Sonuç olarak, FTIR spektroskopisi ile servikal sürüntü örneklerinin analizi, endometrioma tanısı açısından umut vadeden ve invaziv olmayan yöntem olarak öne çıkmaktadır. Özellikle karbonhidrat ve gaz değişimlerine ait biyokimyasal sinyallerin yakalanabilmesi, bu yöntemin hastalığın erken saptanmasında ve invaziv tanı yöntemlerine alternatif olarak kullanılmasında potansiyel taşıdığını göstermektedir. Ancak yöntemin klinik pratikte kullanılabilirliği için, cerrahi doğrulama ve hastalık evrelemesini içeren daha büyük ve çok merkezli çalışmaların yapılması gerekmektedir.

**Anahtar Kelimeler:** Endometrioma, serviks, spektroskopi, karbonhidratlar, tanı teknikleri

## Introduction

Endometriosis is a common chronic disease in women of reproductive age, characterized by symptoms such as pelvic pain, dysmenorrhea, and infertility. The form of this disease that produces cystic lesions in the ovaries is termed "endometrioma". Endometriosis most frequently localizes in the pelvis, especially in the ovaries. However, due to its non-specific symptoms, it is often confused with other causes of chronic pelvic pain, leading to difficulties in diagnosis. The gold-standard diagnostic method, involving surgery and histopathological examination, is associated with a typical delay of 8-12 years in diagnosis[1,2]. This delay adversely affects not only the diagnostic process but also timely access to treatment, causing a substantial decrease in quality of life.

Potential biomarkers found in the biological fluids of women with endometriosis may play an important role not only in diagnosis, but also in evaluating treatment efficacy. Advanced technologies such as proteomics, metabolomics, and genomics have emerged as promising tools for the detection of these biomarkers. Proteomic studies have shown significant changes in the levels of various proteins and peptides in the serum, urine, and cervicovaginal fluids of women with endometriosis[3-6]. Cervicovaginal fluid, in particular, allows for the identification of more specific biomarkers in the diagnosis of gynecological diseases due to its direct exposure to the uterine environment.

In recent years, Fourier transform infrared (FTIR) spectroscopy has attracted attention as a sensitive and rapid technology for analyzing the molecular structure of biological materials. This technology has demonstrated high potential in distinguishing diseased tissues from normal tissues. Successful applications of FTIR spectroscopy have been reported in the existing literature for the diagnosis of breast cancer, prostate cancer, and various gynecological conditions[7-10]. However, evidence of sufficient quality for the routine use of this technology in diagnosing complex diseases such as endometriosis is not yet available[11]. Advantages of FTIR spectroscopy include its rapid and cost-effective nature, the feasibility of non-invasive sampling from biological fluids, and the ability to provide sensitive results at the molecular level. In particular, the use of FTIR technology in samples such as cervicovaginal fluid may allow the detection of more specific biomarkers due to the benefits conferred by exposure to the uterine environment.

This study aimed to evaluate whether FTIR spectroscopy findings in cervical swab samples from women with endometriomas differ from those of healthy controls. In this context, the goal is to investigate changes in band structure and intensity in the FTIR spectra obtained from endometriotic and healthy tissues, which may serve an alternative diagnostic method for endometriosis and introduce an innovative perspective to current diagnostic approaches.

## Materials and Methods

This cross-sectional prospective study was conducted between December 17, 2019, and November 17, 2021, in the Infertility and Gynecology Outpatient Clinics, of Hitit University Training and Research Hospital. The study included patients who presented to the infertility clinic and were diagnosed with endometrioma, as well as healthy women without any gynecological diseases attending solely for routine cervical swab screening. Written informed consent was obtained from all participants before enrollment. The data obtained have not been published elsewhere. This study was approved by the Hitit University Faculty of Medicine Research Ethics Committee with approval number 115, dated 11.12.2019.

Inclusion criteria for the endometrioma group were being between 18 and 45 years of age, having had unprotected intercourse for at least one year without achieving pregnancy, and exhibiting transvaginal ultrasonography findings, consistent with the typical endometrioma definition of the American College of Radiology's O-RADS Ultrasound Risk Stratification System[12]. In the healthy control group, inclusion criteria comprising being between 18 and 45 years of age and having no gynecological complaints or diseases. Exclusion criteria included abnormal cervical swab findings, premalignant or malignant findings, evidence of bleeding or vaginitis, a history of active or chronic vaginitis, intravaginal medication use, use of an intrauterine device, history of chronic disease or long-term medication use. As a result, a total of 104 women participated in the study and were divided into two main groups: (i) 52 patients diagnosed with endometrioma and (ii) 52 healthy controls.

Cervical swab samples were collected from the cervical os following Pap smear sampling. The collected samples were placed in 0.09% sodium chloride solution for 10 minutes and then stored at -20 °C. All samples were preserved under these conditions until the day of analysis. After the optimization process for the cervical swab technique, relatively fewer samples remained for analysis. The spectral data obtained were visually examined for morphological features, and samples with spectra showing similar wave patterns were selected for statistical analysis. The spectra from 24 participants in the endometrioma group and 20 participants in the control group were included in the statistical analyses. Spectra excluded due to differences in wavelength patterns, which may have resulted from variations in biochemical composition, technical factors, biological differences between individuals, or environmental factors.

Spectroscopic analyses were carried out using a Thermo Nicolet 6700 FTIR Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The infrared spectrum was measured based on vibrations in polar covalent bonds of molecules, a method used to analyze the unique "fingerprint" structure of organic compounds. In this study, the attenuated total reflectance technique was employed to record spectra in the range of 4000-600 $cm^{-1}$. The 0.09% sodium chloride solution, used to obtain the background spectrum, was subtracted from the measurements.

### Statistical Analysis

All data were analyzed using IBM Statistical Package for the Social Sciences (SPSS) version 26.0 software. The Kolmogorov-Smirnov test was applied to assess the normality of data distribution. Descriptive statistics for continuous variables were presented as mean, standard deviation, median, minimum, and maximum values. Data with normal distribution were analyzed by the Independent Samples t-test, and the Mann-Whitney U test was used for data not following a normal distribution. A p-value <0.05 was considered statistically significant for all analyses.

## Results

A total of 104 samples-52 from the endometrioma group and 52 from the control group-were analyzed via FTIR spectroscopy. After the optimization process for the cervical swab technique, fewer samples remained; the obtained spectra were examined visually for morphological features. Samples with spectra exhibiting similar wave patterns were selected for statistical analysis. Consequently, the spectra of 24 participants in the endometrioma group and 20 participants in the control group were included in the statistical analyses.

The findings regarding the comparison of demographic and hormonal parameters between the control and endometriosis groups are presented in Table 1. There were no statistically significant differences between the groups in age (p=0.787), body mass index (p=0.368), follicle stimulating hormone (p=0.605), estradiol (p=0.418), or anti-Müllerian hormone (p=0.082). These results indicate that the two groups were largely similar in terms of demographic and hormonal characteristics (p>0.05 all).

FTIR spectra were obtained for all samples in the wavenumber range of 4000-600 $cm^{-1}$. However, due to strong overlapping peaks arising from the sodium chloride solution, the 3800-3000 $cm^{-1}$ region was excluded from the analysis. In line with existing literature, nine peaks were detected in the cervical swab samples, reflecting various bio-organic molecules such as proteins, lipids, nucleic acids, and carbohydrates. These peaks, their wavenumbers, and the corresponding organic components identified in the literature are listed in Table 2.

As shown in Figures 1 and 2, differences in the quantitative properties of the peaks were observed between the two study groups. Upon analysis, the peak area reflecting carbon dioxide ($CO_2$) at 2350 $cm^{-1}$ and the peak area corresponding to oligosaccharides and polysaccharides at 1050 $cm^{-1}$ were found to be significantly higher in the endometrioma group than in the healthy control group (U=151.0, p<0.05, and U=154.0, p<0.05, respectively). These findings are summarized in Table 3.

Furthermore, the peak area at 1250 $cm^{-1}$ was higher in the endometrioma group compared to the control group (mean ranks: 25.46 vs. 18.95); however, this difference was borderline in terms of statistical significance (p=0.094). No statistically significant differences were observed at other wavenumbers (e.g., 2950 $cm^{-1}$ and 1650 $cm^{-1}$), suggesting a similar distribution of

**Table 1.** Comparison of demographic and hormonal parameters between control and endometriosis groups

| Variable | Control group | | Endometriosis group | | t-value | df | p-value | 95% CI lower | 95% CI upper |
|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | | | | |
| Age | 29.83 | 2.98 | 30.00 | 3.28 | -0.27 | 102.00 | 0.787 | -1.38 | 1.05 |
| BMI | 24.47 | 2.13 | 24.07 | 2.33 | 0.90 | 102.00 | 0.368 | -0.47 | 1.27 |
| FSH | 7.78 | 1.19 | 7.65 | 1.19 | 0.52 | 102.00 | 0.605 | -0.34 | 0.58 |
| E2 | 60.26 | 14.20 | 58.19 | 11.70 | 0.81 | 102.00 | 0.418 | -2.98 | 7.11 |
| AMH | 3.36 | 0.59 | 3.57 | 0.58 | -1.76 | 102.00 | 0.082 | -0.43 | 0.03 |

Mean values, standard deviations (SD), t-values, degrees of freedom (df), p-values, and 95% confidence intervals (CI) are provided, BMI: Body mass index, FSH: Follicle stimulating hormone, E2: Estradiol, AMH: Anti-Müllerian hormone

bio-organic molecules between the two groups in those regions. Overall, the FTIR analysis demonstrated distinct differences at 2350 $cm^{-1}$ and 1050 $cm^{-1}$ in the endometrioma group, whereas other wavenumbers showed a relatively stable distribution.

## Discussion

This study aimed to compare the FTIR spectroscopic findings of cervical swab samples from women with endometriomas to those from healthy controls, and in doing so identify potential biochemical alterations that could be used for diagnostic purposes. Our results indicate that the peak areas under 2350 $cm^{-1}$, corresponding to carbon dioxide, and 1050 $cm^{-1}$, reflecting oligosaccharide and polysaccharide content, was significantly higher in the endometrioma group compared to the control group. Conversely, no statistically significant difference was detected between the groups at other wavenumbers known to represent lipids, proteins, phospholipids, and nucleic acids.

The increased peak area at 2350 $cm^{-1}$ in the endometrioma group suggests a specific change in $CO_2$ metabolism. $CO_2$ is a major byproduct of cellular metabolism and may accumulate in states of inflammation, hypoxia, or heightened metabolic activity. Schultz et al.[13] demonstrated that the $CO_2$ peak observed in

**Table 2.** Characteristics of the selected peak wavenumbers for the study

| Peak wavenumber ($cm^{-1}$) | Description | Organic component |
|---|---|---|
| Peak1 (2950 $cm^{-1}$) | $CH_3$ asymmetric stretching | Lipids |
| Peak2 (2350 $cm^{-1}$) | $CO_2$; cellular metabolic rate, atmospheric carbon dioxide | - |
| Peak3 (1650 $cm^{-1}$) | Amide I; 80% C=O stretching, 10% N-H bending, 10% C-N stretching | Proteins |
| Peak4 (1560 $cm^{-1}$) | Amide II; N-H stretching | Proteins |
| Peak5 (1540 $cm^{-1}$) | Amide II; 60% N-H bending, 40% C-N stretching | Proteins |
| Peak6 (1460 $cm^{-1}$) | $CH_3$ bending | Lipids |
| Peak7 (1400 $cm^{-1}$) | COO- symmetric stretching | Fatty acids |
| Peak8 (1250 $cm^{-1}$) | $PO_2$- asymmetric stretching (without hydrogen bonding) | Nucleic acids, phosphoproteins, and phospholipids |
| Peak9 (1050 $cm^{-1}$) | C-O stretching accompanied by C-O bending of carbohydrate C-OH groups | Oligosaccharides and polysaccharides |

The table includes peak wavenumbers ($cm^{-1}$), their corresponding descriptions, and the associated organic components

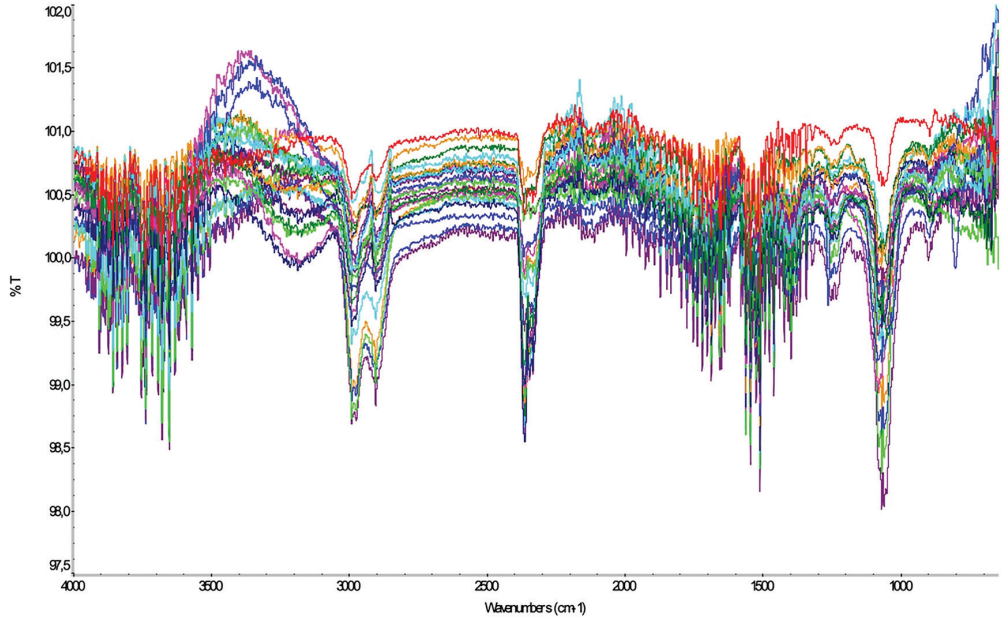


**Figure 1.** FTIR spectra of the endometriosis group, showing the characteristic peaks of biomolecular components

*FTIR: Fourier transform infrared*

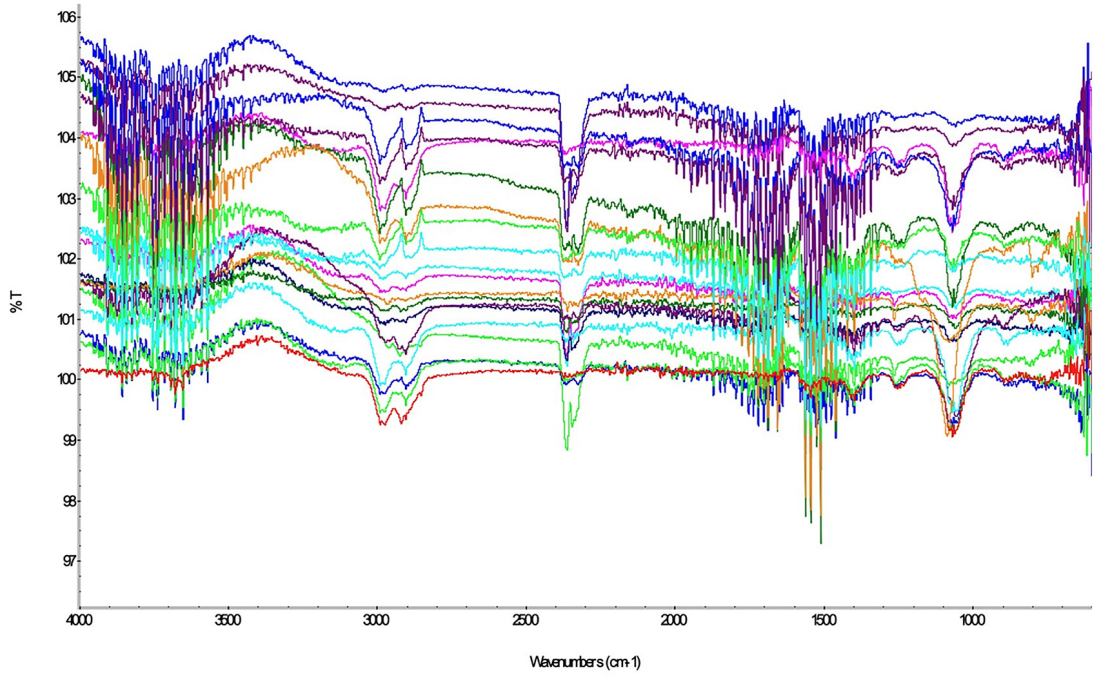


**Figure 2.** FTIR spectra of the control group, displaying the spectral differences compared to the endometriosis group
*FTIR: Fourier transform infrared*

**Table 3.** Comparison of the areas under the peaks between groups using the Mann-Whitney U test

| Peak | Group | Mean rank | Sum of ranks | U | Z-score | p |
|---|---|---|---|---|---|---|
| Peak1 (2950 $cm^{-1}$) | Endometrioma | 25.17 | 604.00 | 176.0 | -1.508 | 0.131 |
| | Control | 19.30 | 386.00 | | | |
| Peak2 (2350 $cm^{-1}$) | Endometrioma | 26.21 | 629.00 | 151.0 | -2.098 | 0.036* |
| | Control | 18.05 | 361.00 | | | |
| Peak3 (1650 $cm^{-1}$) | Endometrioma | 22.42 | 538.00 | 238.0 | -0.047 | 0.962 |
| | Control | 22.60 | 452.00 | | | |
| Peak4 (1560 $cm^{-1}$) | Endometrioma | 23.81 | 571.50 | 208.5 | -0.743 | 0.458 |
| | Control | 20.93 | 418.50 | | | |
| Peak5 (1540 $cm^{-1}$) | Endometrioma | 21.96 | 527.00 | 227.0 | -0.306 | 0.759 |
| | Control | 23.15 | 463.00 | | | |
| Peak6 (1460 $cm^{-1}$) | Endometrioma | 22.29 | 535.00 | 235.0 | -0.118 | 0.906 |
| | Control | 22.75 | 455.00 | | | |
| Peak7 (1400 $cm^{-1}$) | Endometrioma | 22.44 | 538.50 | 238.5 | -0.035 | 0.972 |
| | Control | 22.58 | 451.50 | | | |
| Peak8 (1250 $cm^{-1}$) | Endometrioma | 25.46 | 611.00 | 169.0 | -1.673 | 0.094 |
| | Control | 18.95 | 379.00 | | | |
| Peak9 (1050 $cm^{-1}$) | Endometrioma | 26.08 | 626.00 | 154.0 | -2.027 | 0.043* |
| | Control | 18.20 | 364.00 | | | |

*: P<0.05 values are considered statistically significant. The table presents mean ranks, sum of ranks, U-values, Z-scores, and p-values for each peak

biological materials could be attributed to the intracellular $CO_2$ produced through glucose metabolism. However, it has also been reported that atmospheric $CO_2$ may introduce measurement errors. A study by Kokot et al.[14] investigating serum samples showed that increases in $CO_2$ peaks among individuals with endometriosis were associated with inflammatory processes. This observation aligns with our findings of elevated signal intensity around 2350 $cm^{-1}$, suggesting that this region may serve as both a diagnostic and a prognostic biomarker.

Our investigation revealed that peak areas at 1050 $cm^{-1}$ were significantly higher in the endometrioma group than in the control group. This wavenumber is associated with bending and stretching vibrations of the C-O bond in the C-OH groups of carbohydrates, thereby reflecting oligosaccharides and polysaccharides. In the literature, it has been proposed that this wavenumber could be indicative of molecular alterations linked to endometriosis. In a study by Cheung et al.[15], wavenumbers corresponding to carbohydrate peaks played a pivotal role in distinguishing between eutopic endometrium and ectopic endometriotic tissues. Bozdağ et al.[16] found that in cervical swab samples, carbohydrate bond vibrations at 1153 $cm^{-1}$ and 1035 $cm^{-1}$ were elevated in early-stage endometriosis, but decreased in advanced-stage disease. Moreover, Nsugbe[17], using Raman spectroscopy, reported that wavenumbers reflecting carbohydrate metabolism are key determinants in differentiating between healthy and endometriotic individuals, and that the analysis of Raman data via machine learning algorithms further supports the clinical utility of these wavenumbers. In contrast, Notarstefano et al.[18] found that in granulosa cells, the carbohydrate content at 1053 $cm^{-1}$ was lower in the endometrioma group than in healthy controls.

These discrepancies may stem from factors such as endometriosis stage, lesion site, and the disease's biochemical microenvironment. For example, an increase in carbohydrate metabolism has been associated with early-stage endometriosis, whereas this alteration appears less pronounced in advanced stages. Although our study did not include staging of the endometrioma cases, the elevated carbohydrate peaks observed in the patient group may indicate the predominance of earlier-stage disease. Overall, our findings suggest that endometrioma may affect glycosylation patterns and carbohydrate metabolism, and that spectroscopy offers a powerful method for detecting such molecular changes.

In our study, no significant differences were observed between the groups at 2950, 1460, and 1400 $cm^{-1}$, which represent lipid components. Nonetheless, the literature indicates that endometriosis can influence lipid metabolism. Notarstefano et al.[18] reported a decline in the degree of saturation for lipid alkyl chains and an increase in peroxidized lipid content in endometrioma cases. Furthermore, Bozdağ et al.[16] noted that in advanced endometriosis, lipid-protein vibrations at 1450 $cm^{-1}$ were higher than in controls.

Regarding protein components, Cheung et al.[15] identified the amid-I and amid-II vibrational regions in FTIR spectra of endometriotic tissues as key spectral features associated with endometrial pathologies. Notarstefano et al.[18] observed a decrease in properly folded proteins and an increase in unfolded proteins in granulosa cells. In a serum-based study, Kokot et al.[14] reported that inflammation-related protein levels were significantly elevated in individuals with endometriosis, a finding that could explain why protein peaks showed no differences in our study, given that cervical swab and serum samples reflect different biochemical properties. Taken together, these results imply that endometrioma may influence protein metabolism and secondary protein structures.

However, in our study, no statistically significant difference was detected between the groups in the Amide I and Amide II regions of the FTIR spectra, which reflect proteins. Although changes in protein structure have been reported in samples such as serum or granulosa cells in the literature, cervical swab samples may not fully capture these alterations. This may be explained by two main reasons: (i) FTIR spectroscopy can primarily detect prominent structural changes in complex biological matrices, as it has limited sensitivity to subtle conformational variations; (ii) the cervix is anatomically distant from the peritoneal or ovarian microenvironment where endometriotic foci reside, and thus may not fully reflect local inflammatory protein alterations. Indeed, while increases in peaks originating from lipids and some proteins have been reported in FTIR spectra obtained from cervical swab samples, these signals do not directly indicate specific conformational changes in protein structure. In particular, in the study by Bozdağ et al.[16], increases were observed at 1405 and 1450 $cm^{-1}$ peaks in advanced-stage endometriosis groups. However, second derivative analyses of the Amide I-II regions did not reveal statistically significant differences in protein structure. These findings suggest that the ability of FTIR to detect conformational changes in proteins in cervical swab samples may be limited.

Therefore, future studies should be supported by simultaneous sampling from different anatomical sites and validation with proteomic methods.

A key strength of this study is the ability to analyze cervical swab samples via FTIR spectroscopy without resorting to invasive methods. This approach enables the rapid and precise detection of potential biochemical alterations arising from the presence of endometrioma. Additionally, the similarity of demographic and hormonal characteristics between the patient and control groups enhances the comparability of the findings and thereby strengthens the validity of the results. Another noteworthy advantage lies in the simplicity and speed of the protocol employed, which could facilitate the future integration of FTIR technology into clinical practice.

In recent years, FTIR spectroscopy has gained attention as a non-invasive diagnostic method capable of rapidly and

sensitively detecting biochemical alterations at the molecular level. Several publications have reported successful use of FTIR spectroscopy in identifying molecular changes associated with endometriosis in various biological tissues. Researchers such as Kokot et al.[14] and Nsugbe[17] have proposed that combining FTIR or Raman spectroscopy with machine learning and other advanced analytical methods could accelerate the integration of these techniques into clinical practice. Our study demonstrates that this approach is feasible for cervical swab samples.

### Study Limitations

Nevertheless, an important limitation of our study is the relatively small number of samples included in the final analysis (endometrioma group: 24, control group: 20). This may limit the generalizability and statistical power of the findings. Although the spectral quality control and optimization process was conducted meticulously, larger sample sizes, multicenter studies, and surgical confirmation will be required to validate these findings. Such studies will more clearly demonstrate the potential of this method for clinical application.

Another limitation of our investigation is that the diagnosis of endometrioma was based solely on ultrasonographic evaluation without surgical confirmation. This approach prevented staging and made it challenging to fully compare our findings with those of other studies in the literature. Future research with surgical confirmation and disease staging would allow more precise delineation of the spectral characteristics involved.

## Conclusion

This study demonstrates that the use of FTIR spectroscopy on cervical swab samples can detect significant biochemical differences between women with endometrioma and healthy controls. In particular, the pronounced changes observed at 2350 $cm^{-1}$ and 1050 $cm^{-1}$ suggest alterations in carbohydrate metabolism and $CO_2$ levels associated with endometrioma. Although no significant differences were found in lipid and protein components between the groups-without excluding the clinical importance of these molecular categories-the findings imply that endometrioma may primarily affect carbohydrate metabolism and $CO_2$ balance. Taken together, these data indicate that FTIR analysis of cervical swab samples has the potential to contribute to non-invasive diagnostic processes for endometrioma in the future. Nonetheless, larger-scale studies incorporating surgical confirmation and disease staging will be instrumental in guiding the integration of this method into clinical practice.

### Statement on the Use of Artificial Intelligence (AI) Tools

During the preparation of this work, the authors utilized OpenAI's ChatGPT and Grammarly to enhance language clarity, grammar, and readability of the manuscript. The AI-generated suggestions were carefully evaluated by comparing them with original author-written content, ensuring accuracy, consistency, and scientific correctness. After thorough review and necessary editing, the authors assume full responsibility for the manuscript's final content. This incorporation of AI tool usage primarily impacted the language quality, manuscript readability, and overall editorial refinement.

### Ethics

**Ethics Committee Approval:** This study was approved by the Hitit University Faculty of Medicine Research Ethics Committee with approval number 115, dated 11.12.2019.

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

### Footnotes

### Authorship Contributions

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

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

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## References

1. Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, et al. Clinical diagnosis of endometriosis: a call to action. Am J Obstet Gynecol. 2019;220:354.e1-12.
2. Fassbender A, Burney RO, D'Hooghe T, Giudice L. Update on biomarkers for the detection of endometriosis. Biomed Res Int. 2015;2015:130854.
3. Long X, Jiang P, Zhou L, Zhang W. Evaluation of novel serum biomarkers and the proteomic differences of endometriosis and adenomyosis using MALDI-TOF-MS. Arch Gynecol Obstet. 2013;288:201-5.
4. Wölfler MM, Schwamborn K, Otten D, Hornung D, Liu H, Rath W. Mass spectrometry and serum pattern profiling for analyzing the individual risk for endometriosis: promising insights? Fertil Steril. 2009;91:2331-7.
5. Zheng N, Pan C, Liu W. New serum biomarkers for detection of endometriosis using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. J Int Med Res. 2011;39:1184-92.
6. Dutta M, Joshi M, Srivastava S, Lodh I, Chakravarty B, Chaudhury K. A metabonomics approach as a means for identification of potential biomarkers for early diagnosis of endometriosis. Mol Biosyst. 2012;8:3281-6.
7. Cameron JM, Sala A, Antoniou G, Brennan PM, Butler HJ, Conn JJA, et al. A spectroscopic liquid biopsy for the earlier detection of multiple cancer types. Br J Cancer. 2023;129:1658-66.
8. Delrue C, De Bruyne S, Speeckaert MM. The promise of infrared spectroscopy in liquid biopsies for solid cancer detection. Diagnostics (Basel). 2025;15:368.
9. Delrue C, De Bruyne S, Oyaert M, Delanghe JR, Moresco RN, Speeckaert R, et al. Infrared spectroscopy in gynecological oncology: a comprehensive review of diagnostic potentials and challenges. Int J Mol Sci. 2024;25:5996.
10. Bilgory A, Haimovich S, David L, Malonek D, Dekel BZ, Shechtman L, et al. Mid-infrared spectroscopy as a real-time diagnostic tool for

chronic endometritis: a preliminary study. Spectrochim Acta A Mol Biomol Spectrosc. 2025;330:125670.

11. Schmidt A, Aebersold R. High-accuracy proteome maps of human body fluids. Genome Biol. 2006;7:1-4.
12. Andreotti RF, Timmerman D, Strachowski LM, Froyman W, Benacerraf BR, Bennett GL, et al. O-RADS US risk stratification and management system: a consensus guideline from the ACR ovarian-adnexal reporting and data system committee. Radiology. 2020;294:168-85.
13. Schultz CP, Eysel HH, Mantsch HH, Jackson M. Carbon dioxide in tissues, cells, and biological fluids detected by FTIR spectroscopy. J Phys Chem. 1996;100:6845-8.
14. Kokot I, Mazurek S, Piwowar A, Szostak R, Jędryka M, Kratz EM. ATR-IR spectroscopy application to diagnostic screening of advanced endometriosis. Oxid Med Cell Longev. 2022;2022:4777434.
15. Cheung KT, Trevisan J, Kelly JG, Ashton KM, Stringfellow HF, Taylor SE, et al. Fourier-transform infrared spectroscopy discriminates a spectral signature of endometriosis independent of inter-individual variation. Analyst. 2011;136:2047-55.
16. Bozdağ G, Igci N, Calis P, Ayhan B, Demiralp DO, Mumusoglu S, et al. Examination of cervical swabs of patients with endometriosis using Fourier transform infrared spectroscopy. Arch Gynecol Obstet. 2019;299:1501-8.
17. Nsugbe E. On the use of Raman blood spectroscopy and prediction machines for enhanced care of endometriosis patients. Digit Technol Res Appl. 2023;2:1-12.
18. Notarstefano V, Gioacchini G, Byrne HJ, Zaca C, Sereni E, Vaccari L, et al. Vibrational characterization of granulosa cells from patients affected by unilateral ovarian endometriosis: new insights from infrared and Raman microspectroscopy. Spectrochim Acta A Mol Biomol Spectrosc. 2019;212:206-14.

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# Prediction of oocyte maturity before denudation: Is the assessment of COC morphology a reliable option?

## *Denudasyon öncesi oosit maturasyonunu tahmin etmek için KOK morfolojisi değerlendirilmesi güvenilir bir seçenek midir?*

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### Abstract

**Objective:** We aimed to demonstrate the predictive value of morphological assessment of cumulus-oocyte complexes (COCs) prior to denudation in distinguishing mature and immature oocytes.

**Materials and Methods:** The study consisted of two stages. Five embriologists were enrolled to the first stage of the study and they divided COCs into two groups according to the morphologic features of the COS's: COCs with mature oocytes and COCs with immature oocytes. The process was overseen by one embryologist. Two hours later, COCs were denuded, and the maturity of oocytes was evaluated by another embryologist. The results were recorded. The first stage was terminated when each embryologist had evaluated a minimum of 100 COCs. In the second stage, three embryologists applied the procedure continuously for one more month. At the end of the study, the effects of continuous assessment on the prediction success were evaluated.

**Results:** Eighty patients were enrolled in the study, and a total of 1039 COCs were examined. In the first stage of the study, 69% of immature and 80% of mature oocytes were identified correctly by the embryologists. There was no significant difference among the embryologists in terms of success rates. In the second stage of the study, the success rates of immature oocyte prediction increased for all three embryologists. However, a statistically significant increase was observed for only one embryologist ($p<0.05$). However, the prediction rates of mature oocytes were comparable with the results of the first stage of the study. There was no significant relationship between the number of COCs and the prediction value.

**Conclusion:** Morphological assessment of COCs before denudation does not provide accurate results in identifying mature and immature oocytes.

**Keywords:** Oocyte, maturation, in vitro fertilization

### Öz

**Amaç:** Olgun oositler ile olgun olmayan oositleri denudasyon öncesi ayırt edebilmek amacı ile, denüdasyon öncesi kümülüs-oosit komplekslerinin (KOK) morfolojik değerlendirmesinin öngörü değerini göstermeyi amaçladık.

**Gereç ve Yöntemler:** Çalışma iki aşamadan oluşmaktadır. İlk aşamaya beş embriyolog dahil edildi. Her bir embriyolog denudasyon öncesi, KOK'ları morfolojik özelliklerini göz önüne alarak KOK'ları, olgun ve olgun olmayan oosit içeren KOK'lar olarak iki gruba ayırdı. İşlem bir embriyolog tarafından denetlendi ve iki saat sonra KOK'lar başka bir embriyolog tarafından denude edildi, oositlerin olgun olup olmadıkları not edildi. İkinci aşamada, üç embriyolog işlemi bir ay süre ile kesintisiz olarak uyguladı. Çalışmanın sonunda, KOK morfolojisinin sürekli aynı kişiler tarafından değerlendirilmesinin olgun-olgun olmayan oositi tahmin başarısı üzerindeki etkileri değerlendirildi.

**Bulgular:** Çalışmaya 80 hasta dahil edildi ve toplam 1039 KOK incelendi. Çalışmanın birinci aşamasında embriyologlar tarafından immatür oositlerin %69'u, matür oositlerin ise %80'i doğru olarak tanımlandı. Embriyologlar arasında başarı oranları açısından anlamlı bir fark saptanmadı. Çalışmanın ikinci aşamasında, immatür oositleri tahmin etme başarı oranı üç embriyolog için de artarken, matür oositleri tahmin etme başarısında sadece bir embriyolog için anlamlı artış gözlendi ($p<0,05$). Diğer iki embriyolog için matür oositleri tahmin etme oranları çalışmanın birinci aşamasının sonuçlarıyla karşılaştırılabilir düzeydeydi. Tek seferde değerlendirilen KOK sayısı ile tahmin başarısı arasında istatistiksel olarak anlamlı bir ilişki saptanmadı.

**PRECIS:** Morphological assessment of cumulus-oocyte complexes before denudation does not provide definitive results about oocyte maturity.

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**Sonuç:** Denudasyon öncesi KOK'ların morfolojik değerlendirilmesi olgun ve olgun olmayan oositlerin belirlenmesinde her zaman doğru sonuçlar vermemektedir.

**Anahtar Kelimeler:** Oosit, maturasyon, in vitro fertilizasyon

## Introduction

Diminished ovarian reserve (DOR) is a challenging issue in in vitro fertilization (IVF) treatment, leading to low follicle counts, high cancellation rates, and decreased IVF success. Premature ovarian insufficiency and poor ovarian response are other challenging issues that account for a significant portion of IVF patients with DOR[1-3]. To date, numerous pharmacological add-on treatments have been applied to increase IVF success, including testosterone replacement therapy and growth hormone supplementation[4]. Recently, autologous platelet-rich plasma and exosome injections into ovaries have also been performed, resulting in an increase in antral follicle count, mature oocyte (M2) count, and pregnancy rates[5]. Additionally, in vitro maturation (IVM), which enhances IVF success since it allows for retrieval of mature oocytes from small antral follicles[6,7], is a treatment option that has become increasingly popular in recent years[6].

IVM is defined as an assisted reproductive technology (ART) involving in vitro maturation of immature oocytes collected from small antral follicles. There are numerous IVM regimens introduced in the literature, namely standard IVM, biphasic IVM, human chorionic gonadotrophin (hCG)-primed IVM, and rescue IVM[7]. In all these regimens, the key aim is to achieve IVM of immature oocytes without denudation of cumulus-oocyte complexes (COCs), mainly due to the bidirectional communication between oocyte and cumulus cells that supports oocyte growth and maturation[6,7]. However, this is the case in standard and biphasic IVM; cumulus cells are denuded in rescue IVM in order to distinguish mature and immature oocytes. Therefore, IVM success is significantly lower in rescue IVM. Accordingly, if mature and immature oocytes in small follicles can be differentiated before denudation in IVM, it may be possible to obtain a higher number of mature oocytes in conventional IVF patients.

In this study, we aimed to investigate the predictive value of morphological assessment of COCs prior to denudation in distinguishing mature and immature oocytes in conventional IVF cycles.

## Materials and Methods

This is a single-center prospective observational study conducted at Acıbadem Maslak Hospital IVF department between February 1 and April 1, 2025. The study was approved by Acıbadem University Ethics Committee and was performed in accordance with the ethical standards described in the 1975 Declaration of Helsinki, as revised in 2000 (approval no: 2025-04/157, date: 06.03.2025). Informed consent was obtained from each patient prior to the study. On the 2nd or the 3rd day of the menstrual cycle, the uterus and bilateral adnexa were examined, and antral follicles were counted automatically by vaginal sonography. Estradiol and progesterone levels were also measured for each patient. The antagonist protocol was applied to all patients, and follicles were triggered by hCG, gonadotropin-releasing hormone agonist (GnRHa), or a combination of both. Oocyte retrieval was performed 36 hours after the trigger using transvaginal sonographic guidance, with a 17 G needle, under sedation. Approximately two hours later, COCs were denuded and immature oocytes were discarded, followed by the administration of intracytoplasmic sperm injection (ICSI) to mature oocytes.

The procedure was conducted by five embryologists with at least five years of experience. The study consisted of two stages:

### First Stage

The first stage was undertaken by all five embryologists. After oocyte retrieval, COCs were evaluated under a microscope by one embryologist and they were distinguished as mature or immature based on their morphological appearance. Two hours later, COCs were denuded by another embryologist and the counts of mature and immature oocytes were recorded. The first stage was terminated when each embryologist had evaluated a minimum of 100 COCs.

### Second Stage

The second stage was undertaken by three embryologists. The procedure in this stage was applied continuously for one more month to assess its effect on the success rate. Mature and immature oocytes were distinguished based on the following criteria:

1. COCs consisting of mature oocytes with an appearance of radiating corona cells surrounded by loose granulosa cells (GCs).
2. Cumulus cells showing a bright appearance under microscope.
3. COCs consisting of immature oocytes [germinal vesicles (GVs)] characterized by an unexpanded cumulus with multiple layers of compact GSs and a dark-brown appearance.

### Statistical Analysis

All data were analyzed using SPSS (SPSS-IBM 2.3, Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to assess data normality. Categorical variables were compared using chi-square or Fisher's exact test. Continuous variables were expressed as mean ± a standard deviation (SD). Categorical variables were presented as frequencies (n) and percentages (%). Statistical significance was set at a p value of $<0.05$.

## Results

A total of 80 patients were enrolled in the study, and 1039 COCs were examined, comprising 837 (80.6%) mature and 202 (19.4%) immature oocytes. In the first stage of the study, 69% of immature and 80% of mature oocytes were identified correctly by the embryologists.

Table 1 shows the success rates obtained in the identification of mature and immature oocytes in the first stage of the study. These rates varied between 78.86% to 90.21% and 65.69% to 79.72% for mature and immature oocytes, respectively, and there was no significant difference among the embryologists in terms of the success rates.

Table 2 presents a comparison of the two stages with regard to the success rates obtained by three embryologists. Although the identification rates of immature oocytes according to the COC's morphology increased in the second stage for all three embryologists, the only significant increase was observed for AA ($p<0.05$). In the identification of mature oocytes, however, the success rate for EK increased from 88.55% to 94.01%, while it decreased for the remaining two embryologists, though not significantly.

Table 3 demonstrates the correlation between the number of COCs retrieved and the success rates. Patients were divided into four groups according to the number of COCs retrieved: (i) 1-5, (ii) 6-10, (iii) 11-20, and (iv) >21. There was no significant difference among these groups with regard to success rates.

**Table 1.** Success rates of identifying mature and immature oocytes among embryologists

| Embryologist ID | Immature (%) (median value) | Mature (%) (median value) |
|---|---|---|
| I | 66.88 | 90.21 |
| II | 65.69 | 85.55 |
| III | 79.72 | 88.97 |
| IV | 73.06 | 86.27 |
| V | 68.18 | 78.86 |
| Total | 70.95 | 86.87 |

## Discussion

The world's first IVF baby, Louise Brown, was born in 1978. Although there have been numerous advancements in the field of IVF since then, DOR remains a limiting factor for IVF procedures. To date, numerous treatment options have been used to improve the success rate of IVF in DOR patients; however, no serious progress has yet been made. IVM, particularly rescue IVM, can be a good option for improving pregnancy rates in patients with DOR. IVM is a well-known ART that was developed decades ago. However, it has not been adopted into routine practice and remains underutilized. There are numerous IVM regimens, namely standard IVM, biphasic IVM, hCG-primed IVM, and rescue IVM. In standard and biphasic IVM, follicles are stimulated using follicle-stimulating hormone analogues for an average of three days, and follicles are retrieved without hCG triggering. After retrieval, COCs are cultured in IVM medium for approximately 36 hours and then denuded. Subsequently, mature oocytes are subjected to ICSI[6,7]. During this period, cumulus cells are of paramount importance since COCs are a group consisting of oocytes and specialized GCs that support oocyte growth and maturation, and also protect oocytes from the microenvironment[8].

On the other hand, rescue IVM involves steps similar to those of conventional IVF, except for the IVM of GV or meiosis 1 (MI) oocytes. In rescue IVM, the follicles are stimulated by gonadotropins over an average period of 10-12 days and then triggered by GnRHa, hCG, or a combination of both. After retrieval, COCs are denuded and ICSI is performed on mature oocytes while immature oocytes are kept in IVM medium for approximately 24 hours[6,7]. At the end of this period, if GV or MI oocytes transform into metaphase II (MII) oocytes, ICSI is performed[6,7]. However, it is distinct from classical or biphasic IVM due to the denudation of COCs before IVM[6-8]. Therefore, the success rate of rescue IVM is not notably high because oocytes are subjected to IVM after denudation of COCs[6-8]. In a study by Lee et al.[9], rescue IVM was performed in patients with low functional ovarian reserve. Approximately three hours after oocyte retrieval, COCs were denuded and immature oocytes were matured in vitro. The authors concluded that rescue IVM in patients with low functional ovarian reserve improved the likelihood of pregnancy and delivery. However, recent studies

**Table 2.** Comparison of the predictive value of mature and immature oocytes among three embryologists between the first and the second stage of the study

| Embryologist ID | Immature (%) (first stage of the study) | Immature (%) (second stage of the study) | p-value | Mature (%) (first stage of the study) | Mature (%) (second stage of the study) | p-value |
|---|---|---|---|---|---|---|
| I (MY) | 66.88 | 85.0 | p>0.05 | 90.21 | 81.6 | p>0.05 |
| II (EK) | 65.69 | 81.82 | p>0.05 | 85.55 | 94.01 | p<0.05 |
| III (AA) | 73.06 | 97.14 | p<0.05 | 86.27 | 78.57 | p>0.05 |

suggest a decrease in the success of IVM treatment after COC denudation[10,11]. One of the main purposes of this study was to demonstrate the accuracy of morphological assessment of COCs in identifying mature and immature oocytes before denudation. The morphology of COCs provides valuable information about the maturity of oocytes. In the literature, there are a limited number of studies demonstrating the effectiveness of morphological assessment of COCs in the identification of mature and immature oocytes[12-14]. Our study showed a significant predictive value of morphological assessment of COCs, in identifying mature and immature oocytes. Specifically, mature oocytes were characterized by a bright appearance of COCs and loosely arranged GCs surrounding radiate cells, while immature oocytes exhibited a darker appearance with multilayered GCs. On the other hand, oocyte diameter may also be used to improve the accuracy of this identification. In a study with Pors et al.[12], the association between oocyte diameter and maturation rate was assessed. The authors classified the COCs according to the diameter of oocytes and reported that the diameter was positively associated with a higher incidence of MII. They also suggested that the diameter of MII oocytes was significantly larger than the diameter of GV oocytes[12]. A recent study by Batsry et al.[13] investigated the accuracy in predicting oocyte maturity before denudation and, in a similar way to our study, evaluated the success rate of experienced embryologists in differentiating MII and GV oocytes before denudation. They found that the embryologists correctly identified 90% of MII oocytes and 72.7% of GV oocytes. In a similar study, Hammitt et al.[14] assessed the ability of three embryologists to identify the maturity of oocytes before denudation and reported the rates of accuracy as 74%, 64%, and 47%. Unlike in previous studies, this study evaluated the accuracy rates of the embryologists over an extended period. The study noted that there was no significant change in the accuracy rates for the first embryologist, while the rate for the second embryologist increased over the first nine months before reaching a plateau. As for the third embryologist, the rate showed a continuous increase throughout the study and reached 72% at the end of 17 months[14]. Although we obtained similar findings, our study consisted of two stages of identification in a similar way to the study by Hammitt et al.[14]. Our findings also showed that embryologists who consistently made the distinction between MII and GV oocytes demonstrated greater success.

**Table 3.** The relationship between the number of cumulus-oocyte complexes and the prediction success

| Oocyte category | Immature (%) | Mature (%) |
|---|---|---|
| Category I | 83.33 | 95.0 |
| Category II | 91.29 | 81.07 |
| Category III | 77.58 | 86.55 |
| Category IV | 61.20 | 84.11 |
| Total | 79.00 | 85.70 |

### Study Limitations

The first limitation of our study was its short duration. Particularly in the second phase, the study could have been planned for three months, or longer. The second limitation of our study was that there was a relatively small number of COCs evaluated by the embryologists.

## Conclusion

Morphological assessment of COCs before denudation does not provide definitive results about oocyte maturity, but the success rate may be higher if the procedure is performed by the same embryologist. In conclusion, this method does not seem to have sufficient accuracy in identifying mature and immature oocytes before denudation and thus further studies are needed to explore a more accurate procedure.

### Ethics

**Ethics Committee Approval:** The study was approved by Acıbadem University Ethics Committee and was performed in accordance with the ethical standards described in the 1975 Declaration of Helsinki, as revised in 2000 (approval no: 2025-04/157, date: 06.03.2025).

**Informed Consent:** Informed consent was obtained from each patient prior to the study.

### Footnotes

### Authorship Contributions

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

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

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## References

1. Cohen J, Chabbert-Buffet N, Darai E. Diminished ovarian reserve, premature ovarian failure, poor ovarian responder--a plea for universal definitions. J Assist Reprod Genet. 2015;32:1709-12.
2. Yun BH, Kim G, Park SH, Noe EB, Seo SK, Cho S, et al. *In vitro* fertilization outcome in women with diminished ovarian reserve. Obstet Gynecol Sci. 2017;60:46-52.
3. Reig A, Garcia-Velasco JA, Seli E. Bologna vs. POSEIDON criteria as predictors of the likelihood of obtaining at least one euploid embryo in poor ovarian response: an analysis of 6,889 cycles. Fertil Steril. 2023;120:605-14.

4. Conforti A, Carbone L, Di Girolamo R, Iorio GG, Guida M, Campitiello MR, et al. Therapeutic management in women with a diminished ovarian reserve: a systematic review and meta-analysis of randomized controlled trials. Fertil Steril. 2025;123:457-76.
5. Cakiroglu Y, Yuceturk A, Karaosmanoglu O, Kopuk SY, Korun ZEU, Herlihy N, et al. Ovarian reserve parameters and IVF outcomes in 510 women with poor ovarian response (POR) treated with intraovarian injection of autologous platelet rich plasma (PRP). Aging (Albany NY). 2022;14:2513-23.
6. Sanchez F, Le AH, Ho VNA, Romero S, Van Ranst H, De Vos M, et al. Biphasic in vitro maturation (CAPA-IVM) specifically improves the developmental capacity of oocytes from small antral follicles. J Assist Reprod Genet. 2019;36:2135-44.
7. Gilchrist RB, Smitz J. Oocyte in vitro maturation: physiological basis and application to clinical practice. Fertil Steril. 2023;119:524-39.
8. Turathum B, Gao EM, Chian RC. The function of cumulus cells in oocyte growth and maturation and in subsequent ovulation and fertilization. Cells. 2021;10:2292.
9. Lee HJ, Barad DH, Kushnir VA, Shohat-Tal A, Lazzaroni-Tealdi E, Wu YG, et al. Rescue in vitro maturation (IVM) of immature oocytes in stimulated cycles in women with low functional ovarian reserve (LFOR). Endocrine. 2016;52:165-71.
10. De Vos M, Grynberg M, Ho TM, Yuan Y, Albertini DF, Gilchrist RB. Perspectives on the development and future of oocyte IVM in clinical practice. J Assist Reprod Genet. 2021;38:1265-80.
11. Morato ALC, Verruma CG, Furtado CLM, Dos Reis RM. In vitro maturation of oocytes: what is already known?†. Biol Reprod. 2025;112:18-30. Erratum in: Biol Reprod. 2025;112:415.
12. Pors SE, Nikiforov D, Cadenas J, Ghezelayagh Z, Wakimoto Y, Jara LAZ, et al. Oocyte diameter predicts the maturation rate of human immature oocytes collected ex vivo. J Assist Reprod Genet. 2022;39:2209-14.
13. Batsry L, Orvieto R, Mor-Hadar D, Yung Y, Gitman H, Shimon C, et al. Can we predict oocyte maturation prior to denudation for intracytoplasmic sperm injection? Gynecol Endocrinol. 2020;36:265-7.
14. Hammitt DG, Syrop CH, Van Voorhis BJ, Walker DL, Miller TM, Barud KM, et al. Prediction of nuclear maturity from cumulus-coronal morphology: influence of embryologist experience. J Assist Reprod Genet. 1992;9:439-46.

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# Diagnostic and prognostic value of transaminase complex-platelet ratio in intrahepatic cholestasis of pregnancy: A novel composite index based on routine blood tests

## *Gebeliğin intrahepatik kolestazında transaminaz kompleksi-trombosit oranının tanısal ve prognostik değeri: Rutin kan testlerine dayalı yeni bir bileşik endeks*

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### Abstract

**Objective:** The study aims to evaluate the transaminase complex-platelet ratio (TACPR), a novel composite biomarker derived from routine laboratory parameters, in its ability to serve as a predictor of intrahepatic cholestasis of pregnancy (ICP) and related adverse perinatal outcomes.

**Materials and Methods:** This retrospective study included 98 pregnant women diagnosed with ICP and 100 matched healthy controls at a tertiary referral center between January 2024 and March 2025. TACPR was calculated as (alanine aminotransferase x aspartate aminotransferase)/platelet count. Groups were compared in terms of clinical characteristics, TACPR values (first trimester and diagnosis), and perinatal outcomes. Receiver operating characteristic analysis and multivariate logistic regression were used to assess predictive performance and independent risk factors for ICP and composite adverse perinatal outcomes (CAPO).

**Results:** TACPR values were significantly higher in the ICP group at both time points ($p<0.001$). In the first trimester, a TACPR >1.35 predicted ICP [area under curve (AUC)=0.806], while a TACPR >1.81 predicted CAPO (AUC=0.759). At diagnosis, a TACPR >27.7 predicted severe ICP and a TACPR >7.15 predicted CAPO. TACPR >1 in the first trimester was independently associated with ICP [odds ratio (OR)=5.49, $p<0.001$], and TACPR >50 at diagnosis was independently associated with CAPO (OR=4.38, $p=0.009$). A weak yet statistically significant correlation was identified between first trimester TACPR and peak serum bile acid levels ($r=0.325$, $p=0.001$).

**Conclusion:** TACPR is a novel, cost-effective biomarker for early identification and risk stratification of ICP and associated perinatal complications. Its integration into routine prenatal screening may enhance timely diagnosis and intervention, particularly in resource-limited settings.

**Keywords:** Composite biomarker, intrahepatic cholestasis of pregnancy, liver enzymes, perinatal outcomes, TACPR

### Öz

**Amaç:** Bu çalışmanın amacı rutin laboratuvar parametrelerinden elde edilen yeni bir bileşik biyomarker olan transaminaz kompleksi-trombosit oranının (TAKPO), gebeliğin intrahepatik kolestazı (GİHK) ve ilgili perinatal advers sonuçları öngörmede tanısal ve prognostik değerini değerlendirmektir.

**Gereç ve Yöntemler:** Bu retrospektif çalışmaya, Ocak 2024 ile Mart 2025 tarihleri arasında bir üçüncü basamak sevk merkezinde GİHK tanısı alan 98 hamile kadın ve 100 eşleştirilmiş sağlıklı kontrol dahil edildi. TAKPO, (alanin aminotransferaz x aspartat aminotransferaz)/platelet sayısı olarak hesaplandı. Gruplar klinik özellikler, TAKPO değerleri (ilk trimester ve tanı) ve perinatal sonuçlar açısından karşılaştırıldı. Alıcı işletim karakteristiği analizi ve çok

**PRECIS:** Transaminase complex-platelet ratio, a novel biomarker derived from routine blood tests, effectively predicts the onset, severity, and adverse perinatal outcomes of intrahepatic cholestasis of pregnancy.

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değişkenli lojistik regresyon, GİHK ve kompozit perinatal olumsuz sonuçlar (KAPO) için prediktif performansı ve bağımsız risk faktörlerini değerlendirmek için kullanıldı.

**Bulgular:** TAKPO değerleri her iki zaman noktasında da GİHK grubunda anlamlı olarak daha yüksekti (p<0,001). İlk trimesterde TAKPO >1,35 GİHK'yi [eğri altındaki alan (AUC)=0,806], >1,81 ise KAPO'yu (AUC=0,759) öngördü. Tanı anında, TAKPO >27,7 şiddetli GİHK'yi, >7,15 ise KAPO'yu öngörmüştür. İlk trimesterde TAKPO >1, GİHK ile bağımsız olarak ilişkiliydi [risk oranı (OR)=5,49, p<0,001] ve tanı anında TAKPO >50, KAPO ile bağımsız olarak ilişkiliydi (OR=4,38, p=0,009). İlk trimester TAKPO ile pik serum safra asidi düzeyleri arasında zayıf ancak anlamlı bir korelasyon gözlendi (r=0,325, p=0,001).

**Sonuç:** TAKPO, GİHK ve ilişkili perinatal komplikasyonların erken tanısı ve risk sınıflandırması için yeni ve maliyet-etkin bir biyomarkerdir. Rutin prenatal taramaya dahil edilmesi, özellikle kaynakların sınırlı olduğu ortamlarda, zamanında tanı ve müdahaleyi artırabilir.

**Anahtar Kelimeler:** Bileşik biyomarker, gebeliğin intrahepatik kolestazı, karaciğer enzimleri, perinatal sonuçlar, TAKPO

## Introduction

The most prevalent prenatal liver disease is known as intrahepatic cholestasis of pregnancy (ICP)[1]. The incidence of ICP varies by geographical region, ethnic origin of the population, and accepted diagnostic criteria. It ranges from 0.3% to 5.6%[2], with an approximate rate of 0.9% in our country[3]. This disease manifests clinically in the second trimester and later stages of pregnancy, and is characterized by elevated serum bile acid (SBA) concentrations (>10 µmol/L) or abnormal liver function tests. It is frequently accompanied by pruritus, particularly on the palms or soles, without cutaneous rash. These symptoms and clinical findings usually resolve rapidly after birth[2,4].

The ICP is related to poor perinatal outcomes such as preterm birth, low birth weight (LBW), meconium-stained amniotic fluid, fetal asphyxia, and intrauterine death[2,5]. Although uncertainties remain regarding its pathogenesis, multifactorial processes (genetic predisposition, hormonal and environmental factors) are thought to play a role. An increase in bile acid concentrations in amniotic fluid, which is associated with altered expression of hepatobiliary transport proteins due to increased estrogen and progesterone levels during pregnancy, may be one of the underlying mechanisms of these complications[2,6-8].

The biomarkers of hepatocellular injury, aspartate transaminase (AST) and alanine transaminase (ALT), catalyze the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid. This process is involved in gluconeogenesis, resulting in the formation of oxaloacetic acid and pyruvic acid. AST, which is found in the cytosol and mitochondria of cells, is present in the liver as well as in heart muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes, and red blood cells. Consequently, as compared to ALT, AST is less sensitive and specific for evaluating hepatic function. An increase in AST may also be due to non-hepatic causes[9]. ALT, a cytosolic enzyme found in high levels in the liver, is more likely than AST to increase in the blood in most liver diseases associated with hepatocyte cytosol pathology (e.g., acute viral hepatitis). Even in cases of hepatocellular damage without cell death, these enzymes tend to be released into the circulation[9,10].

The ICP is frequently diagnosed in the latter stages of pregnancy. Additionally, SBA level measurement, a valuable diagnostic tool, is costly and not readily accessible. These factors have precipitated the necessity for more accessible and cost-effective techniques, such as routine blood tests, to predict this disease in the early weeks of pregnancy. In line with these objectives, the APRI score obtained by dividing the AST by platelet (PLT) count[11]; has been associated with many pathologies previously linked to liver damage[10,12,13] has recently been introduced as a valuable and easy-to-use routine blood test index for the early prediction of ICP and adverse perinatal outcomes[4,14]. In this study, considering that ALT is a highly sensitive biomarker for liver diseases, we present the transaminase complex-platelet ratio (TACPR), obtained by adding the multiplying value of ALT to the APRI score, as a novel and more robust predictor index in ICP and its outcomes.

## Materials and Methods

This retrospective study was carried out at the Perinatology Clinic of Ankara Bilkent City Hospital between January 2024 and March 2025. The study was reviewed by the Ethics Committee of the Republic of Türkiye Ministry of Health, Ankara Bilkent City Hospital, and approved by the Institutional Review Board (approval number: TABED 2-25-1116, date: 30.04.2025). Every part of the study followed the rules of the Declaration of Helsinki.

Women aged 18-45 were included in the study. The case group consisted of 98 pregnant women who were hospitalized due to ICP during the study period and gave birth in the maternity ward of our hospital. A control group of 100 healthy, low-risk pregnant women was included and matched with the case group based on demographic characteristics and median gestational weeks at blood sampling (Figure 1). The diagnosis of ICP was based on the presence of pruritus and maternal SBA concentrations >10 µmol/L following exclusion of other hepatobiliary causes[15]. Multiple gestations, pregnancies with known multisystemic diseases (malignancies, hepatobiliary, rheumatological, or cardiovascular diseases), obstetric pathologies other than ICP (gestational diabetes, hypertensive disorders of pregnancy, placental abruption), active viral or bacterial infections, and congenital anomalies were excluded from the study. No medications or medical/surgical interventions were administered to any pregnant women in the study population prior to blood sampling.

The researchers obtained the medical records of the study groups from the hospital database retrospectively. The recorded data included maternal age, body mass index (BMI) (calculated by dividing weight in kilograms by the square of height in

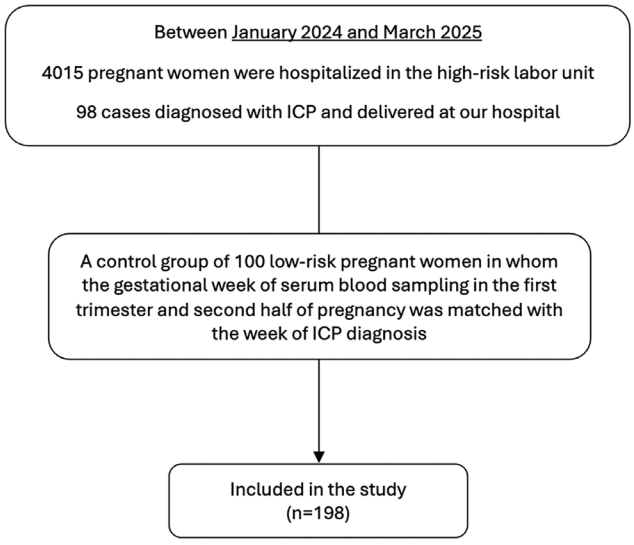


**Figure 1.** Flowchart of the study population

*ICP: Intrahepatic cholestasis of pregnancy*

meters), gravidity, parity, C-section rate, gestational age at ICP diagnosis, highest SBA concentration (µmol/L) in the case group, and first trimester and diagnosis-time AST, ALT, PLT, and TACPR values, as well as perinatal outcomes. Information on birth week and weight, 1- and 5-minute APGAR scores, preterm birth and LBW rates, presence of meconium-stained amniotic fluid, neonatal intensive care unit (NICU) admission, and stillbirth were recorded as perinatal outcomes. TACPR was calculated by dividing the product of AST and ALT values by PLT [TACPR=AST (IU/L) x ALT (IU/L)/PLT (10/L)]. While births occurring before the 37th week of pregnancy are considered preterm[16], births weighing less than 2500 grams are considered LBW[17]. Composite adverse perinatal outcomes (CAPO) are defined as stillbirth alone or the presence of at least two of the following: preterm birth, LBW, APGAR score of less than seven at 1 minute and 5 minutes, meconium-stained amniotic fluid, and admission to the NICU. The ICP and control groups were compared in terms of clinical and demographic characteristics, first trimester and diagnosis week, TACPR indices, perinatal outcomes, and CAPO.

The ICP cases were divided into two groups according to SBA levels at diagnosis: mild ICP (SBA: 10-40 µmol/L) and severe ICP (SBA >40 µmol/L)[18]. These groups, classified according to clinical severity, were compared in terms of parameters measured between the ICP and control groups, as well as the highest SBA concentrations, the number of patients receiving ursodeoxycholic acid (UDCA) treatment, and the average UDCA dose (mg/day).

### Statistical Analysis

The required sample size was calculated using the G*Power software (version 3.1; Heinrich-Heine-Universität, Düsseldorf, Germany). Assuming a medium effect size ($f^2$=0.15), a significance level of 0.05, and 80% statistical power, the minimum sample needed was 76 participants per group. All statistical procedures were carried out with the Statistical Package for Social Sciences (SPSS, version 26.0; IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation if normally distributed, or as median with interquartile range or with minimum-maximum values when not normally distributed. Categorical variables were summarized as frequencies and percentages. Normality of the data was tested using the Kolmogorov-Smirnov test. For group comparisons, normally distributed continuous data were analyzed with the Independent Samples t-test, while non-normally distributed data were compared using the Mann-Whitney U test. Associations between categorical variables were assessed using the Pearson chi-square test or Fisher's exact test where appropriate.

In the ICP group, correlations between TACPR values (measured during the first trimester and at diagnosis) and peak SBA levels were examined using Pearson's correlation analysis, and the correlation coefficient (r) was reported. Receiver operating characteristic (ROC) curve analysis was applied to determine cut-off points of TACPR indices for predicting ICP development and severity, as well as CAPO occurrence, with the Youden index used to optimize sensitivity and specificity.

Multivariable logistic regression was performed to identify independent predictors of ICP and CAPO. Candidate variables with p<0.10 in univariate analysis were included in the multivariable model using the enter (forced entry) method. The model's calibration and goodness of fit were evaluated with the Hosmer–Lemeshow test. Results are presented as odds ratios (OR) with 95% confidence intervals CIs. A two-sided p<0.05 was considered statistically significant for all analyses.

## Results

During the study period, a total of 4015 pregnant women were hospitalized in the high-risk labor unit, and the incidence of ICP was 2.44%. The median diagnosis week in the ICP group was at 33, and higher C-section rates were observed in this group (p=0.007). The TACPR value during the first trimester and at the diagnosis week was significantly higher in cases of ICP (p<0.001, all). Preterm birth, LBW, meconium-stained amniotic fluid, and NICU admission rates were significantly higher in the ICP group (p<0.05, all). Although stillbirth rates were higher in the ICP group, the difference was not statistically significant (p=0.244). When these adverse perinatal outcomes were evaluated, the CAPO rate was 30.6% in cases of ICP and was found to be statistically significant (p<0.001). Detailed data on the comparison of clinical and demographic characteristics, TACPR, and perinatal outcomes between the study groups are presented in Table 1.

No statistically significant disparity was not identified between mild and severe ICP with regard to demographic characteristics, C-section rates, and median gestational weeks at diagnosis

**Table 1.** Comparison of clinical and demographic data, TACPR, and perinatal outcomes between study groups

| | ICP group (n=98) | Controls (n=100) | p-value* |
|---|---|---|---|
| Age | 28.62±5.14 | 29.28±5.30 | 0.377[a] |
| BMI (kg/m$^2$) | 27.76±2.72 | 27.05±2.47 | 0.059[a] |
| Gravidity | 2 (1) | 2 (2) | **0.017**[b] |
| Parity | 0 (1) | 1 (1) | **0.042**[b] |
| GA at diagnosis (weeks) | 33 (5) | 33 (4)# | 0.925[b] |
| C-section rates | 44 (44.9%) | 26 (26%) | **0.007**[c] |
| **First trimester blood parameters** | | | |
| AST (IU/L) | 22 (11) | 16.35±5.60 | **<0.001**[b] |
| ALT (IU/L) | 27.5 (19.5) | 14.85±5.67 | **<0.001**[b] |
| PLT (10$^9$/L) | 260.21±58.96 | 244.27±64.74 | 0.072[a] |
| TACPR | 2.41 (3.05) | 0.86 (0.95) | **<0.001**[b] |
| **Blood parameters at the diagnosis week#** | | | |
| AST (IU/L) | 55 (64) | 17.90±5.30 | **<0.001**[b] |
| ALT (IU/L) | 85.5 (112.5) | 17.17±6.41 | **<0.001**[b] |
| PLT (10$^9$/L) | 255 (94) | 243.88±64.35 | **0.039**[b] |
| TACPR | 20.67 (47.38) | 1.24 (1.01) | **<0.001**[b] |
| **Perinatal outcomes** | | | |
| GA at delivery (week) | 37 (1) | 38 (2) | **<0.001**[b] |
| Preterm birth | 32 (32.7%) | 5 (5%) | **<0.001**[c] |
| Low birth weight (<2500 g) | 15 (15.3%) | - | **<0.001**[c] |
| Birth weight (g) | 2969.5±504.3 | 3327.45±382.27 | **<0.001**[a] |
| APGAR score (1$^{st}$ min.) | 7 (1) | 7 (1) | 0.972[b] |
| APGAR score (5$^{th}$ min.) | 9 (1) | 9 (0) | 0.277[b] |
| Meconium-stained amniotic fluid | 10 (10.2%) | 2 (2%) | **0.018**[c] |
| NICU admission | 25 (25.5%) | 3 (3%) | **<0.001**[c] |
| Stillbirth | 2 (2.04%) | - | 0.244[c] |
| CAPO | 30 (30.6%) | 2 (2%) | **<0.001**[c] |

ALT: Alanine transaminase, AST: Aspartate transaminase, BMI: Body mass index, CAPO: Composite adverse perinatal outcomes, GA: Gestational age, ICP: Intrahepatic cholestasis of pregnancy, NICU: Neonatal intensive care unit, PLT: Platelet, TACPR: Transaminase complex-to-platelet ratio

**Data Presentation:** Continuous variables are expressed as **mean ± standard deviation** or **median with interquartile range**, depending on distribution. Categorical data are shown as **frequency and percentage**.

#**Control Group Note:** The median GA at the time of sample collection in the control group was aligned with the diagnostic week of ICP in the case group.

*Significance testing was performed using:

[a]: Independent samples t-test

[b]: Mann-Whitney U test

[c]: Fisher's exact test

A p-value below 0.05 was regarded as statistically significant. Outcomes meeting this threshold are **emphasized in bold.**

(p>0.05, all). Although there was no significant difference in the TACPR value between the mild and severe ICP groups for the first trimester (p=0.204), the TACPR value at diagnosis was significantly higher for severe ICP cases (p<0.001). The highest SBA concentration, the percentage of patients receiving UDCA treatment, and the daily UDCA dose were significantly higher in severe ICP cases (p<0.05, all). The CAPO rate (38.5%) was also found to be significantly higher in severe ICP due to higher rates of preterm delivery, meconium-stained amniotic fluid, and admission to the NICU (p<0.05, all). Detailed data on the comparison of clinical and demographic characteristics, TACPR, and perinatal outcomes according to groups based on the clinical severity of ICP are presented in Table 2.

**Table 2.** Comparison of clinical and demographic characteristics, TACPR, and perinatal outcomes in groups based on the clinical severity of ICP

| | Mild ICP (n=72) | Severe ICP (n=26) | p-value* |
|---|---|---|---|
| Age | 28.34±5.44 | 28.92±5.10 | 0.763[a] |
| BMI (kg/m$^2$) | 27.73±2.91 | 28.15±2.08 | 0.275[a] |
| Gravidity | 2 (2) | 2 (3) | 0.292[b] |
| Parity | 0 (1) | 0 (2) | 0.320[b] |
| GA at diagnosis (weeks) | 33 (4) | 33 (6) | 0.238[b] |
| C-section rates | 29 (40.3%) | 15 (57.7%) | 0.168[c] |
| **First trimester blood parameters** | | | |
| AST (IU/L) | 22.08±7.83 | 31.12±17.24 | **0.020**[a] |
| ALT (IU/L) | 28.5 (20.5) | 29 (38) | 0.275[b] |
| PLT (10$^9$/L) | 254.96±59.98 | 271.48±58.05 | 0.275[a] |
| TACPR | 2.26 (2.95) | 3.11 (7.7) | 0.204[b] |
| **Blood parameters at the diagnosis week** | | | |
| AST (IU/L) | 53.5 (58) | 96 (104.5) | **<0.001**[b] |
| ALT (IU/L) | 88 (84.25) | 230.84 (227.5) | **<0.001**[b] |
| PLT (10$^9$/L) | 255 (74) | 265.36±76.82 | 0.971[b] |
| TACPR | 17.39 (32.1) | 59.1 (201.27) | <0.001[b] |
| **Clinical characteristics** | | | |
| Highest SBA concentration (µmol/L) | 19.68±7.08 | 80.70±31.67 | **<0.001**[a] |
| Cases treated with UDCA | 56 (77.8%) | 25 (96.2%) | **0.037**[c] |
| Dose of UDCA (mg/day) | 750 (187.5) | 750 (250) | **0.002**[b] |
| Perinatal outcomes | | | |
| GA at delivery (week) | 37 (1) | 36 (2) | **0.016**[b] |
| Preterm birth | 17 (23.6%) | 15 (57.7%) | **0.003**[c] |
| Low birth weight (<2500 g) | 11 (15.3%) | 4 (15.4%) | 1.000[c] |
| Birth weight (g) | 2952.5±506.42 | 2879.8±461 | 0.430[a] |
| APGAR score (1$^{st}$ min.) | 7 (1) | 7 (1) | **0.049**[b] |
| APGAR score (5$^{th}$ min.) | 9 (1) | 9 (1) | 0.245[b] |
| Meconium-stained amniotic fluid | 3 (4.2%) | 7 (26.9%) | **0.003**[c] |
| NICU admission | 10 (13.9%) | 15 (57.7%) | **<0.001**[c] |
| Stillbirth | 1 (1.4%) | 1 (3.8%) | 0.462[c] |
| CAPO | 14 (19.4%) | 16 (38.5%) | **<0.001**[c] |

ALT: Alanine transaminase, AST: Aspartate transaminase, BMI: Body mass index, CAPO: Composite adverse perinatal outcomes, GA: Gestational age, ICP: Intrahepatic cholestasis of pregnancy, NICU: Neonatal intensive care unit, PLT: Platelet, SBA: Serum bile acid, TACPR: Transaminase complex-to-platelet ratio, UDCA: Ursodeoxycholic acid

**Data Presentation**: Values are expressed as mean ± standard deviation for normally distributed continuous variables, median with interquartile range for non-normally distributed data, or count (percentage) for categorical variables.

*Significance levels were assessed using the following tests:

[a]: Independent sample t-test,

[b]: Mann-Whitney U test,

[c]: Fisher's exact test.

A p-value below 0.05 was regarded as statistically significant. Outcomes meeting this threshold are **emphasized in bold.**

In cases of ICP, a weak positive correlation was observed between TACPR in the first trimester and the highest SBA concentration (r=0.325, p=0.001), while also no significant correlation between TACPR at the time of diagnosis and the highest SBA concentration (p=0.060). In the study population, ROC analyses were performed to calculate the optimal cut-off values of first trimester TACPR for predicting ICP and CAPO, and these values were determined as 1.35 [68.4% sensitivity, 68% specificity, (AUC) 0.806, p<0.001] and 1.81 [65.6% sensitivity, 65.7% specificity, (AUC) 0.759, p<0.001], respectively. The ROC curves for these analyses are shown in Figure 2A, 2B. In cases of ICP, the optimal cut-off value of TACPR at diagnosis for predicting severe ICP was determined to be 27.7 [69.2% sensitivity, 70.8% specificity, (AUC) 0.774, p<0.001]. In the study population, the optimal cut-off value of TACPR at diagnosis for predicting CAPO was determined to be 7.15 [75% sensitivity, 74.1% specificity, (AUC) 0.807, p<0.001]. The ROC curves for these analyses are shown in Figure 3A, 3B. A summary of ROC analyses showing the cut-off values of the first trimester and the diagnosis week TACPR indices in predicting clinical outcomes associated with ICP and CAPO is presented in Table 3.

Multivariate logistic regression analysis was conducted to identify independent predictors of ICP in the study population (n=198). The model demonstrated a good fit (Nagelkerke $R^2$=0.426; Hosmer and Lemeshow test, $\chi^2$=8.606, df=8, p=0.377). Three variables exhibited statistical significance as independent predictors of ICP development. First trimester ALT levels were positively associated with the development of ICP (OR=1.16, 95% CI: 1.09-1.24, p<0.001), indicating that for each unit increase in ALT, the odds of developing ICP increased by 16%. In contrast, first trimester AST levels were inversely associated with ICP risk (OR=0.84, 95% CI: 0.78-0.90, p<0.001). Furthermore, patients with a TACPR greater than 1 in the first trimester had significantly higher odds of developing ICP (OR=5.49, 95% CI: 2.48-12.18, p<0.001) (Table 4).

Among patients diagnosed with ICP (n=98), multivariate logistic regression was performed additionally to evaluate predictors of CAPO. The model fit was robust (Nagelkerke $R^2$=0.477; Hosmer and Lemeshow test, $\chi^2$=1.632, df=4, p=0.803). TACPR remained a consistent risk indicator in this subgroup analysis. A TACPR value >5 in the first trimester was associated with a threefold increase in the odds of CAPO (OR=3.06, 95% CI: 1.06-8.83, p=0.038), while a TACPR >50 at the time of ICP diagnosis was associated with a greater than fourfold increased risk (OR=4.38, 95% CI: 1.44-13.30, p=0.009). Additionally, severe ICP was independently associated with a significantly elevated risk of CAPO (OR=4.08, 95% CI: 1.39-11.93, p=0.010) (Table 5).

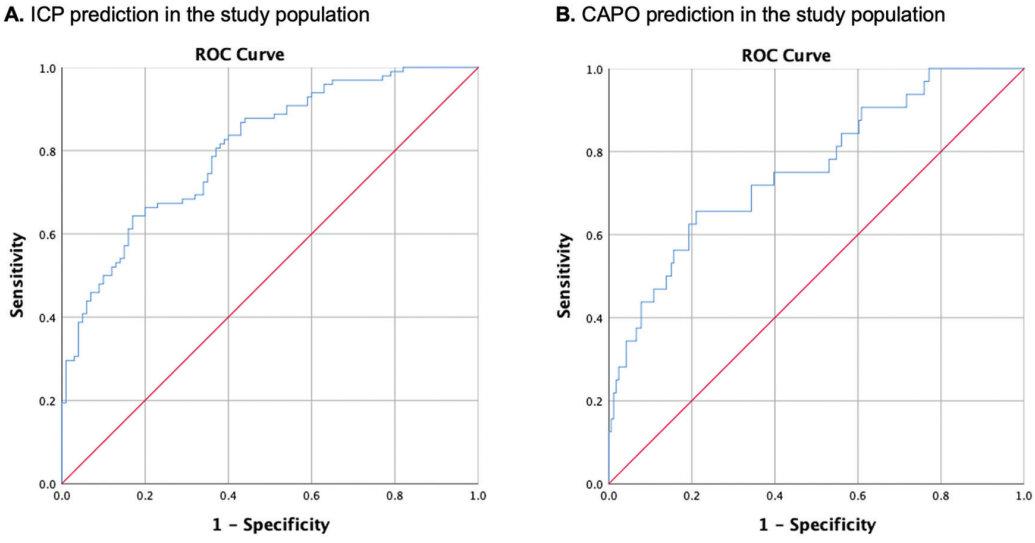


**Figure 2.** Receiver operating characteristic curves of first trimester TACPR in predicting ICP (A) and CAPO (B) in the study population

*ROC: Receiver operating characteristic, CAPO: Composite adverse perinatal outcomes, ICP: Intrahepatic cholestasis of pregnancy, TACPR: Transaminase complex-platelet ratio*

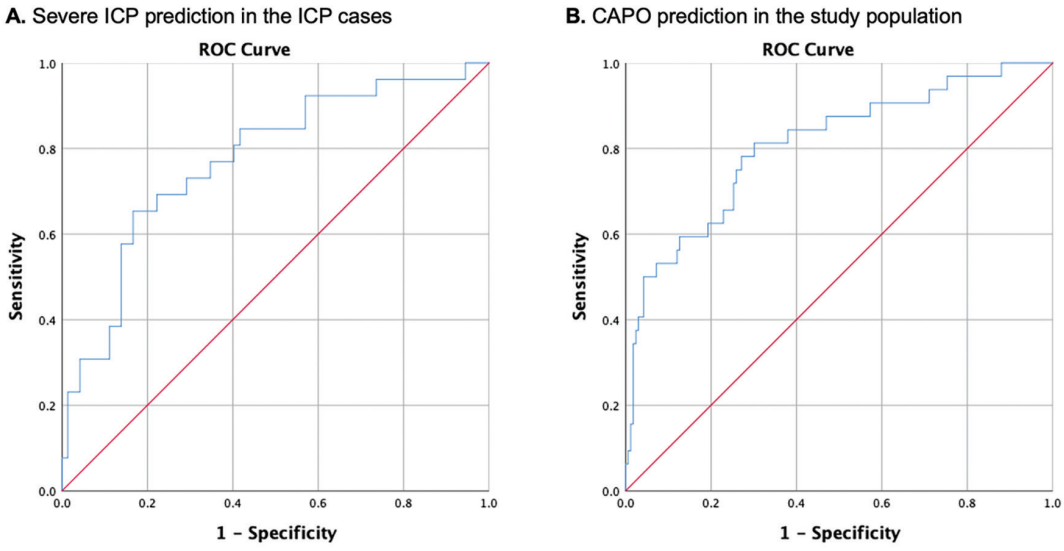


**Figure 3.** Receiver operating characteristic curves of TACPR at diagnosis week in predicting severe ICP in the case group (A) and CAPO in the study population (B)

*ROC: Receiver operating characteristic, CAPO: Composite adverse perinatal outcomes, ICP: Intrahepatic cholestasis of pregnancy, TACPR: Transaminase complex-platelet ratio*

**Table 3.** Receiver operating characteristics analysis table showing cut-off values of first trimester and diagnosis week TACPR indices in predicting clinical outcomes associated with ICP and CAPO

| Variable | Group | Outcome | AUC | Std. error | Sensitivity | Specificity | Asymp. Sig* | 95% CI | | Cut-off value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Lower | Upper | |
| First trimester TACPR | All | ICP | 0.806 | 0.030 | 68.4% | 68% | <0.001 | 0.747 | 0.864 | 1.35 |
| First trimester TACPR | All | CAPO | 0.759 | 0.048 | 65.6% | 65.7% | <0.001 | 0.665 | 0.853 | 1.81 |
| TACPR at diagnosis week | ICP | Severe ICP | 0.774 | 0.055 | 69.2% | 70.8% | <0.001 | 0.666 | 0.881 | 27.7 |
| TACPR at diagnosis week | All | CAPO | 0.807 | 0.045 | 75% | 74.1% | <0.001 | 0.719 | 0.896 | 7.15 |

AUC: Area under the curve, CI: Confidence interval, ICP: Intrahepatic cholestasis of pregnancy, TACPR: Transaminase complex-platelet ratio, CAPO: Composite adverse perinatal outcomes
*Significance threshold: p<0.05

**Table 4.** Multivariate logistic regression analysis table showing independent risk factors associated with ICP development in the study population

| Outcome: ICP (group: all, n=198) | | | |
|---|---|---|---|
| Independent risk factors | OR | 95% CI | p-value* |
| First trimester AST (IU/L) | 0.84 | 0.78-0.90 | <0.001 |
| First trimester ALT (IU/L) | 1.16 | 1.09-1.24 | <0.001 |
| TACPR >1 at first trimester | 5.49 | 2.48-12.18 | <0.001 |

ALT: Alanine transaminase, AST: Aspartate transaminase, CI: Confidence interval, ICP: Intrahepatic cholestasis of pregnancy, OR: Odds ratio, TACPR: Transaminase complex-to-platelet ratio. Nagelkerke $R^2$=0.426; Hosmer and Lemeshow test: $\chi 2$=8.606; df=8; p=0.377.
*Significance threshold: p<0.05

**Table 5.** Multivariate logistic regression analysis table showing independent risk factors associated with the occurrence of CAPO in cases of ICP

| Outcome: CAPO (group: ICP, n=98) | | | |
|---|---|---|---|
| Independent risk factors | OR | 95% CI | p-value* |
| TACPR >5 at first trimester | 3.06 | 1.06-8.83 | 0.038 |
| TACPR >50 at diagnosis week | 4.38 | 1.44-13.30 | 0.009 |
| Severe ICP | 4.08 | 1.39-11.93 | 0.010 |

CAPO: Composite adverse perinatal outcomes, CI: Confidence interval, ICP: Intrahepatic cholestasis of pregnancy, OR: Odds ratio, TACPR: Transaminase complex–platelet ratio. Nagelkerke $R^2$=0.477; Hosmer and Lemeshow test: $\chi 2$=1.632; df=4; p=0.803.
*Significance threshold: p<0.05

## Discussion

This study evaluated the clinical utility of the TACPR as a novel, composite biomarker for the early prediction and severity assessment of ICP and related adverse perinatal outcomes. The results support TACPR as a significantly elevated marker in ICP cases both in the first trimester and at the time of diagnosis, with notable correlations to disease severity and poor perinatal outcomes. To the best of our knowledge, this study is the first to propose and clinically validate TACPR as a meaningful predictor of ICP based on the combination of existing biomarkers, including APRI and ALT.

The observed incidence of ICP in our study population (2.44%) falls within the previously reported global range of 0.3% to 5.6%[2], confirming its clinical relevance in high-risk pregnancy cohorts. However, this incidence rate is higher than expected in our country[3]; which may be due to the hospital being a referral center and only high-risk pregnancies monitored in the maternity ward. In line with prior research, ICP was associated with an increased risk of preterm birth, LBW, NICU admission, and meconium-stained amniotic fluid, consistent with documented outcomes[19-21]. Although stillbirth rates were also elevated in the ICP group, the lack of statistical significance may be due to limited sample size or timely intervention in delivery planning. In addition, the CAPO rate in severe ICP (38.5%) was double that of mild cases (19.4%), further emphasizing the clinical relevance of risk stratification at diagnosis.

The ROC curve analysis confirmed TACPR's robust predictive ability for both ICP (AUC: 0.806) and CAPO (AUC: 0.759-0.807). Notably, a first-trimester TACPR >1.35 was significantly predictive of ICP, while a cut-off >1.81 was associated with CAPO. At diagnosis, a TACPR >7.15 predicted CAPO, and a value >27.7 predicted severe ICP. These findings align with previous studies emphasizing the prognostic value of early liver function tests in ICP[4,22,23]. However, our study goes further by offering a composite score that could be integrated into routine screening.

Multivariate analysis further solidified ALT and TACPR as independent predictors of ICP, echoing similar findings in non-pregnant liver disease models[11,24]. TACPR retained its predictive power even when adjusted for gestational age and BMI. Specifically, TACPR >5 in the first trimester tripled the risk of CAPO, and TACPR >50 at diagnosis increased this risk more than fourfold. These associations may reflect worsening hepatic inflammation and impaired placental function, which are hallmarks of more severe disease forms[25].

From a pathophysiological standpoint, the hepatocellular disruption seen in ICP is influenced by hormonal, genetic, and environmental factors, many of which modulate bile acid metabolism and hepatobiliary transport[20]. Previous work has shown that ALT and AST levels increase in liver disease due to hepatocyte membrane damage, even in the absence of overt necrosis[24]. ALT, being more liver-specific, showed a stronger correlation with ICP in our multivariate model, while AST, due to its broader tissue distribution and lower specificity, showed an inverse correlation in the results of the study.

No significant differences were observed in demographic or baseline clinical characteristics, such as mother's age, BMI, gravida, or parity, when comparing mild with severe ICP. This aligns with earlier studies suggesting that biochemical markers, rather than maternal characteristics, are the primary differentiators in disease severity[25]. However, in the first trimester, patients who later developed severe ICP already exhibited significantly elevated AST levels (p=0.020), hinting at an underlying subclinical hepatocellular injury early in pregnancy.

At the time of diagnosis, severe ICP cases displayed a significantly more deranged liver profile; ALT and AST values were nearly 2-3 times higher than those in the mild group, consistent with the concept that progressive cholestasis exacerbates hepatocellular stress[24]. Notably, the TACPR value at diagnosis was over threefold higher in severe ICP (median 59.1 vs. 17.39; p<0.001), reinforcing its value as a severity marker. Interestingly, while UDCA treatment was more commonly administered in severe ICP cases, and at a higher dose, this did not appear sufficient to equalize outcomes between groups. This suggests that early detection using biomarkers like TACPR may be necessary to initiate treatment before severe liver dysfunction manifests, rather than as a response to elevated SBA.

The study revealed a weak yet statistically significant positive correlation between first trimester TACPR and the highest recorded SBA concentration during pregnancy (r=0.325, p=0.001). This finding suggests that hepatic stress or dysfunction may begin well before clinical manifestations of

ICP or SBA elevation become apparent. The absence of a similar correlation at the time of diagnosis (p=0.060) could reflect confounding influences such as disease-modifying treatments (e.g., UDCA), physiological compensations, or non-linear dynamics of bile acid accumulation. These findings highlight the potential of TACPR as an early biomarker: not necessarily for directly quantifying bile acid burden, but for flagging hepatocellular vulnerability in early pregnancy. This allows clinicians to identify at-risk pregnancies before cholestasis fully manifests. This early predictive utility aligns with the increasing demand for cost-effective, non-invasive tools to optimize timing of surveillance and intervention in obstetric hepatology.

### Study Limitations

Despite promising results, this study has some limitations. The retrospective, single-center design may limit generalizability, and external validation is necessary to confirm TACPR thresholds in different populations. Furthermore, while TACPR appears promising, clinical implementation would require harmonization of lab measurement units and prospective studies assessing real-world effectiveness.

## Conclusion

This study demonstrated that the TACPR is a valuable, novel biomarker for the early prediction and severity assessment of ICP. First-trimester elevated TACPR values were significantly associated with subsequent ICP development and adverse perinatal consequences, such as preterm birth, NICU admission, and meconium-stained amniotic fluid. Furthermore, TACPR values at diagnosis were markedly higher in severe ICP cases and independently predicted poor perinatal outcomes.

Importantly, TACPR showed a weak but significant correlation with the highest SBA concentrations when measured in early pregnancy, suggesting its utility as an early indicator of subclinical hepatocellular stress before cholestasis becomes clinically apparent. As a simple, cost-effective index derived from routine laboratory parameters, TACPR represents a promising alternative or adjunct to SBA testing, particularly in low-resource settings where SBA assays may not be readily accessible.

Incorporating TACPR into standard prenatal screening protocols could improve the timely identification of at-risk pregnancies, allowing for closer monitoring, earlier interventions, and potentially better maternal and fetal outcomes. The necessity of future prospective studies is indicated to validate its predictive thresholds and evaluate its integration into broader clinical workflows.

### Ethics

**Ethics Committee Approval:** This retrospective study was carried out at the Perinatology Clinic of Ankara Bilkent City Hospital between January 2024 and March 2025. The study was reviewed by the Ethics Committee of the Republic of Turkey Ministry of Health, Ankara Bilkent City Hospital, and approved by the Institutional Review Board (approval number: TABED 2-25-1116, date: 30.04.2025).

**Informed Consent:** Retrospective study.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: G.O., Concept: D.Ş., Design: D.Ş., Data Collection or Processing: G.O., Analysis or Interpretation: D.Ş., Literature Search: G.O., D.Ş., Writing: G.O., D.Ş.

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

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## References

1. Clinical updates in women's health care summary: liver disease: reproductive considerations. Obstet Gynecol. 2017;129:236.
2. Bicocca MJ, Sperling JD, Chauhan SP. Intrahepatic cholestasis of pregnancy: review of six national and regional guidelines. Eur J Obstet Gynecol Reprod Biol. 2018;231:180-7.
3. Odabaş RK, Sökmen Y, Dünder E, Taşpınar A. The incidence of intrahepatic cholestasis of pregnancy and its maternal, fetal, and neonatal adverse outcomes: a systematic review and meta-analysis. J Midwifery Womens Health. 2024;69:370-82.
4. Peker A, Tanaçan A, İpek G, Ağaoğlu Z, Şahin D. Role of aspartate aminotransferase to platelet ratio in the prediction and prognosis of intrahepatic cholestasis of pregnancy: a case-control study from a tertiary center. Int J Gynaecol Obstet. 2024;164:656-61.
5. Li L, Chen YH, Yang YY, Cong L. Effect of intrahepatic cholestasis of pregnancy on neonatal birth weight: a meta-analysis. J Clin Res Pediatr Endocrinol. 2018;10:38-43.
6. Meng LJ, Reyes H, Palma J, Hernandez I, Ribalta J, Sjövall J. Effects of ursodeoxycholic acid on conjugated bile acids and progesterone metabolites in serum and urine of patients with intrahepatic cholestasis of pregnancy. J Hepatol. 1997;27:1029-40.
7. Abu-Hayyeh S, Ovadia C, Lieu T, Jensen DD, Chambers J, Dixon PH, et al. Prognostic and mechanistic potential of progesterone sulfates in intrahepatic cholestasis of pregnancy and pruritus gravidarum. Hepatology. 2016;63:1287-98.
8. Williamson C, Geenes V. Intrahepatic cholestasis of pregnancy. Obstet Gynecol. 2014;124:120-33.
9. Lala V, Zubair M, Minter D. Liver function tests. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-.
10. Kasarala G, Tillmann HL. Standard liver tests. Clin Liver Dis (Hoboken). 2016;8:13-8.
11. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology. 2003;38:518-26.
12. Gonsalkorala ES, Cannon MD, Lim TY, Penna L, Willliamson C, Heneghan MA. Non-invasive markers (ALBI and APRI) predict pregnancy outcomes in women with chronic liver disease. Am J Gastroenterol. 2019;114:267-75.

13. Lin ZH, Xin YN, Dong QJ, Wang Q, Jiang XJ, Zhan SH, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. Hepatology. 2011;53:726-36.
14. Bozbay N, Kılıç Ş, Yıldırım O, Pekin AT, Örgül G. Role of aspartate aminotransferase to platelet ratio in prediction of intrahepatic cholestasis of pregnancy. Clin Exp Hepatol. 2024;10:285-90.
15. Lee RH, Mara G, Metz TD, Pettker CM. Society for maternal-fetal medicine consult series #53: intrahepatic cholestasis of pregnancy: replaces consult #13, April 2011. Am J Obstet Gynecol. 2021;224:B2-9.
16. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371:75-84.
17. Cutland CL, Lackritz EM, Mallett-Moore T, Bardají A, Chandrasekaran R, Lahariya C, et al. Low birth weight: case definition & guidelines for data collection, analysis, and presentation of maternal immunization safety data. Vaccine. 2017;35:6492-500.
18. Maternal SA. Neonatal Community of Practice. Policy clinical guideline: hepatitis B in pregnancy adelaide, Australia: government of South Australia, SA health. 2016.
19. Brouwers L, Koster MP, Page-Christiaens GC, Kemperman H, Boon J, Evers IM, et al. Intrahepatic cholestasis of pregnancy: maternal and fetal outcomes associated with elevated bile acid levels. Am J Obstet Gynecol. 2015;212:100.e1-7.
20. Bacq Y, Sentilhes L. Intrahepatic cholestasis of pregnancy: diagnosis and management. Clin Liver Dis (Hoboken). 2014;4:58-61.
21. Lee RH, Goodwin TM, Greenspoon J, Incerpi M. The prevalence of intrahepatic cholestasis of pregnancy in a primarily Latina Los Angeles population. J Perinatol. 2006;26:527-32.
22. Zhang X, Yu C, Stephen S, Yi L, Libin Z, Xiaoxi Z, et al. Prediction of intrahepatic cholestasis of pregnancy in the first 20 weeks of pregnancy. J Matern Fetal Neonatal Med. 2022;35:6329-35.
23. Luo M, Wang L, Yao H, Wen Y, Cao D, Shen W, et al. Diagnostic and prognostic value of blood inflammation and biochemical indicators for intrahepatic cholestasis of pregnancy in Chinese pregnant women. Sci Rep. 2022;12:20833.
24. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. Cmaj. 2005;172:367-79.
25. Ovadia C, Seed PT, Sklavounos A, Geenes V, Di Ilio C, Chambers J, et al. Association of adverse perinatal outcomes of intrahepatic cholestasis of pregnancy with biochemical markers: results of aggregate and individual patient data meta-analyses. Lancet. 2019;393:899-909.

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# Factor deficiency in pregnancy and the role of the delta hemoglobin indices

## *Gebelikte kalıtsal faktör eksiklikleri ve delta hemoglobin indekslerinin rolü*

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### Abstract

**Objective:** To evaluate the bleeding degree with objective indices and treatment interventions in the delivery of inherited factor deficiency pregnancies.

**Materials and Methods:** The presented case-control study was conducted with pregnancies with factor deficiencies. Maternal obstetrical history, disease characteristics (factor levels, duration of disease, and bleeding history), and treatment features during pregnancy were evaluated. Obstetric (delivery mode, antepartum/postpartum bleedings) and neonatal outcomes (birth weights, birth weeks, APGAR scores) of the study group were compared to those of the control group. The Delta hemoglobin/hematocrit (prepartum - postpartum hemoglobin/hematocrit), and hemoglobin and hematocrit % change [(prepartum - postpartum hemoglobin/hematocrit)/prepartum hemoglobin/hematocrit] indices were used to assess the extent of bleeding during delivery.

**Results:** None of the patients had an early postpartum hemorrhage. The delta hemoglobin and hematocrit values were increased in the factor deficiency group, with p-values of 0.019 and <0.001. The hemoglobin and hematocrit percentage changes were also found to increase, associated with p-values of <0.001 and 0.010. Three of the patients (16.7%) had postpartum complications. Gestational age at birth, APGAR scores at 1 and 5 minutes were lower in the factor deficiency group with p-values of 0.016, <0.001, and <0.001, respectively. There was one stillbirth. Most patients received peripartum tranexamic acid treatment, with factor derivatives and desmopressin in required cases.

**Conclusion:** Hemoglobin/hematocrit delta and change rate indices were increased, although none of the patients were recorded as having early peripartum hemorrhage or needing transfusion. New delta bleeding indices are promising for objectively identifying bleeding and regulating treatment in clinical practice. The experience of this clinical study might guide future studies.

**Keywords:** Inherited factor deficiency, postpartum hemorrhage, obstetric complications, von Willebrand factor

### Öz

**Amaç:** Bu çalışmanın amacı kalıtsal faktör eksikliği olan gebeliklerin doğumunda kanama derecesini objektif indeksler ve tedavi müdahaleleriyle değerlendirmektir.

**Gereç ve Yöntemler:** Sunulan olgu kontrol çalışması faktör eksikliği olan gebeliklerle yürütülmüştür. Annenin obstetrik öyküsü, hastalık özellikleri (faktör düzeyleri, hastalık süresi ve kanama öyküsü) ve gebelik sırasındaki tedavi özellikleri değerlendirilmiştir. Obstetrik (doğum şekli, doğum öncesi/doğum sonrası kanamalar) ve neonatal sonuçlar (doğum ağırlıkları, doğum haftaları, APGAR skorları) kontrol grubuyla karşılaştırılmıştır. Delta hemoglobin/

**PRECIS:** We thoroughly evaluated inherited factor deficiency during pregnancy and objectively assessed the degree of bleeding during delivery using objective indices.

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hematokrit (doğum öncesi - doğum sonrası hemoglobin/hematokrit), hemoglobin ve hematokrit % değişimi [(doğum öncesi - doğum sonrası hemoglobin/hematokrit)/doğum öncesi hemoglobin/hematokrit] indeksleri doğumdaki kanama derecesini değerlendirmek için kullanılmıştır.

**Bulgular:** Hastaların hiçbiri erken postpartum kanama yaşamadı. Delta hemoglobin ve hematokrit değerleri faktör eksikliği grubunda artmıştı, p-değerleri 0,019 ve <0,001 idi. Hemoglobin ve hematokrit % değişimlerinin de p-değerleri <0,001 ve 0,010 değerleri ile arttığı bulundu. Hastaların üçünde (%16,7) doğum sonrası komplikasyonlar görüldü. Doğum haftası, APGAR 1 ve 5 faktör eksikliği grubunda daha düşüktü, p-değerleri sırasıyla 0,016, <0,001 ve <0,001 idi. Bir hasta intrauterin eksitus oldu. Çoğu hasta peripartum traneksamik asit tedavisi aldı, gerekli durumlarda faktör türevleri ve desmopressin aldı.

**Sonuç:** Hemoglobin/hematokrit delta ve değişim oranı indeksleri artmıştı, ancak hastaların hiçbirinde erken postpartum kanama gelişmedi veya transfüzyon ihtiyacı olmadı. Yeni delta kanama indeksleri klinik uygulamada kanamayı objektif olarak belirlemek ve tedaviyi düzenlemek için umut vericidir. Bu klinik çalışmanın gelecekteki ileri çalışmalara rehberlik edebileceğine inanıyoruz.

**Anahtar Kelimeler:** Kalıtsal faktör eksikliği, doğum sonrası kanama, obstetrik komplikasyonlar, von Willebrand faktör

## Introduction

Inherited factor deficiencies increase bleeding complications in the obstetric patient. Women with inherited factor deficiency have an increased risk of pregnancy-related obstetric and neonatal complications such as peripartum hemorrhage (PPH), placental abruption, retained placenta, abortion, and stillbirth[1]. Studies on prophylactic or therapeutic interventions in pregnancy for this valuable group are limited, and guidance protocols are heavily based on the results of case reports and a small number of studies.

Von Willebrand disease (VWD) is the most common inherited bleeding disorder, and it may be accompanied by mild thrombocytopenia. Its clinical presentation varies according to the degree of deficiency of von Willebrand factor (VWF), factor VIII, and platelet levels[1]. Mild thrombocytopenia could result from biological stressors such as inflammation and pregnancy[2]. The risk of PPH with VWD was reported to be 15-60% in the literature[3]. VWF levels physiologically increase and reach about 50-60% above baseline at the time of delivery and decline rapidly after, reaching a nadir one to three weeks after delivery[4]. The recommended target plasma VWF level is accepted as greater than >50 IU/dL for delivery. However, physiological increases in levels of VWF during pregnancy and mean plasma VWF levels of healthy pregnancies, which were found to be >150 IU/dL, make this recommendation questionable[5]. Factor VIII levels also physiologically increase in pregnancy. However, patients with factor VIII deficiency have an increased risk for PPH, with a reported incidence of about 20% in the literature[6].

Factor V, VII, and XI deficiencies are rare conditions. Factor V deficiency incidence is 1 in 1,000,000, and the actual risk of bleeding in this population remains undetermined[7]. Some studies reported high PPH, reaching 60% in vaginal deliveries, although other studies reported lower bleeding rates[8,9]. Factor VII deficiency incidence is 1 in 500,000, and levels increase during pregnancy[10]. The literature reported the PPH rate as 10-13%[11]. Factor XI deficiency incidence is 1 in 1,000,000 individuals, and levels are mostly variable in pregnancy. Although there is a high risk for PPH, bleeding symptoms do not correlate with factor XI levels[7,12].

Peripartum bleeding evaluations in the literature were mainly subjective or based on only postpartum hemoglobin/hematocrit values. Therefore, since these evaluations had not adequately captured clinical details, indices showing the amount of bleeding were defined as relatively new methods. Delta hemoglobin/hematocrit calculations and hemoglobin/hematocrit change rate indices were used in various studies to predict PPH[13-18].

In the presented study, obstetric and neonatal outcomes were evaluated. The delta hemoglobin/hematocrit and hemoglobin/hematocrit change rate indices were used to assess the tendency to bleed in pregnancies with inherited factor deficiencies. The study aimed to evaluate the usefulness of these new indices in an objective context.

## Materials and Methods

The presented case-control study was conducted on pregnant women diagnosed with congenital factor deficiencies and delivered between October 2019 and 2023. Patient data were obtained retrospectively from the hospital database and patient files. All patients who met the inclusion criteria for the study were consecutively included, and the control group consisted of low-risk pregnant women who delivered during the same period. Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. The study was approved by the Ankara Bilkent City Hospital Ethics Committee (approval number: E2-23-5850, date: 06.12.2023).

Eligibility criteria for the study included having a singleton pregnancy, maternal age between 18 and 45 years, and no chronic systemic diseases except for congenital factor deficiencies. Patients with oncological diagnoses, liver failure, and anticoagulant use were not included in the study, and other diagnoses of coagulopathies were carefully excluded. Low-risk pregnancy was defined as having no chronic disease and not using any anticoagulant medication.

Maternal obstetrical history (gravidity, parity, abortion, and living children), age, disease characteristics (factor levels, duration of disease, and bleeding history), and treatment received in pregnancy (medications, treatment trimesters, and transfusions) were recorded for all participants. The treatments of all patients were arranged by the hematology department.

During pregnancy, complete blood count and coagulation parameter tests were done monthly or in cases of obstetric necessity, such as antepartum vaginal bleeding, epistaxis, and gum bleeding. All patients had their first, second, and third

trimesters and postpartum blood parameters were recorded and compared with the control group. The last hematological variables recorded before labor were considered third-trimester blood parameters.

All participants had prophylactic carbetosine administration at birth. The lowest hemoglobin/hematocrit parameters 24-48 hours postpartum were recorded as postpartum blood parameter measurements since hemoglobin concentration in the early period after bleeding could be measured falsely higher[19]. Delta hemoglobin (prepartum - postpartum hemoglobin) and delta hematocrit (prepartum - postpartum hematocrit) indices were used to compare peripartum blood loss between the factor deficiency group and control group[13-16]. Hemoglobin % change was calculated as (prepartum - postpartum hemoglobin)/prepartum hemoglobin, and hematocrit % change was calculated as (prepartum - postpartum hematocrit)/prepartum hematocrit, as defined in the recent studies[17,18].

Birth weights, gestational ages at birth, APGAR scores at the first and fifth minutes, neonatal intensive care unit admissions, delivery mode, cesarean section (c/s) indications, antepartum bleeding, and postpartum bleeding events were recorded as neonatal and obstetric outcomes. All patients were kept under close observation for the occurrence of late PPH for 4-6 weeks. Early PPH was defined as more than 500 mL bleeding in vaginal delivery and more than 1000 mL bleeding for cesarean section. Experienced midwives and doctors evaluated PPH[20]. Delivery mode and time decisions were based on obstetric indications and hematology department advice.

The main hematological parameters were determined: VWF, factor levels, and platelet levels for factor deficiency subgroups. These hematological parameters were measured at baseline and in the third trimester, and level changes were reported according to the literature study[1].

### Statistical Analysis

The statistical analyses used the Statistical Package for the Social Sciences version 23. Due to the inconsistency with a normal distribution, descriptive statistics were presented as the median and interquartile range. The Mann-Whitney U test was used to compare the parameters between the groups. The chi-square test was used to compare independent variables between groups. Wilcoxon signed-rank test was used for dependent variables. Statistical significance was defined as a two-tailed p-value of 0.05.

## Results

Demographic parameters were similar between groups, except for the history of abortions, which were found to be higher in the factor deficiency group with a p-value of 0.037. The rate of bleeding history before pregnancy was 22.2% in the factor deficiency group.

Hemoglobin and hematocrit levels were statistically lower in the factor deficiency group than the control group, in the first trimester, with p-values of 0.002 and 0.023, respectively. Postpartum-24-48 hours hemoglobin and hematocrit counts were lower in the factor deficiency group than in the control group, with p-values of 0.028 and 0.013, respectively. Hemoglobin and hematocrit counts were similar in both the postpartum first week and the first 48 hours.

The delta hemoglobin and hematocrit values, the hemoglobin and hematocrit % changes were found to be increased in the factor deficiency group (p-values of 0.019, <0.001, <0.001, 0.010, respectively) (Table 1).

Gestational age at birth, APGAR scores at 1 minute and 5 minutes were found statistically significant and lower in the factor deficiency group, with p-values of 0.016, <0.001, and <0.001, respectively (Table 2). There was only one stillbirth.

**Table 1.** Demographic and laboratory parameters of factor deficiency and control groups

| | Factor deficiency group (n=18) | Control group (n=80) | p-value |
|---|---|---|---|
| Age | 29 (11) | 27 (13) | 0.390 |
| Gravida | 2 (2) | 1 (1) | 0.116 |
| Parity | 1 (1) | 0 (1) | 0.669 |
| Abortion | 0 (1) | 0 (0) | **0.037** |
| Living child | 1 (1) | 0 (1) | 0.598 |
| **First trimester** | | | |
| Hemoglobin (g/dL) | 12.35 (1.75) | 13.15 (1.07) | **0.002** |
| Hematocrit (%) | 37.10 (4.70) | 39.00 (3.08) | **0.023** |
| Platelet ($10^9$/L) | 268.50 (97.75) | 267.00 (66.50) | 0.670 |
| **Second trimester** | | | |
| Hemoglobin (g/dL) | 11.00 (1.55) | 11.50 (1.97) | **0.049** |
| Hematocrit (%) | 32.20 (4.92) | 34.35 (6.30) | 0.219 |

**Table 1.** Continued

| | Factor deficiency group (n=18) | Control group (n=80) | p-value |
|---|---|---|---|
| Platelet ($10^9$/L) | 266.00 (99.50) | 227.00 (97.50) | 0.214 |
| **Third trimester** | | | |
| Hemoglobin (g/dL) | 11.90 (1.73) | 11.70 (2.07) | 0.579 |
| Hematocrit (%) | 36.55 (6.85) | 35.10 (6.23) | 0.480 |
| Platelet ($10^9$/L) | 238.50 (104.00) | 240.00 (106.25) | 0.491 |
| **Postpartum 24-48 hours** | | | |
| Hemoglobin (g/dL) | 9.85 (2.48) | 11.10 (1.82) | **0.028** |
| Hematocrit (%) | 31.00 (7.45) | 34.25 (5.45) | **0.013** |
| Platelet ($10^9$/L) | 250.50 (93.50) | 235.50 (112.00) | 0.909 |
| **Indices** | | | |
| Delta hemoglobin | 1.05 (1.58) | 0.65 (0.95) | **0.019** |
| Hemoglobin % change | 0.11 (0.14) | 0.03 (0.10) | **<0.001** |
| Delta hematocrit | 3.85 (5.23) | 1.10 (3.75) | **<0.001** |
| Hematocrit % change | 0.09 (0.12) | 0.06 (0.07) | **0.010** |
| **Factor deficiency group postpartum parameters** | **Postpartum first 48 hours** | **Postpartum first week** | |
| Hemoglobin (g/dL) | 9.85 (2.48) | 10.30 (2.40) | 0.689 |
| Hematocrit (%) | 31.00 (7.45) | 32.80 (10.08) | 0.556 |
| Platelet ($10^9$/L) | 250.50 (93.50) | 282.00 (90.50) | 0.206 |

*Due to the inconsistency with a normal distribution, descriptive statistics were presented as the median and interquartile range. The Mann-Whitney U test was used to compare the parameters between the groups. The chi-square test was used to compare independent variables between groups. Wilcoxon analysis was used for dependent variables

**Table 2.** Neonatal and obstetric outcomes of factor deficiency and control groups

| | Factor deficiency group (n=18) | Control group (n=80) | p-value |
|---|---|---|---|
| Gestational age at birth | 38 (1) | 39 (2) | **0.016** |
| Birth weight | 3160 (795) | 3210 (438) | 0.769 |
| APGAR 1st. minute | 7 (1) | 8 (1) | **<0.001** |
| APGAR 5th. minute | 9 (1) | 9 (0) | **<0.001** |
| NICU (%) | 2 (11.8) | 2 (2.5) | 0.081 |
| **Method of delivery (%)** | | | |
| -Vaginal | 7 (38.9) | 52 (65) | **0.041** |
| -Cesarean | 11 (61.1) | 28 (35) | |
| **Cesarean indications (%)** | | | |
| -Previous cesarean | 6 (54.5) | 18 (64.3) | **0.001** |
| -Fetal distress | 2 (18.2) | 4 (14.3) | |
| -Non-vertex presentation | 0 (0) | 6 (21.4) | |
| -Hematology suggestion | 3 (27.3) | 0 (0) | |

**Table 2.** Continued

| | Factor deficiency group (n=18) | Control group (n=80) | p-value |
|---|---|---|---|
| Antepartum bleeding (%) | 3 (16.7) | | |
| Antepartum treatment (%) | 4 (22.2) | | |
| Postpartum bleeding complication (%) | 3 (16.7) | | |
| **Prepartum treatment (%)** | 10 (55.6) | | |
| - VWF/fVIII concentrate | 2 (20) | | |
| -Tranexamic acid | 3 (30) | | |
| -FFP | 1 (10) | | |
| -VWF/fVIII concentrate + tranexamic acid | 3 (30) | | |
| -VWF/fVIII concentrate + FFP | 1 (10) | | |
| **Postpartum treatment (%)** | 15 (83.3) | | |
| -Tranexamic acid | 9 (60) | | |
| -VWF/fVIII concentrate + tranexamic acid | 4 (26.8) | | |
| -FFP+VWF/fVIII concentrate + tranexamic acid | 1 (6.6) | | |
| -Tranexamic acid + desmopressin | 1 (6.6) | | |

*Continuous variables without a normal distribution were presented as medians and interquartile ranges. Categorical variables were presented as numbers (percentages). VWF: Von Willebrand factor, FFP: Fresh frozen plasma, NICU: Neonatal intensive care unit

Three patients (16.7%) had antepartum bleeding, and four patients (22.2%) needed treatment in the antenatal period. Two of them received tranexamic acid treatment in the third trimester, one patient received factor VIII replacement, and another one received factor VIII with VWF replacement in all three trimesters.

Three of the patients (16.7%) had postpartum bleeding complications; one had a postpartum incisional hematoma, one had placental abruption, and the other one had late postpartum bleeding two months postpartum. Prepartum treatment necessities and detailed treatments of patients were given in Table 2. Three patients (16.7%) did well and did not need treatment in the peripartum period.

The factor deficiency subgroups' patient numbers and their hemostatic laboratory parameters are shown in Table 3.

## Discussion

Delta hemoglobin/hematocrit and hemoglobin/hematocrit change rate indices increased in the factor deficiency group compared to the control group; however, none of the patients had early PPH or needed transfusion. Although obstetric bleeding complications and the need for transfusion can be reduced by close follow-up of pregnant patients with factor deficiency, there is still an increased bleeding tendency.

Third-trimester hemoglobin and hematocrit results of pregnant women with factor deficiency and the control group were similar, in line with the literature[21]. Postpartum hemoglobin and hematocrit values were lower in the factor deficiency group than in the control group, although major PPH was not reported as a consequence of factor deficiency. Decreased postpartum hemoglobin and hematocrit values in the factor deficiency group did not require a blood transfusion, and none of the patients were clinically symptomatic.

In the literature, PPH was mostly evaluated subjectively, and rates changed between 32-58% in factor deficiency studies[22]. A recent cohort study reported increased PPH and antepartum hemorrhage rates associated with factor levels that were considered safe in the third trimester[23]. In recent literature, new indices were used for the objective evaluation of bleeding in obstetric groups with an increased risk of PPH. In a new study conducted on pregnancies with immune thrombocytopenia, the level of PPH in well-managed cases was similar to that of average pregnant women[24]. In the evaluation of objective bleeding degree in various conditions such as estimated blood loss in c/s, induction of labor, PPH evaluation in intrahepatic cholestasis of pregnancy; delta hemoglobin/hematocrit and hemoglobin/hematocrit change rate indices were used and shown to be more reliable than only postpartum hemoglobin or hematocrit values[14-16].

All patients in the factor deficiency group were followed closely for 4-6 weeks postpartum due to the decrease in factor levels and high bleeding risk. In the literature, late PPH rates, up to 12 weeks postpartum, were reported in the range of 2-66%[22]. In the presented study, there was one patient with late postpartum bleeding and one incisional hematoma. In the first postpartum week, the hemoglobin and hematocrit values for the factor

**Table 3.** Hemostatic laboratory parameters of factor deficiency subgroups

| | Platelet count (x$10^9$) | VWF:RCo (%) | VWF:Ag (%) | Factor VIII (%) | Factor VII (%) | Factor V (%) | Factor XI (%) |
|---|---|---|---|---|---|---|---|
| **Baseline (n)** | | | | | | | |
| VWF (7) | 263 (127-326) | 46 (3-122) | 52 (2-129) | 66 (1-170) | | | |
| VWF+f8 (4) | 255 (220-273) | 15.0 (10-19) | 28.0 (15-36) | 30.0 (2-54) | | | |
| f7 (2) | 335 (305-365) | | | | 30.0 (12-48) | | |
| f8 (2) | 365 (360-370) | | | 50.0 (44-56) | | | |
| VWF+f8+f5 (1) | 300 | 101.2 | 98.0 | 6.0 | | 3.0 | |
| f5+f7 (1) | 316 | | | | 57.0 | 25.0 | |
| f11 (1) | 184 | | | | | | 32.0 |
| **Third trimester (n)** | | | | | | | |
| VWF (7) | 186 (95-321) | 71.50 (3-144) | 73 (2-150) | 81 (2-211) | | | |
| VWF+f8 (4) | 247 (190-272) | 18 (15-75) | 45.5 (15-78) | 33.50 (3-80) | | | |
| f7 (2) | 284 (250-312) | | | | 52.5 (18-87) | | |
| f8 (2) | 268 (256-280) | | | 77.5 (30-125) | | | |
| VWF+f8+f5 (1) | 227 | 148.9 | 129.0 | 8.0 | | 3.0 | |
| f5+f7 (1) | 205 | | | | 124.0 | 26.0 | |
| f11 (1) | 150 | | | | | | 10.5 |

*Continuous variables without a normal distribution were presented as medians and interquartile ranges. VWF: Von Willebrand factor, VWF:RCo: Von Willebrand factor ristocetin cofactor, VWF:Ag: Von Willebrand factor antigen

deficiency group were similar to the early postpartum values of other patients. Although there is a high bleeding risk due to the decrease in factor levels, the similarity of the parameters might be explained by the close follow-up of patients and their receipt of necessary prophylactic and therapeutic treatments.

In the presented study, VWF and factor VIII levels were increased, and platelet counts decreased in the third-trimester evaluation compared to the baseline levels. A recent review of VWD studies also reported similar results in line with physiological pregnancy changes in factor levels[1]. The Factor VII levels were found to increase, Factor V levels were unchanged, and XI levels were found to be decreased, which is in line with the literature[22].

Most of the patients received peripartum medical treatment: Few received prepartum tranexamic acid treatment, and all received postpartum treatment, with factor derivatives and desmopressin in required cases. In the literature, the administration of the antifibrinolytic tranexamic acid as an adjunctive treatment is recommended to reduce the risk of late PPH[25]. In a study, the PPH ratio was significantly increased in the group not using tranexamic acid compared with the group that received it[26]. Desmopressin and factor derivatives were also used in a limited number of studies, mostly in addition to tranexamic acid. In a study, the use of tranexamic acid for an extended period postpartum was recommended as VWF and factor VIII levels fall to baseline after delivery. Tranexamic acid could be used for prophylactic cases, and factor concentrates or desmopressin could be preserved for severe bleeding cases[27]. In our study, tranexamic acid was used primarily for prophylaxis, factor concentrate was preserved for patients with low factor levels, and bleeding histories, and FFP was used for moderate coagulation parameters in accordance with the literature.

In this study, gestational weeks and APGARs were significantly lower in the factor deficiency group, but this difference was not clinically significant. There was only one stillbirth in the factor deficiency group. In the literature, c/s indications, c/s ratios, APGAR, and birth weights were reported similarly to this study[1,21].

### Study Limitations

The strengths of our study were: the bleeding degrees of the patients were evaluated through objective indices; the patients were followed up for all three trimesters and postpartum for four to six weeks in a multidisciplinary manner in the tertiary center; and both obstetric and neonatal results were available. Possible limitations of the study were the relatively small number of patients since factor deficiency is a very rare disease group, and the retrospective design of the study.

## Conclusion

The inherited factor deficiency group requires careful follow-up in pregnancy to avoid and manage undesired obstetric and neonatal complications. Delta and change rate indices are

promising for objectively identifying patients at high risk of bleeding and regulating treatment in obstetrics practice.

### Ethics

**Ethics Committee Approval:** The study was approved by the Ankara Bilkent City Hospital Ethics Committee (approval number: E2-23-5850, date: 06.12.2023).

**Informed Consent:** Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

### Footnotes

### Authorship Contributions

Concept: G.İ., G.P., D.Ş., Design: G.İ., A.T., F.D.Y.Y., Data Collection or Processing: A.A.B., F.D.Y.Y., İ.D., E.B., Analysis or Interpretation: A.T., Literature Search: G.İ., A.A.B., F.D.Y.Y., İ.D., E.B., G.P., Writing: G.İ., A.T., G.P., D.Ş.

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

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## References

1. Miljic P, Noureldin A, Lavin M, Kazi S, Sanchez-Luceros A, James PD, et al. Challenges in the management of women with type 2B von Willebrand disease during pregnancy and the postpartum period: evidence from literature and data from an international registry and physicians' survey-communication from the Scientific and Standardization Committees of the International Society on Thrombosis and Haemostasis. J Thromb Haemost. 2023;21:154-63.
2. Ranger A, Manning RA, Lyall H, Laffan MA, Millar CM. Pregnancy in type 2B VWD: a case series. Haemophilia. 2012;18:406-12.
3. Punt MC, Waning ML, Mauser-Bunschoten EP, Kruip M, Eikenboom J, Nieuwenhuizen L, et al. Maternal and neonatal bleeding complications in relation to peripartum management in women with Von Willebrand disease: a systematic review. Blood Rev. 2020;39:100633.
4. Sood SL, James AH, Ragni MV, Shapiro AD, Witmer C, Vega R, et al. A prospective study of von Willebrand factor levels and bleeding in pregnant women with type 1 von Willebrand disease. Haemophilia. 2016;22:e562-e4.
5. James AH, Konkle BA, Kouides P, Ragni MV, Thames B, Gupta S, et al. Postpartum von Willebrand factor levels in women with and without von Willebrand disease and implications for prophylaxis. Haemophilia. 2015;21:81-7.
6. Chi C, Lee CA, Shiltagh N, Khan A, Pollard D, Kadir RA. Pregnancy in carriers of haemophilia. Haemophilia. 2008;14:56-64.
7. Bannow BS, Konkle BA. Inherited bleeding disorders in the obstetric patient. Transfus Med Rev. 2018;32:237-43.
8. Noia G, De Carolis S, De Stefano V, Ferrazzani S, De Santis L, Carducci B, et al. Factor V deficiency in pregnancy complicated by Rh immunization and placenta previa. A case report and review of the literature. Acta Obstet Gynecol Scand. 1997;76:890-2.
9. Girolami A, Scandellari R, Lombardi AM, Girolami B, Bortoletto E, Zanon E. Pregnancy and oral contraceptives in factor V deficiency: a study of 22 patients (five homozygotes and 17 heterozygotes) and review of the literature. Haemophilia. 2005;11:26-30.
10. Kulkarni AA, Lee CA, Kadir RA. Pregnancy in women with congenital factor VII deficiency. Haemophilia. 2006;12:413-6.
11. Baumann Kreuziger LM, Morton CT, Reding MT. Is prophylaxis required for delivery in women with factor VII deficiency? Haemophilia. 2013;19:827-32.
12. Chi C, Kulkarni A, Lee CA, Kadir RA. The obstetric experience of women with factor XI deficiency. Acta Obstet Gynecol Scand. 2009;88:1095-100.
13. Kahr MK, Franke D, Brun R, Wisser J, Zimmermann R, Haslinger C. Blood group O: a novel risk factor for increased postpartum blood loss? Haemophilia. 2018;24:e207-e12.
14. Saoud F, Stone A, Nutter A, Hankins GD, Saade GR, Saad AF. Validation of a new method to assess estimated blood loss in the obstetric population undergoing cesarean delivery. Am J Obstet Gynecol. 2019;221: 267.e1-267.e6.
15. Brun R, Meier L, Kapfhammer E, Zimmermann R, Ochsenbein-Kölble N, Haslinger C. Intramyometrial and intravenous oxytocin compared to intravenous carbetocin for prevention of postpartum hemorrhage in elective cesarean section-A quasi-randomized controlled phase IV non-inferiority interventional trial. Acta Obstet Gynecol Scand. 2024;103:1838-46.
16. Furrer R, Winter K, Schäffer L, Zimmermann R, Burkhardt T, Haslinger C. Postpartum blood loss in women treated for intrahepatic cholestasis of pregnancy. Obstet Gynecol. 2016;128:1048-52.
17. Spolverato G, Kim Y, Ejaz A, Frank SM, Pawlik TM. Effect of Relative Decrease in Blood Hemoglobin Concentrations on Postoperative Morbidity in Patients Who Undergo Major Gastrointestinal Surgery. JAMA Surg. 2015;150:949-56.
18. Fidan U, Keskin U, Ulubay M, Efendi Karaca R, Burçin Fıratlıgil F, Göksen Kocagöz G, et al. The effect of the use of oxytocin on blood loss during different postpartum periods. J Perinat Med. 2015;43:461-5.
19. Kahr MK, Brun R, Zimmermann R, Franke D, Haslinger C. Validation of a quantitative system for real-time measurement of postpartum blood loss. Arch Gynecol Obstet. 2018;298:1071-7.
20. Tanacan A, Fadiloglu E. Importance of shock index in the evaluation of postpartum hemorrhage cases that necessitate blood transfusion. Women Health. 2020;60:1070-8.
21. Wilson E, Hanna F, Hyppa A, Orlowski E, Dawar HU, Sanga Z, et al. Assessment of von Willebrand disease and pregnancy outcomes at regional Australian hospitals. Eur J Haematol. 2021;106:456-66.
22. Byrne B, Ryan K, Lavin M. Current challenges in the peripartum management of women with von Willebrand disease. Semin Thromb Hemost. 2021;47:217-28.
23. Alam AU, Wu C, Kaul P, Jain V, Sun HL. Impact of inherited bleeding disorders on maternal bleeding and other pregnancy outcomes: a population-based cohort study. Haemophilia. 2024;30:478-89.
24. İpek G, Tanaçan A, Demet I, Ağaoğlu Z, Yıldız EG, Şahin D. Obstetric and neonatal outcomes of immune thrombocytopenic purpura in pregnancy and the role of delta hemoglobin index: a case-control study from a tertiary center. J Obstet Gynaecol Res. 2024;50:2246-54.
25. Connell NT, Flood VH, Brignardello-Petersen R, Abdul-Kadir R, Arapshian A, Couper S, et al. ASH ISTH NHF WFH 2021 guidelines on the management of von Willebrand disease. Blood Adv. 2021;5:301-25.
26. Kruse-Jarres R, Johnsen JM. How I treat type 2B von Willebrand disease. Blood. 2018;131:1292-300.
27. Sadler B, Castaman G, O'Donnell JS. von Willebrand disease and von Willebrand factor. Haemophilia. 2022;28(Suppl 4):11-7.

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# Routine antenatal toxoplasmosis screening, is it necessary?

## *Rutin antenatal toksoplazmozis taraması gerekli midir?*

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### Abstract

**Objective:** Toxoplasmosis is an intracellular parasite and one of the most common congenital infections. Currently, there is no clear consensus on routine screening for toxoplasma infection during pregnancy. This study aimed to discuss the results of antenatal toxoplasma screening in a tertiary center.

**Materials and Methods:** A retrospective study including the data of the antenatal toxoplasmosis screening test results over four years. Toxoplasma immunoglobulin M (IgM), toxoplasma immunoglobulin G (IgG), anti-IgG avidity test results, amniocentesis, and toxoplasma polymerase chain reaction (PCR) were obtained from the hospital records. Patients with missing toxoplasma IgM, IgG, anti-IgG avidity, test results were excluded from the study. In addition, the fetal outcomes and follow-up information for the newborns of pregnant women who gave birth in our hospital were recorded.

**Results:** During the study period, a total of 49,292 toxoplasma IgM tests were examined. Fifty pregnant women whose toxoplasma IgM was positive with a low-anti-toxoplasma IgG avidity index were enrolled in the study group. Forty percent of the pregnant women are expected to have amniocentesis. There was only one termination of pregnancy with specific ultrasonographic findings. Toxoplasma PCR was found to be negative in the other pregnant women. Of the pregnant women who were followed up, 23 gave birth in our hospital and the Sabin Feldman test was positive in 65.2 percent (15/23) of the newborns.

**Conclusion:** Antenatal toxoplasmosis screening should be preserved for pregnant women with fetal ultrasonographic findings which may be related to toxoplasmosis. Further studies are needed.

**Keywords:** Toxoplasmosis, antenatal screening, avidity, high risk pregnancy, fetal infection

### Öz

**Amaç:** Toksoplazmozis, hücre içi bir parazittir ve genellikle en yaygın konjenital enfeksiyonlardan biridir. Günümüzde, gebelikte toksoplazma enfeksiyonu için rutin tarama konusunda net bir fikir birliği söz konusu değildir. Bu çalışma, üçüncü basamak bir merkezde antenatal toksoplazma taramasının sonuçlarını tartışmayı amaçlamaktadır.

**Gereç ve Yöntemler:** Bu çalışma dört yıl boyunca antenatal toksoplazmoz tarama testi sonuçlarının verilerini içeren retrospektif bir çalışmadır. Toksoplazma immünoglobulin M (IgM), toksoplazma immünoglobulin G (IgG), anti-IgG avidite test sonuçları, amniyosentez ve toksoplazma polimeraz zincir reaksiyonu (PCR) sonuçları hastane kayıtlarından elde edildi. Toksoplazma IgM, IgG, anti-IgG avidite test sonuçları eksik olan hastalar çalışmanın dışında tutuldu. Ayrıca, hastanemizde doğum yapan gebe kadınların yenidoğanlarının fetal sonuçları ve takip bilgileri de kaydedildi.

**Bulgular:** Çalışma süresince toplam 49.292 toksoplazma IgM testi incelendi. Toksoplazma IgM'si pozitif olan ve anti-toksoplazma IgG avidite indeksi düşük olan 50 gebe kadın çalışma grubuna dahil edildi. Gebe kadınların %40'ı amniyosentez yaptırdı. Sadece bir gebeliğin spesifik ultrasonografik bulgularla sonlandırılması oldu. Diğer gebe kadınlarda toksoplazma PCR negatif bulundu. Takibi yapılan gebe kadınlardan 23'ü hastanemizde doğumunu gerçekleştirdi ve Sabin Feldman testi yenidoğanların %65,2'sinde (15/23) pozitifti.

**Sonuç:** Toksoplazmozla ilişkili olabilecek fetal ultrasonografik bulguları olan gebe kadınlar için doğum öncesi toksoplazmoz taraması yapılmalıdır. Gelecekteki yönetimin standardize edilmesi için çok daha fazla çalışmaya ihtiyaç vardır.

**Anahtar Kelimeler:** Toksoplasmosis, antenatal tarama, avidite, yüksek riskli gebelik, fetal enfeksiyonlar

**PRECIS:** Toxoplasmosis is an intracellular parasite and one of the most common congenital infections. Currently, there is no clear consensus on routine screening for toxoplasma infection during pregnancy. This study aimed to discuss the results of antenatal toxoplasma screening in a tertiary center.

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## Introduction

Toxoplasma gondii is an intracellular parasite. It is transmitted to humans by eating infected undercooked meat or by inhaling oocysts left by cats[1,2]. Congenital infection occurs due to transplacental transmission of tachyzoites after primary infection of the pregnant woman[3].

In pregnancy, there is an inverse relationship between maternal-fetal transmission rates and fetal complication rates as the gestational week increases[4]. Whereas the rate of transmission increases as the gestational week increases, the rate of fetal complications decreases[5]. Fetal ventriculomegaly, intracranial and intrahepatic calcifications, hepatomegaly, ascites, and pleural effusion can be seen due to toxoplasmosis during pregnancy[6]. After birth, it can lead to serious conditions such as chorioretinitis, hydrocephalus, mental disorder, psychomotor retardation, and hearing impairment in the newborn[7].

The higher rates of toxoplasma infection are detected in countries in which the population is exposed to contaminated water, undercooked, or raw meat[8]. Changes in eating habits and improved hygiene have been shown to reduce the incidence of toxoplasmosis infections[9].

Currently, there is no clear consensus on routine screening for toxoplasma infection during pregnancy[10]. Although screening for toxoplasma is not recommended by most obstetrician societies, it is held for free in some countries such as France and Italy[11,12]. Lack of treatment, low prevalence of congenital toxoplasmosis disease, and the cost were the main causes against screening[13].

Amniocentesis is performed prenatally for the diagnosis of toxoplasma infection. Amniosentesis is conducted later than the 18$^{th}$ week of pregnancy and at least 4 weeks after the presumed time of maternal toxoplasma infection[14]. This study aimed to discuss whether routine antenatal toxoplasmosis screening is necessary or if it leads to unnecessary interventions during pregnancy.

## Materials and Methods

This is a retrospective study investigating pregnant women who had toxoplasma screening at a tertiary hospital between 2019 and 2023. The study approval was obtained from the Ankara Bilkent City Hospital Institutional Review Board (no: 1-24-234, date: 05/2024). The patient data were obtained from the hospital records.

Toxoplasma screening is performed routinely and free of charge in our hospital. There is a pregnancy counseling school in the hospital. Routinely, all pregnant women are counseled about hygienic measures and the possibility of fetal infections. For the current study, antenatal toxoplasma screening results were obtained from pregnant women. The pregnant women were accepted as seropositive if both toxoplasma immunoglobulin G (IgG) and immunoglobulin M (IgM) were positive, and, in these cases, an acute infection was suspected.

Pregnant women with positive toxoplasma IgG and IgM test results accompanied by low IgG avidity were included in the study group. LIAISON diagnostic system kits were used to test for quantitative detection of IgM and IgG antibodies to toxoplasma gondii. The VIDAS automated analyzer system was used to perform an IgG avidity test. Pregnant women with missing data for either toxoplasma IgM, IgG, or avidity testing were excluded.

All pregnant women with positive toxoplasma IgM test results and low avidity were referred to Perinatology Outpatient Clinics. Detailed ultrasound examination was performed. Amniocentesis was offered. The amniotic fluid was sent to the molecular laboratory for toxoplasma polymerase chain reaction (PCR). Ultrasound examinations were carried out every 4 weeks by the Perinatology Clinic.

In addition, fetal outcomes and follow-up information of the newborns born to the pregnant women who gave birth in our hospital were recorded.

### Statistical Analysis

The data were analyzed by SPSS 20.0 statistical software (SPSS, Inc., Chicago, IL, USA). While mean ± standard deviation was used to present normally distributed data, median (minimum-maximum) was used to present non-normally distributed data. Number (%) was used to present the categorical data.

## Results

Between the years 2019 and 2023, 49,292 toxoplasma IgM screenings were performed antenatally. A total of 50 pregnant women whose toxoplasma IgM and IgG were positive, accompanied by a low anti-toxoplasma IgG avidity index, were eligible to be enrolled in the study. All the pregnant women were counseled from both the Infectious Diseases Department and the Perinatology Department. The pregnant women received oral spiramycin three times a day until delivery.

Of the 50 pregnant women, 30 refused to have amniocentesis. Twenty pregnant women agreed to have amniocentesis. Among the patients who consented to amniocentesis, there was one termination of pregnancy due to toxoplasmosis infection (Figure 1). In one of the pregnant women, trisomy 18 was detected, and the fetus was shown to be deceased during pregnancy. Toxoplasma PCR was negative in the remaining patients (Figure 2).

There were 23 women who gave birth in our hospital. Unfortunately, data regarding the neonatal period and early childhood outcomes of all fetuses were not available. The postnatal follow-up results of the fetuses who were born in our hospital showed that the Sabin Feldman test was performed. The Sabin Feldman test was positive in 65.2 percent (15/23) of the newborns. These newborns were monitored by the pediatric infection department.

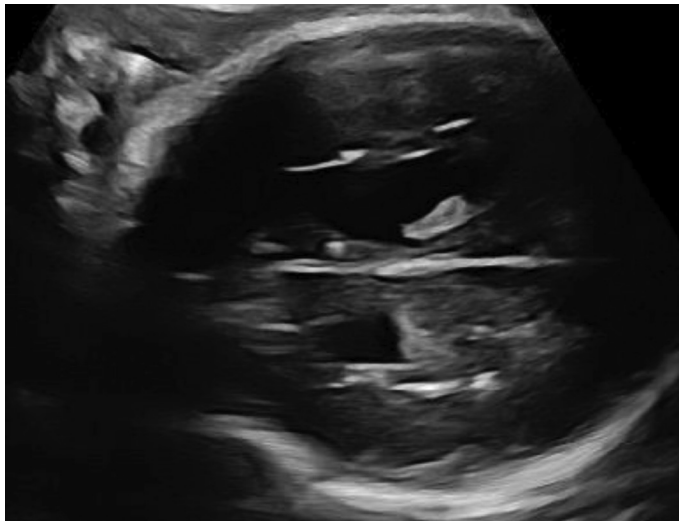

**Figure 1.** Fetal toxoplasmosis - intracranial ultrasonographic findings

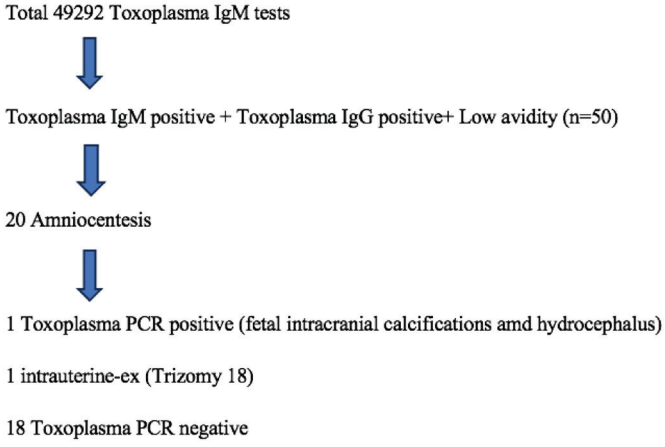


**Figure 2.** Toxoplasma screening flowchart

*IgM: Immunoglobulin M, IgG: Immunoglobulin G, PCR: Polymerase chain reaction*

## Discussion

Toxoplasma gondii is an intracellular parasite with protean clinical manifestations. In pregnant women, the important issue is to determine whether the acute infection occurred during pregnancy and therefore was transmitted to the fetus[15,16]. This study aimed to discuss antenatal toxoplasma screening, which is conducted routinely in one of the biggest tertiary hospitals in Turkey. In the current study, fewer than half of the pregnant women whose toxoplasma IgM and IgG were positive, accompanied by low anti-toxoplasma IgG avidity, agreed to have amniocentesis, and only 1 fetus was terminated due to toxoplasmosis infection[17].

Antenatal toxoplasma screening is not recommended by the societies, and has been questioned due to decreased incidence of infection as a result of increased hygiene measures and prenatal classes for pregnant women[18]. The debate about toxoplasma screening involves not only the cost and decreased incidence but also other factors. The increased number of invasive procedures held during pregnancy, such as amniocentesis, is another issue[19].

In the patient whose toxoplasma diagnosis was confirmed by amniocentesis and whose pregnancy was terminated, intrauterine ultrasonographic findings such as fetal intracranial calcification and hydrocephalus were observed in the fetus[20]. This result suggests that toxoplasma screening should be reserved for pregnant women with intrauterine ultrasonographic findings[20]. However, we believe that the high number of amniocenteses in our study (n=20) was because the amniocentesis procedure was free of charge. The right to have amniocentesis was granted to every patient who was enrolled in this study with toxoplasma IgM positivity accompanied by low anti-toxoplasma IgG avidity.

Prevention of toxoplasmosis infection is based on hygiene measures[9]. Pregnant women are counseled in antenatal classes about hygiene. They were given education on avoiding the use of contaminated water, cooking food well, avoiding eating raw meat, and washing hands.

In a study conducted between the years 2008 and 2017, the frequency of toxoplasma IgM seropositivity was found to be 0.64% in Türkiye[21]. Congenital toxoplasmosis was not documented in that study. In our study, a total of 49,292 toxoplasma IgM tests were performed. Fifty out of 49,292 pregnant women were shown to be toxoplasma IgM seropositive. Syrian refugees may not have been able to receive health services, and hygiene conditions were not suitable during the period when that study was conducted. Indeed, in the study by Halici-Ozturk et al.[22], seropositivity rates were found to be higher for Syrian refugees compared to natives. Over the years, it has been observed that toxoplasma positivity has decreased with the developments in health services. Additionally, we think that toxoplasma positivity has decreased in our hospital thanks to antenatal classes.

### Study Limitations

The main limitation of the current study was that the number of patients who were examined in the outpatient clinics but did not give birth in our hospital was high. The study had a retrospective design. Therefore, the outcomes during the neonatal period and early childhood for all fetuses were not available. This study was not a cost-effectiveness study. Therefore, we cannot conclude that antenatal routine toxoplasma screening is not cost-effective.

## Conclusion

Antenatal toxoplasma screening should be recommended for pregnant women with ultrasonographic findings and/or in the case of suspicion for primary infection during pregnancy to prevent unnecessary intervention during the fetal period.

### Ethics

**Ethics Committee Approval:** The study approval was obtained from the Ankara Bilkent City Hospital Institutional Review Board (no: 1-24-234, date: 05/2024).
**Informed Consent:** Retrospective study.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: G.T.E., D.Ş., Concept: G.T.E., D.Ş., Design: G.T.E., D.Ş., Data Collection or Processing: G.T.E., D.Ş., Analysis or Interpretation: G.T.E., D.Ş., Literature Search: G.T.E., D.Ş., Writing: G.T.E., D.Ş.
**Conflict of Interest:** No conflict of interest was declared by the authors.
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## References

1. Parigi M. Toxoplasma gondii in animals and the environment. 2014.
2. Parlak M, Çim N, Nalça Erdin B, Güven A, Bayram Y, Yıldızhan R. Seroprevalence of toxoplasma, rubella, and cytomegalovirus among pregnant women in Van. Turk J Obstet Gynecol. 2015;12:79-82.
3. de Noya BA, Ruiz-Guevara R. Pregnancy as a risk factor to disease and the vertical transmission to the fetus, of a host of parasitic ailments. CientMed. 2020;1:01-16.
4. Silasi M, Cardenas I, Kwon JY, Racicot K, Aldo P, Mor G. Viral infections during pregnancy. Am J Reprod Immunol. 2015;73:199-213.
5. Cheong JN, Wlodek ME, Moritz KM, Cuffe JS. Programming of maternal and offspring disease: impact of growth restriction, fetal sex and transmission across generations. J Physiol. 2016;594:4727-40.
6. Feldman DM, Keller R, Borgida AF. Toxoplasmosis, parvovirus, and cytomegalovirus in pregnancy. Clin Lab Med. 2016;36:407-19.
7. Zinjani S. Common medical conditions in the neonates, in Clinical anesthesia for the newborn and the neonate. 2023, Springer. p. 49-70.
8. Ducrocq J, Simon A, Lemire M, De Serres G, Lévesque B. Exposure to toxoplasma gondii through consumption of raw or undercooked meat: a systematic review and meta-analysis. Vector Borne Zoonotic Dis. 2021;21:40-9.
9. Wehbe K, Pencole L, Lhuaire M, Sibiude J, Mandelbrot L, Villena I, et al. Hygiene measures as primary prevention of toxoplasmosis during pregnancy: a systematic review. J Gynecol Obstet Hum Reprod. 2022;51:102300.
10. Peyron F, L'ollivier C, Mandelbrot L, Wallon M, Piarroux R, Kieffer F, et al. Maternal and congenital toxoplasmosis: diagnosis and treatment recommendations of a french multidisciplinary working group. Pathogens. 2019;8:24.
11. Bobić B, Villena I, Stillwaggon E. Prevention and mitigation of congenital toxoplasmosis. Economic costs and benefits in diverse settings. Food Waterborne Parasitol. 2019;16:e00058.
12. Tomasoni LR, Messina G, Genco F, Scudeller L, Prestia M, Spinoni V, et al. Risk of congenital toxoplasmosis in women with low or indeterminate anti-toxoplasma IgG avidity index in the first trimester of pregnancy: an observational retrospective study. Clin Microbiol Infect. 2019;25:761.e9-761.e13.
13. Binquet C, Lejeune C, Seror V, Peyron F, Bertaux AC, Scemama O, et al. The cost-effectiveness of neonatal versus prenatal screening for congenital toxoplasmosis. PLoS One. 2019;14:e0221709.
14. Antsaklis A, Theodora M. Amniocentesis, in clinical maternal-fetal medicine. 2021, CRC Press. p. 63.1-63.10.
15. Racicot K, Mor G. Risks associated with viral infections during pregnancy. J Clin Invest. 2017;127:1591-9.
16. Dasa TT, Geta TG, Yalew AZ, Abebe RM, Kele HU. Toxoplasmosis infection among pregnant women in Africa: a systematic review and meta-analysis. PLoS One. 2021;16:e0254209.
17. Teimouri A, Mohtasebi S, Kazemirad E, Keshavarz H. Role of toxoplasma gondii IgG avidity testing in discriminating between acute and chronic toxoplasmosis in pregnancy. J Clin Microbiol. 2020;58:e00505-20.
18. Elsheikha HM. Congenital toxoplasmosis: priorities for further health promotion action. Public Health. 2008;122:335-53.
19. Robert-Gangneux F, Dardé ML. Epidemiology of and diagnostic strategies for toxoplasmosis. Clin Microbiol Rev. 2012;25:264-96.
20. Khalil A, Sotiriadis A, Chaoui R, da Silva Costa F, D'Antonio F, Heath PT, Jet al. ISUOG practice guidelines: role of ultrasound in congenital infection. Ultrasound Obstet Gynecol. 2020;56:128-51.
21. Sert UY, Ozgu-Erdinc AS, Gokay S, Engin-Ustun Y. Toxoplasma screening results of 84587 pregnant women in a tertiary referral center in Turkey. Fetal Pediatr Pathol. 2019;38:307-16.
22. Halici-Ozturk F, Yakut K, Öcal FD, Erol A, Gökay S, Çağlar AT, et al. Seroprevalence of toxoplasma gondii infections in Syrian pregnant refugee women in Turkey. Eur J Obstet Gynecol Reprod Biol. 2021;256:91-4.

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# Composition of the vaginal microbiota in relation to cervical intraepithelial lesions

## *Servikal intraepitelyal lezyonlarla ilişkili vajinal mikrobiyota kompozisyonu*

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### Abstract

**Objective:** This study aimed to investigate the relationship between cervical intraepithelial neoplasia associated with human papillomavirus (HPV) infection and the vaginal microbiome.

**Materials and Methods:** In this study, the vaginal microbiota profile was compared among three groups of women: those with HPV infection and no cervical intraepithelial neoplasia (NILM, n=35), those with low-grade squamous intraepithelial lesions (LSIL, n=28), and those with high-grade squamous intraepithelial lesions (HSIL, n=24). Vaginal bacterial diversity was analyzed by deep sequencing of the V3-4 region of the barcoded *16S rRNA* gene using the Illumina MiSeq platform, considering alpha diversity, beta diversity, and taxon classifications. Statistical significance was set at $p<0.05$.

**Results:** In the analyses performed using Chao1, Inverse Simpson, Shannon, and Observed indices, statistically significant differences were found among the groups in terms of all indices ($p<0.05$). Among groups, beta diversity did not show any notable differences. According to the "Linear Discriminant Analysis Effect Size" analysis, the taxa enriched in the HSIL group were *Roseburia inulinivorans* (p=0.0308), *Micromonosporaceae* family (p=0.0331), and *Pirellula* genus and species, (Planctomycetes), (p=0.0165); the taxa enriched in the LSIL group were *Actinobaculum* genus and species (p=0.0183). *Lactobacillus helveticus* and *Faecalibacterium prausnitzii* were more abundant in the NILM group, while *Prevotella copri*, *Akkermansia muciniphila*, and *Fusobacterium species* were more abundant in the LSIL and HSIL groups.

**Conclusion:** Our findings indicate that variations in the severity of cervical lesions are associated with notable alterations in vaginal microbiota composition. Further research is required to conclude which contribute to the formation of the cervical lesion and which are a consequence, among those that cause changes in the vaginal microbiota, of the lesion.

**Keywords:** Microbiota, cervical intraepithelial neoplasia, human papillomavirus

### Öz

**Amaç:** Bu çalışmanın amacı insan papilloma virüsü (HPV) enfeksiyonu ile ilişkili servikal intraepitelyal neoplazi ve vajinal mikrobiyom arasındaki ilişkiyi araştırmaktır.

**Gereç ve Yöntemler:** Bu çalışmada, vajinal mikrobiyota profili üç grup kadın arasında karşılaştırıldı: HPV enfeksiyonu olan ve servikal intraepitelyal neoplazisi olmayanlar (NILM, n=35), düşük dereceli skuamöz intraepitelyal lezyonu olanlar (LSIL, n=28) ve yüksek dereceli skuamöz intraepitelyal lezyonu olanlar (HSIL, n=24). Vajinal bakteri çeşitliliği, Illumina MiSeq platformu kullanılarak barkodlanmış *16S rRNA* geninin V3-4 bölgesinin derin sekanslanmasıyla alfa çeşitliliği, beta çeşitliliği ve takson sınıflandırmaları dikkate alınarak analiz edilmiştir. İstatistiksel anlamlılık $p<0,05$ olarak belirlenmiştir.

**PRECIS:** Comparison of human papillomavirus genotype distribution and microbiota profiles in women with varying grades of cervical epithelial abnormalities.

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**Bulgular:** Chao1, Inverse Simpson, Shannon ve Observed indeksleri kullanılarak yapılan analizlerde, tüm indeksler açısından gruplar arasında istatistiksel olarak anlamlı farklılıklar bulunmuştur (p<0,05). Beta çeşitliliği açısından gruplar arasında önemli bir farklılık gözlenmemiştir. "Lineer Diskriminant Analizi Etki Büyüklüğü" analizine göre, HSIL grubunda zenginleşen taksonlar: *Roseburia inulinivorans* (p=0,0308), *Micromonosporaceae* familyası (p=0,0331), *Pirellula* cinsi ve türleri (Planctomycetes) (p=0,0165); LSIL grubunda zenginleşen taksonlar: *Actinobaculum* cinsi ve türleri (p=0,0183). *Lactobacillus helveticus* ve *Faecalibacterium prausnitzii* NILM grubunda daha bol bulunurken, *Prevotella copri*, *Akkermansia muciniphila* ve *Fusobacterium* türleri LSIL ve HSIL gruplarında daha bol bulunmuştur.

**Sonuç:** Bulgularımız, farklı derecelerde servikal lezyonları olan kadınların vajinal mikrobiyota profillerinde önemli değişiklikler olduğunu ortaya koymaktadır. Vajinal mikrobiyotadaki bu değişikliklere neden olan biyolojik ajanlardan hangilerinin servikal lezyon oluşumuna katkıda bulunduğu ve hangilerinin lezyonun bir sonucu olduğu sonucuna varmak için daha fazla araştırma yapılması gerekmektedir.

**Anahtar Kelimeler:** Mikrobiyota, servikal intraepitelyal neoplazi, insan papilloma virüsü

## Introduction

Human papilloma virus (HPV) is the most common infection among sexually transmitted infections, and the lifetime infection rate in women who continue their sexual life can reach 70% and above[1]. Epidemiological data show that most of these infections disappear spontaneously in the short term thanks to the body's own immune mechanisms. However, in 10% to 20% of cases, the infection becomes persistent, and a long-term clinical course is observed[1,2]. Persistent infections predispose the development of cervical squamous intraepithelial lesions (SIL), which can develop into low-grade squamous intraepithelial lesions (LSIL) or high-grade intraepithelial lesions (HSIL). HSIL carries a high risk of carcinogenesis and requires early intervention against the risk of viral DNA integration into the cell genome. In contrast, LSIL cases are usually associated with transient HPV infections, and approximately 60% of cases tend to resolve naturally within one year[3].

The vaginal microbiome has long been identified as playing a critical role in maintaining vaginal health. Next-generation sequencing technologies provide more detailed and in-depth information about the structure and functions of bacterial communities in the vagina[4,5]. Dysbiosis in the vagina causes damage at the cellular and DNA level by producing bacterial genotoxins, thus triggering the formation of a chronic inflammatory environment favorable for cancer development[6]. Chronic inflammation triggers the production of nitrosamines, paving the way for metabolomic profiles considered to be associated with cancer to play a decisive role in the interaction between cervical inflammation, HPV infection, and the local microbiome[7,8].

Previous studies have revealed that *Lactobacillus* dominance decreased and bacterial diversity increased in the vaginal microbiome of women diagnosed with HPV persistence, LSIL, and HSIL[9,10]. In addition, although specific bacterial species associated with HPV and SIL have been identified, no consensus has been reached on this issue[11]. Therefore, in this study, only the differences between vaginal dysbiosis and the normal microbiome of women diagnosed with LSIL and HSIL were analysed.

## Materials and Methods

### Ethical Approval

This is a prospective multi-center study of adult women admitted to the colposcopy units of Bozok University Research and Application Hospital and Etlik Zübeyde Hanım Gynecology Training and Research Hospital. Participants were enrolled between January 2023 and January 2024. The research protocol received ethical clearance from the Clinical and Interventional Research Ethics Committee of Yozgat Bozok University Research and Application Hospital, with official approvals granted on 25 August 2022 and 22 September 2022 (decision no: 2017-KAEK-189_2022.08.25_10, date: 28.08.2022 and decision no: 2017-KAEK-189_2022.09.22_06, date: 22.09.2022). All authors fully complied with the ethical principles of the Declaration of Helsinki.

### Study Design and Sample Collection

Pre-menopausal, non-pregnant women over the age of 30 years who presented to the Clinic of Colposcopy, Yozgat Bozok University Research and Application Hospital and Etlik Zübeyde Hanım Training and Research Hospital, with a diagnosis of HPV positivity, were included in the study. In this study, we excluded individuals who met the following criteria: 1) those who had received antibiotic treatment in the last 7 days, 2) those who had used suppositories, vaginal medication or vaginal cleaning products in the 48 hours before the visit, 3) those who had a history of coitus within the last 48 hours, 4) those currently diagnosed with a sexually transmitted infection or vaginal infection, 5) those with diseases affecting the immune system, 6) those who had undergone cervical treatment in the past, 7) those using hormone replacement therapy or oral contraceptives, and 8) active smokers. All participants provided written informed consent.

A total of 87 women who participated in the study were divided into three groups according to their cervical pathological findings based on the Bethesda 2001 system[12] from the histopathological evaluation of the biopsy samples taken under colposcopy: No intraepithelial lesion or malignancy (NILM) (n=35), LSIL (n=28), and HSIL (n=24). The presence of HPV was detected by Linear Array HPV Genotyping Assays developed

by Roche (Indianapolis, IN) and the subjects were divided into three categories as follows: 1) HPV 16 and 18 positive, 2) Other high-risk subtypes positive, and 3) Low-risk subtypes positive (Table S1). Other high-risk HPV types include HPV26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, and 82. The low-risk HPV comprises HPV6, 11, 40, 42, 43, 44, 54, 61, and 70. Some demographic characteristics of all included patients were also recorded. It is shown in Table 1.

Two gynecologists from each of the two centers used eNATTM kits (606CS01L, Copan Group, Copan Italia), to collect vaginal swabs without lubrication after inserting the vaginal speculum. Each vaginal sample was collected using a different swab and collecting tube, and it was promptly stored at -80 °C until DNA extraction.

### DNA Isolation

After the samples were collected, they were all sent to the Diagen lab in Ankara, Türkiye, on dry ice so that DNA could be extracted. As directed by the manufacturer, vaginal swab samples were examined using the Kurabo QuickGene DNA tissue kit S (Japan).

### DNA Amplification and Sequencing of *16S RNA* Gene Fragments

Total genomic DNA was extracted from vaginal samples with the CTAB/SDS technique. DNA concentration and purity were evaluated via electrophoresis on a 1% agarose gel, after which the samples were adjusted to a final concentration of 1 µg/µL using sterile water. The V3-V4 hypervariable portions of the bacterial 16S rRNA gene were subsequently amplified according to the designated polymerase chain reaction (PCR) conditions. In this procedure, the primers 515F: 5'-GTGCCAGCMGCCGCGGTAA-3' and 806R: 5'-GGACTACHVGGGTWTCTAAT-3' were utilized. DNA amplification was conducted following the Illumina technique for 16S Metagenomic Sequencing Library Preparation (Part No. 15044223 Rev. A, Illumina, CA, USA). PCR processes commenced with an initial denaturation at 98 °C for 1 minute, succeeded by 30 cycles including denaturation at 98 °C for 10 seconds, annealing at 50 °C for 30 seconds, and elongation at 72 °C for 30 seconds. PCR products were assessed using 2% agarose gel electrophoresis and subsequently purified using the Qiagen Gel Extraction Kit (Qiagen, Germany). The PCR products were indexed using the Nextera XT Index Kit (Illumina), followed by the multiplexing step. Purified DNA libraries were constructed utilizing TruSeq DNA PCR-free sample preparation kits (Illumina, USA) and sequenced to acquire 250 bp paired-end reads using the Illumina NovaSeq platform. Following sequencing, barcodes were eliminated from the arrays, and the data were analyzed using the QIIME V.2.0 analytical pipeline. The amplicon sequence variations (ASVs) for each sample were limited to 10,000 reads through random selection[13,14]. The ASV taxonomy was established using the Silva 138 SSURef NR99 16S rRNA gene reference database[15]. Reliability was maintained throughout the experimental process by employing negative controls in DNA extraction, PCR, and sequencing. The precision of the studies was enhanced by employing an internal standard, which included an internal control for 16S and 1007 reads, during sequencing.

### Bioinformatics Analysis of Sequences

First, a quality evaluation was conducted using the Prinseq-lite (v0.20.4) software (http://prinseq.sourceforge.net) to begin bioinformatic analysis of the raw read data. The quality control step was conducted using the following parameters: 50 for min length, 30 for trim qual right, 20 for trim qual window, and mean for trim qual type. The FLASH software was used with the default parameters to merge the quality-controlled forward and reverse read sequences. After merging, human-derived sequences were identified by aligning individual reads to the human genome (GRCh38.p11, December 2013) using Bowtie2 software (very-sensitive mode: -D 20 -R 3 -N 0 -L 20 -i S,1,0.50) and subsequently removed.

**Table 1.** Clinical characteristics of subjects in each group

| Characteristics | NILM group (n=35) | LSIL group (n=28) | HSIL group (n=24) | p value |
|---|---|---|---|---|
| **Age (years)** | 41.44±10.506 | 41.18±9.851 | 42.58±9.297 | 0.955# |
| **Height (m)** | 1.60±0.739 | 1.60±0.526 | 1.61±0.665 | 0.657* |
| **Weight (kg)** | 70.85±10.59 | 69.36±10.302 | 71.50±7.530 | 0.696* |
| **BMI** | 22.11±2.770 | 21.98±3.254 | 22.24±3.110 | 0.420* |
| **Gravida** | 2.91±1.640 | 2.46±1.401 | 3.33±1.810 | 0.070* |
| **Parity** | 2.50±1.308 | 2.91±1.640 | 2.82±1.416 | 0.182* |
| **Duration of marriage** | 19.29±10.823 | 18.5±12.670 | 24.17±12.240 | 0.067* |
| **Number of previous miscarriages** | 0.35±0.485 | 0.46±0.508 | 0.37±0.495 | 0,65# |

Values are mean ± SD for continuous variables. NILM: No intraepithelial lesion or malignancy, LSIL: Low-grade squamous intraepithelial lesions, HSIL: High-grade intraepithelial lesions, BMI: Body mass index, SD: Standard deviation, *: ANOVA, #: Kruskal-Wallis

Using the Bayesian-based ribosomal database project (RDP) Classifier (v2.12) algorithm, reads that did not match the human genome were taxonomically categorized after they were aligned to the RDP. A contingency table with taxonomic counts was created once each sample's taxonomic distribution was established. QIIME (Quantitative Insights Into Microbial Ecology) v1.9.0 software was used to convert this table to Biom format, and the ecological diversity, abundance, and composition of microorganisms were analyzed[16].

The diversity within samples was measured using the alpha diversity assessment. With 9,500 random readings, 1,000 dilutions were made for every sample. The Shannon diversity index, Chao1 index, observed index, and inverse Simpson index were used per sample to determine diversity. The statistical software R (v3.1.0) was used to create box plots to visualize the results of the alpha diversity analysis.

The microbiological diversity of the samples was examined using beta diversity assessments. To do this, Bray-Curtis distance index matrices were used in principal coordinate analysis. Utilizing Adonis nonparametric analysis of variance, statistical differences across groups were assessed. Additionally, the non-parametric Wilcoxon test was used in R scripts to analyze groups and sample types.

### Statistical Analysis

Data analysis was performed using IBM SPSS Statistics for Windows (version 27.0; Armonk, NY, USA). For continuous variables, descriptive statistics such as mean, standard deviation, and median were calculated. Categorical variables were presented as frequencies and percentages. The Student's t-test was applied to compare numerical data between two groups. For comparisons involving more than two groups, either the Kruskal-Wallis H test or one-way ANOVA was utilized, depending on the normality of the data distribution. A p-value of less than 0.05 was considered statistically significant.

## Results

There were no statistically significant differences among the groups in their demographic profiles, including age, height, weight, body mass index, gravida, parity, duration of marriage, or the number of prior abortions ($p>0.05$) (Table 1).

In the study, analyses of vaginal microbiota alpha diversity between NILM, LSIL, and HSIL groups using Chao1, Inverse Simpson, Shannon, and Observed indices, showed statistically significant differences between the groups in terms of all indices ($p<0.05$). The Chao1 index (Figure 1A) showed that the LSIL group had significantly higher species richness than the other groups ($p<0.001$). The Inverse Simpson index (Figure 1B), a measure that emphasizes the distribution of dominant species within the community, indicated higher diversity in the LSIL group; a statistically significant difference was found between the groups ($p<0.001$). The Shannon index (Figure 1C) was evaluated considering both species richness and even distribution. Alpha diversity analysis revealed significantly increased richness in the LSIL and HSIL groups relative to the NILM group ($p=0.009$). As shown in Figure 1D, the LSIL group exhibited the highest observed species count, whereas the NILM group demonstrated the lowest ($p<0.001$).

Bray-Curtis non-metric multidimensional scaling (NMDS) analyses (Figure 2) revealed that the groups did not differ significantly in terms of beta diversity. No significant difference was observed between the LSIL, HSIL, and NILM (normal) groups. Unweighted UniFrac, and weighted UniFrac NMDS analyses (Figure 2) also showed similar results. There was no significant difference in microbiota composition between the groups. Stress plot analyses showed that the NMDS model was reliable ($R^2>0.96$) (Figure 3). The high model fit indicates that the analyses were technically correct, but there was no significant segregation between the groups (Figures 2 and 3).

According to linear discriminant analysis effect size (LEfSe) analysis used to determine the enriched microbiota species specific to the groups, the enriched taxa in the HSIL group were *Roseburia inulinivorans* ($p=0.0308$, LDA score=2.50), Micromonosporaceae family ($p=0.0331$, LDA score=2.07), Pirellula genus and species (Planctomycetes) ($p=0.0165$, LDA score=2.07); in the LSIL group, the enriched taxa were *Actinobaculum* genus and species ($p=0.0183$, LDA score=3.16); and in the NILM group, the enriched taxa were *Deinococcoci* class ($p=0.0301$, LDA score=2.34), Thermus genus and Thermaceae family ($p=0.0301$, LDA score=2.34) (Figure 4 and S1).

In this study, the distribution of vaginal microbiota at phylum, class, order, family, genus, and species levels among NILM, LSIL, and HSIL groups was analyzed.

When analyzed at the phylum level, Firmicutes emerged as the predominant phylum across all groups. In contrast, the relative abundances of *Bacteroidetes* and *Actinobacteria* were reduced in both LSIL and HSIL groups. Meanwhile, phyla like *Fusobacteria* and *Proteobacteria* showed a marked increase, particularly in the HSIL group (Figure 5A).

At the class level, *Bacilli* and *Clostridia* were observed as dominant in all groups. *Gammaproteobacteria* and *Actinobacteria* had higher proportions in LSIL and HSIL groups; and especially *Verrucomicrobiae* and *Synergistia* increased in the lesion group (Figure S2). *Lactobacillales*, the most common bacterial group at the order level, had the highest proportion in all groups. *Bacteroidales* and *Clostridiales* were more prominent in the HSIL and LSIL groups, while *Fusobacteriales* and *Campylobacterales* were significantly increased, especially in the HSIL group (Figure S2). At the family level, *Lactobacillaceae* was the most common family in all groups. *Prevotellaceae* and *Leptotrichiaceae* were more prevalent in the HSIL group, while *Bifidobacteriaceae* and *Ruminococcaceae* were more prevalent in the LSIL and NILM groups. *Fusobacteriaceae* and *Veillonellaceae* were among the other important families showing an increase in the HSIL group (Figure S2). At the genus level, *Lactobacillus* was the dominant genus in all groups. *Prevotella* and *Gardnerella* were found at higher rates in both the LSIL and HSIL groups, while *Atopobium*

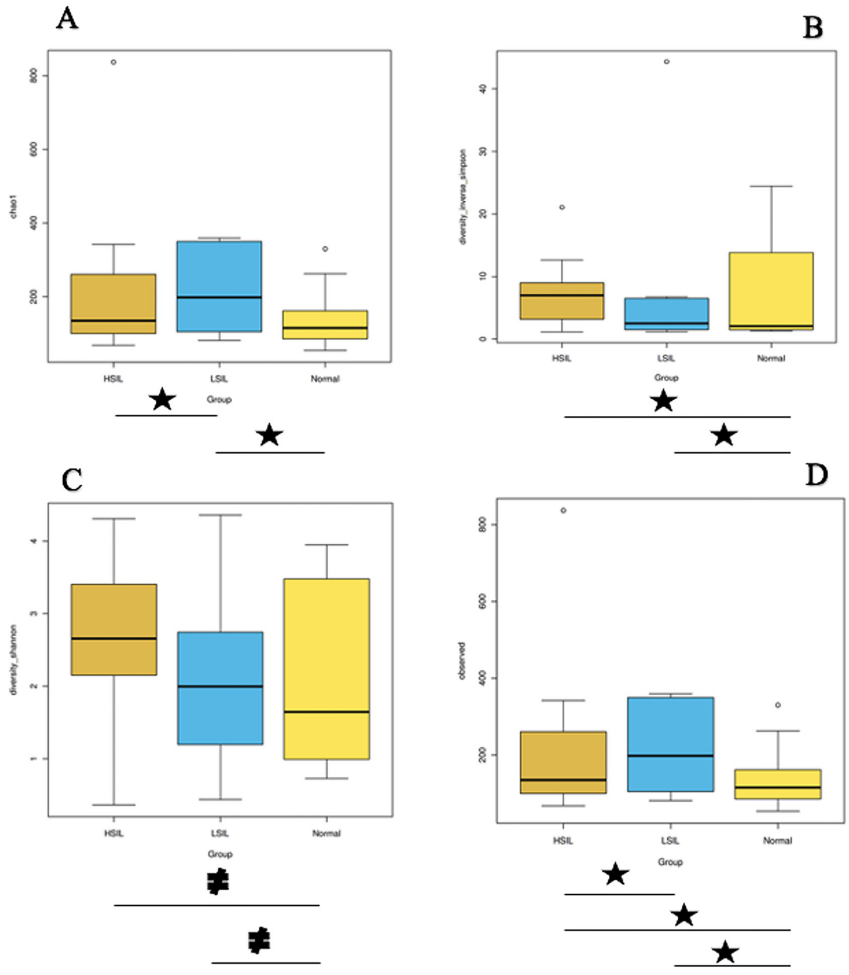


**Figure 1.** Comparison of groups in terms of alpha diversity. 1A: Species richness assessment with Chao1 index. A significant increase was observed in the LSIL group, 1B: Analysis of dominant species distribution by Inverse Simpson index. The highest diversity was observed in the HSIL group, while a wider distribution was observed in the NILM group, 1C: Analysis of species diversity and even distribution with Shannon index. Higher diversity was found in LSIL and HSIL groups, 1D: Box plot showing differences between groups for number of observed species. Star (*): p<0.001; hash (#) p=0.009

*NILM: No intraepithelial lesion or malignancy, LSIL: Low-grade squamous intraepithelial lesions, HSIL: High-grade intraepithelial lesions*

and *Fusobacterium* appeared more frequently in the HSIL group. *Streptococcus* and *Bifidobacterium* were more common in the NILM group (Figure S2).

At the species level, *Lactobacillus iners* was the most dominant species in all groups. *Lactobacillus helveticus* and *Faecalibacterium prausnitzii* were more abundant in the NILM group, while *Prevotella copri*, *Akkermansia muciniphila,* and *Fusobacterium species* were more abundant in the LSIL and HSIL groups. Species such as *Roseburia faecis* and *Dorea formicigenerans* also showed higher abundance in the NILM group compared to the other groups (Figure 5B).

## Discussion

Maintaining the balance of the cervicovaginal microbiota is essential for modulating the acquisition, persistence, and eventual elimination of HPV from the female reproductive tract. An imbalance in this microbial ecosystem, referred to as dysbiosis, can contribute to the initiation of pathological processes that may lead to carcinogenesis. These processes are mediated through disruptions such as impairment of epithelial integrity, metabolic dysfunction, irregular cellular proliferation, genomic instability, chronic inflammatory responses, and enhanced angiogenesis[17-19].

The vaginal microbiome is a constantly changing ecosystem that can be influenced by several factors[20]. Ravel et al.[5] conducted a study where they categorized the vaginal microbiome of healthy females into four distinct community status types (CST), based on the dominating species of Lactobacillus and the corresponding pH values. The four CST types are CST-I, CST-II, CST-III, and CST-V, which correspond to the presence of *Lactobacillus crispatus* (26.2%), *L. gasseri* (6.3%), *L. iners* (34.1%), and *L. jensenii* (5.3%), respectively. In subsequent

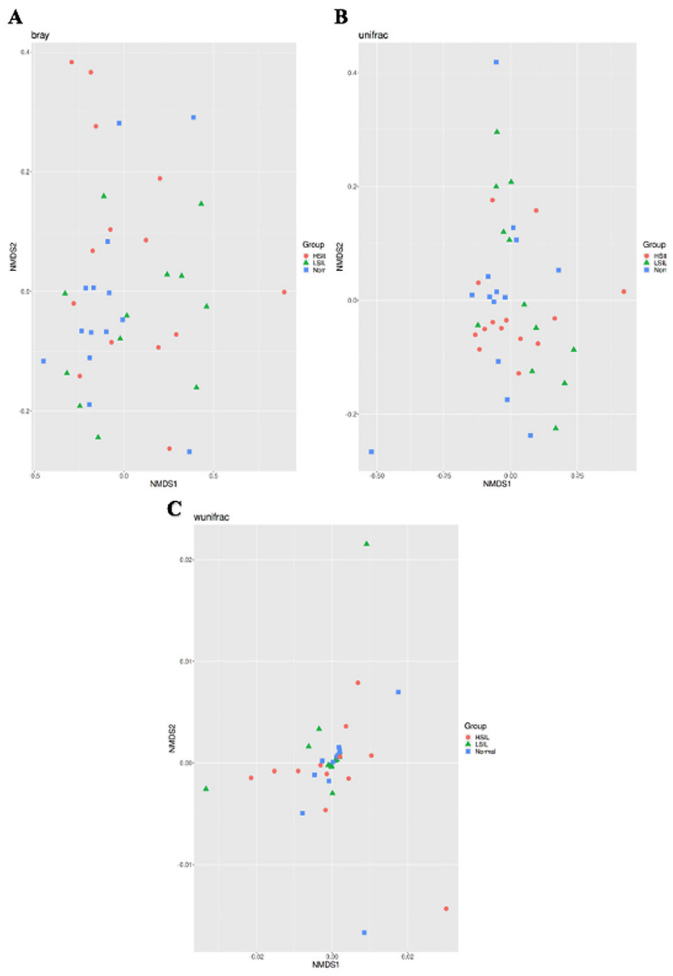


**Figure 2.** NMDS graphs. 2A: Bray-Curtis NMDS graph. No obvious segregation between groups was observed; 2B: Unweighted UniFrac NMDS plot. No significant segregation was found between the groups; 2C: Weighted UniFrac NMDS plot. Groups were similar in terms of microbiota composition

*NMDS: Non-metric multidimensional scaling*

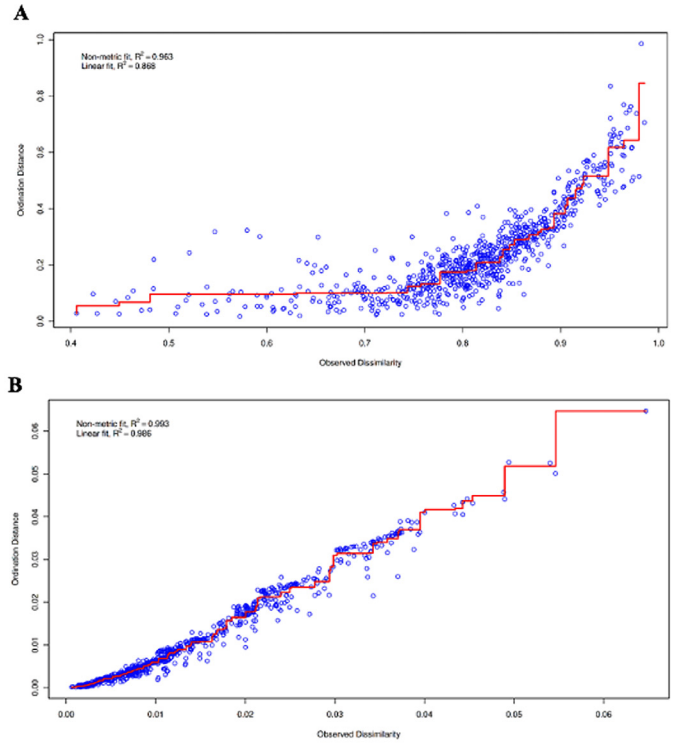


**Figure 3.** Stress plot analyses. A-B: While showing that the NMDS model is reliable, it supports that there is no significant difference between the groups in terms of beta variation

*NMDS: Non-metric multidimensional scaling*

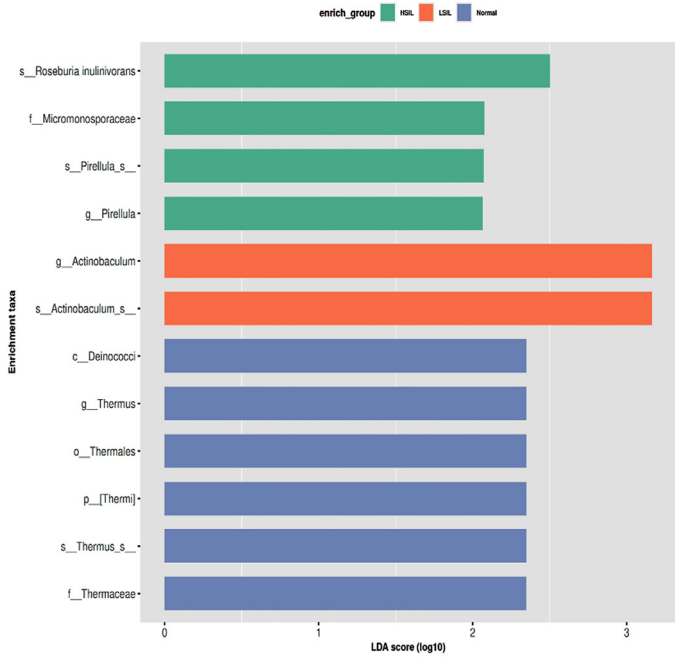


**Figure 4.** Linear discriminant analysis effect size analysis

years, by assembling the largest dataset of human vaginal microbiota profiles, CST-IV was divided into seven subgroups according to the dominant *non-lactobacillus* species: CST IV-A; *Candidatus lachnocurva vagina* high rate *G. vaginalis* a medium rate, CST IV-B; *G. vaginalis* at a high rate, *L. vaginae* at a low rate, CST IV-C0; an equal community with moderate amounts of *Prevotella*, CST IV-C1; *Streptococcus* dominant, CST IV-C2; *Enterococcus* dominant, CST IV-C3; *Bifidobacterium* dominant, CST IV-C4; and *Staphylococcus* dominant[21]. These members of the flora defend against harmful bacteria by generating hydrogen peroxide ($H_2O_2$) and bacteriocins, specifically generated by *Lactobacillus* species to maintain an acidic vaginal pH[22,23]. They produce hemolysin to enhance cytotoxicity (*Prevotella*)[24] and facilitate adhesion for epithelial colonization (*Atopobium, Bifidobacteria, Gardnerella*)[25].

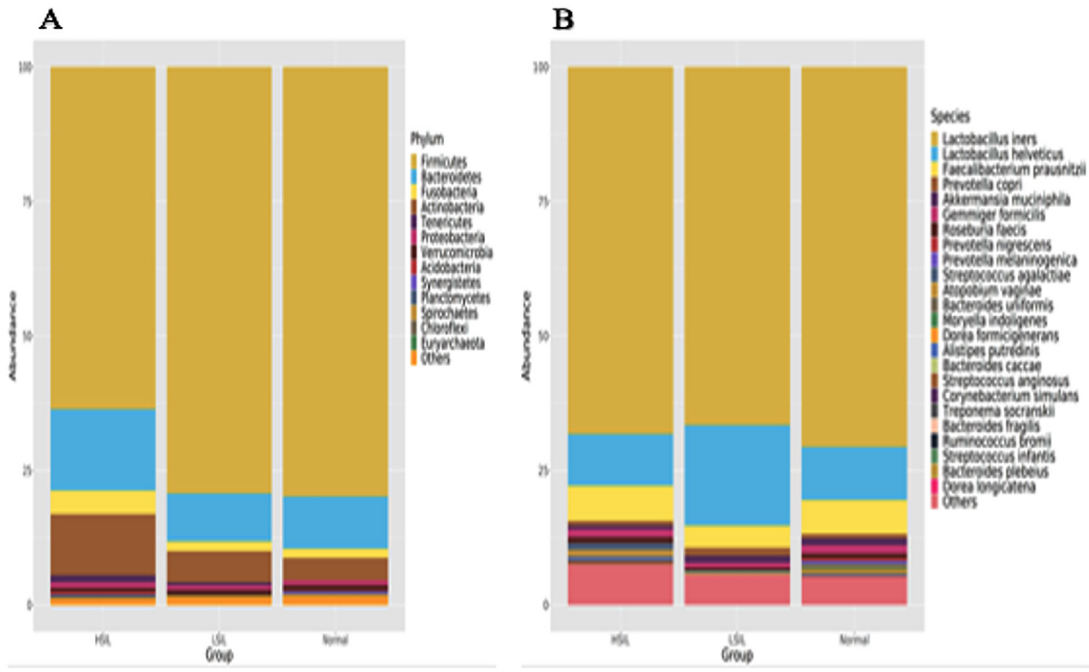


**Figure 5.** Taxa bar plots A. Bacteria abundance distribution at phylum level, B. Bacteria abundance distribution at species level

CST III and IV have frequently been linked to the emergence of premalignant cervical lesions and the progression to invasive cervical cancer, particularly in the presence of HPV infection. Furthermore, *Lactobacillus gasseri*, the predominant strain of CST II, has been suggested to be associated with the fastest clearance of acute HPV infection in HPV-positive women[26,27]. Some studies report that HPV alters the composition of the vaginal microbiota by shifting it from CST III to CST IV, and that CST IV contributes to the progression and increased severity of cervical neoplasms[28]. In the present study, Lactobacillus iners increased more in the LSIL group than in the HSIL and in the normal group. *Lactobacillus gasseri* was not detected in HSIL and LSIL groups. In accordance with the report by Lee et al.[29], which stated that *Prevotella* species are associated with HPV, Prevotella species were more prevalent in HSIL groups in our study. *Fusobacteria* were the predominant phylum, especially in the HSIL group. This is consistent with other studies emphasizing the association between *Fusobacteria* and cervical cancer so far[9,29,30].

LEfSe analysis conducted in our study revealed several candidate biomarkers that were linked to distinct stages of cervical pathology. *Roseburia inulinivorans* and members of the *Micromonosporaceae* family were found to be significantly more abundant in the HSIL group, while *Actinobaculum* was predominantly detected in LSIL samples. *Roseburia inulinivorans* is a motile bacterial species classified under the phylum *Firmicutes*, typically residing in the human colon, where it aids in butyrate synthesis through the fermentation of dietary polysaccharides[31]. On the other hand, *Actinobaculum* is known to be a common pathogen in urogenital tract infections[32]. These two agents are of interest for HSIL and LSIL, considering the exclusion criteria and meticulous sampling.

*Akkermansia muciniphila* was expressed at higher levels in HSIL and LSIL groups compared to other groups. Considering the reports in previous studies that *Akkermansia muciniphila* has the potential to prevent obesity, diabetes, and atherosclerosis and increase the efficacy of cancer immunotherapy, this dysbiosis member raises the question "Could it be a repair function for HSIL and LSIL?"[33,34].

Our findings regarding alpha diversity mirrored the previously reported pattern of changes in species richness and community composition of the vaginal microbiota along the progression of cervical lesions[35]. In contrast, the absence of a statistically significant difference in beta diversity across the groups could be attributed to the application of the NMDS model in our analysis. To assess the robustness of this model, we also performed stress plot evaluations. We also included stress plot analyses in our research to understand the reliability of this model.

### Study Limitations

Given the relatively small sample size, there is a possibility that the statistical power may have been insufficient. Therefore, the results should be approached and interpreted cautiously. Among the patients included in the study, only specific HPV types -HPV6, 16, 18, 31, 35, 42, 45, 51, 52, 53, 54, 56, 58, 59, 61, 66, and 68- were detected (Table S1), while other HPV types were not observed. This restricted HPV spectrum may limit the generalizability of our results and could hinder a comprehensive understanding of the relationship between vaginal microbiota composition and cervical intraepithelial

neoplasia. Future research with larger, more diverse cohorts, encompassing a broader range of HPV genotypes, is warranted to clarify whether the observed associations persist across different viral subtypes and to provide a more complete insight into this interaction.

## Conclusion

The results of this study demonstrate that alterations in vaginal microbiota composition are associated with the severity of cervical lesions. However, further investigations are necessary to determine whether these microbial shifts contribute to the development of cervical lesions or arise as a consequence of the disease process.

### Ethics

**Ethics Committee Approval:** The research protocol received ethical clearance from the Clinical and Interventional Research Ethics Committee of Yozgat Bozok University Research and Application Hospital, with official approvals granted on 25 August 2022 and 22 September 2022 (decision no: 2017-KAEK-189_2022.08.25_10, date: 28.08.2022 and decision no: 2017-KAEK-189_2022.09.22_06, date: 22.09.2022).
**Informed Consent:** All participants provided written informed consent.

### Footnotes

### Authorship Contributions

**Surgical and** Medical Practices: F.K., S.K., D.A.K., Concept: E.S.Y., Ç.A., M.B., Design: E.S.Y., Ç.A., M.B., A.E., Data Collection or Processing: Ç.A., M.B., E.Y.Ş., F.K., S.K., A.E., D.A.K., Analysis or Interpretation: M.B., F.K., S.K., D.A.K., T.O., Literature Search: Ç.A., M.B., E.Y.Ş., A.E., D.A.K., T.O., Writing: Ç.A., F.K., S.K., D.A.K., T.O.
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## References

1. Kilic D, Guler T, Atigan A, Avsaroglu E, Karakaya YA, Kaleli I, et al. Predictors of human papillomavirus (HPV) persistence after treatment of high grade cervical lesions; does cervical cytology have any prognostic value in primary HPV screening? Ann Diagn Pathol. 2020;49:151626.
2. Williamson AL. Recent developments in human papillomavirus (HPV) vaccinology. Viruses. 2023;15.
3. Guan P, Howell-Jones R, Li N, Bruni L, de Sanjosé S, Franceschi S, et al. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer. 2012;131:2349-59.
4. Leitich H, Bodner-Adler B, Brunbauer M, Kaider A, Egarter C, Husslein P. Bacterial vaginosis as a risk factor for preterm delivery: a meta-analysis. Am J Obstet Gynecol. 2003;189:139-47.
5. Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci U S A. 2011;108(Suppl 1):4680-7.
6. Fernandes JV, Cobucci RN, Jatobá CA, Fernandes TA, de Azevedo JW, de Araújo JM. The role of the mediators of inflammation in cancer development. Pathol Oncol Res. 2015;21:524-34.
7. Bartsch H, Montesano R. Relevance of nitrosamines to human cancer. Carcinogenesis. 1984;5:1381-93.
8. Ilhan ZE, Łaniewski P, Thomas N, Roe DJ, Chase DM, Herbst-Kralovetz MM. Deciphering the complex interplay between microbiota, HPV, inflammation and cancer through cervicovaginal metabolic profiling. EBioMedicine. 2019;44:675-90.
9. Mitra A, MacIntyre DA, Lee YS, Smith A, Marchesi JR, Lehne B, et al. Cervical intraepithelial neoplasia disease progression is associated with increased vaginal microbiome diversity. Sci Rep. 2015;5:16865.
10. Brusselaers N, Shrestha S, van de Wijgert J, Verstraelen H. Vaginal dysbiosis and the risk of human papillomavirus and cervical cancer: systematic review and meta-analysis. Am J Obstet Gynecol. 2019;221:9-18.e8.
11. Godoy-Vitorino F, Romaguera J, Zhao C, Vargas-Robles D, Ortiz-Morales G, Vázquez-Sánchez F, et al. Cervicovaginal fungi and bacteria associated with cervical intraepithelial neoplasia and high-risk human papillomavirus infections in a hispanic population. Front Microbiol. 2018;9:2533.
12. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 bethesda system: terminology for reporting results of cervical cytology. JAMA. 2002;287:2114-9.
13. Valles-Colomer M, Bacigalupe R. Variation and transmission of the human gut microbiota across multiple familial generations. Nat Microbiol. 2022;7:87-96.
14. Hall M, Beiko RG. 16S rRNA gene analysis with QIIME2. Methods Mol Biol. 2018;1849:113-29.
15. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41:D590-6.
16. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010;7:335-6.
17. Sharifian K, Shoja Z, Jalilvand S. The interplay between human papillomavirus and vaginal microbiota in cervical cancer development. Virol J. 2023;20:73.
18. Castanheira CP, Sallas ML, Nunes RAL, Lorenzi NPC, Termini L. Microbiome and cervical cancer. Pathobiology. 2021;88:187-97.
19. Kyrgiou M, Moscicki AB. Vaginal microbiome and cervical cancer. Semin Cancer Biol. 2022;86:189-98.
20. Srinivasan S, Liu C, Mitchell CM, Fiedler TL, Thomas KK, Agnew KJ, et al. Temporal variability of human vaginal bacteria and relationship with bacterial vaginosis. PLoS One. 2010;5:e10197.
21. France MT, Ma B, Gajer P, Brown S, Humphrys MS, Holm JB, et al. VALENCIA: a nearest centroid classification method for vaginal microbial communities based on composition. Microbiome. 2020;8:166.
22. Gupta S, Kakkar V, Bhushan I. Crosstalk between vaginal microbiome and female health: a review. Microb Pathog. 2019;136:103696.
23. Miko E, Barakonyi A. The role of hydrogen-peroxide (H(2)O(2)) produced by vaginal microbiota in female reproductive health. Antioxidants (Basel). 2023;12:1055.

24. Dong B, Huang Y, Cai H, Chen Y, Li Y, Zou H, et al. Prevotella as the hub of the cervicovaginal microbiota affects the occurrence of persistent human papillomavirus infection and cervical lesions in women of childbearing age via host NF-κB/C-myc. J Med Virol. 2022;94:5519-34.
25. Machado A, Cerca N. Influence of biofilm formation by gardnerella vaginalis and other anaerobes on bacterial vaginosis. J Infect Dis. 2015;212:1856-61.
26. Brotman RM, Shardell MD, Gajer P, Tracy JK, Zenilman JM, Ravel J, et al. Interplay between the temporal dynamics of the vaginal microbiota and human papillomavirus detection. J Infect Dis. 2014;210:1723-33.
27. Audirac-Chalifour A, Torres-Poveda K, Bahena-Román M, Téllez-Sosa J, Martínez-Barnetche J, Cortina-Ceballos B, et al. Cervical microbiome and cytokine profile at various stages of cervical cancer: a pilot study. PLoS One. 2016;11:e0153274.
28. Chen Y, Qiu X, Wang W, Li D, Wu A, Hong Z, et al. Human papillomavirus infection and cervical intraepithelial neoplasia progression are associated with increased vaginal microbiome diversity in a Chinese cohort. BMC Infect Dis. 2020;20:629.
29. Lee JE, Lee S, Lee H, Song YM, Lee K, Han MJ, et al. Association of the vaginal microbiota with human papillomavirus infection in a Korean twin cohort. PLoS One. 2013;8:e63514.
30. Łaniewski P, Barnes D, Goulder A, Cui H, Roe DJ, Chase DM, et al. Linking cervicovaginal immune signatures, HPV and microbiota composition in cervical carcinogenesis in non-hispanic and hispanic women. Sci Rep. 2018;8:7593.
31. Scott KP, Martin JC, Chassard C, Clerget M, Potrykus J, Campbell G, et al. Substrate-driven gene expression in Roseburia inulinivorans: importance of inducible enzymes in the utilization of inulin and starch. Proc Natl Acad Sci U S A. 2011;108(Suppl 1):4672-9.
32. Yu Y, Tsitrin T, Singh H, Doerfert SN, Sizova MV, Epstein SS, et al. Actinobaculum massiliense proteome profiled in polymicrobial urethral catheter biofilms. Proteomes. 2018;6:52.
33. Fan S, Jiang Z, Zhang Z, Xing J, Wang D, Tang D. Akkermansia muciniphila: a potential booster to improve the effectiveness of cancer immunotherapy. J Cancer Res Clin Oncol. 2023;149:13477-94.
34. Hasani A, Ebrahimzadeh S, Hemmati F, Khabbaz A, Hasani A, Gholizadeh P. The role of Akkermansia muciniphila in obesity, diabetes and atherosclerosis. J Med Microbiol. 2021;70.
35. Wei B, Chen Y, Lu T, Cao W, Tang Z. Correlation between vaginal microbiota and different progression stages of cervical cancer. Genet Mol Biol. 2022;45:e20200450.

**Table S1.** Distribution of HPV genotypes by cervical pathology groups

| Cervical pathology group | HPV 16/18 positive | Other high-risk HPV positive | Low-risk HPV positive | n |
|---|---|---|---|---|
| **NILM** | 11 | 16 | 8 | 35 |
| **LSIL** | 11 | 9 | 8 | 28 |
| **HSIL** | 6 | 7 | 11 | 24 |
| **Total** | 87 | 32 | 27 | 87 |

NILM: No intraepithelial lesion or malignancy, LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, HPV: Human papillomavirus

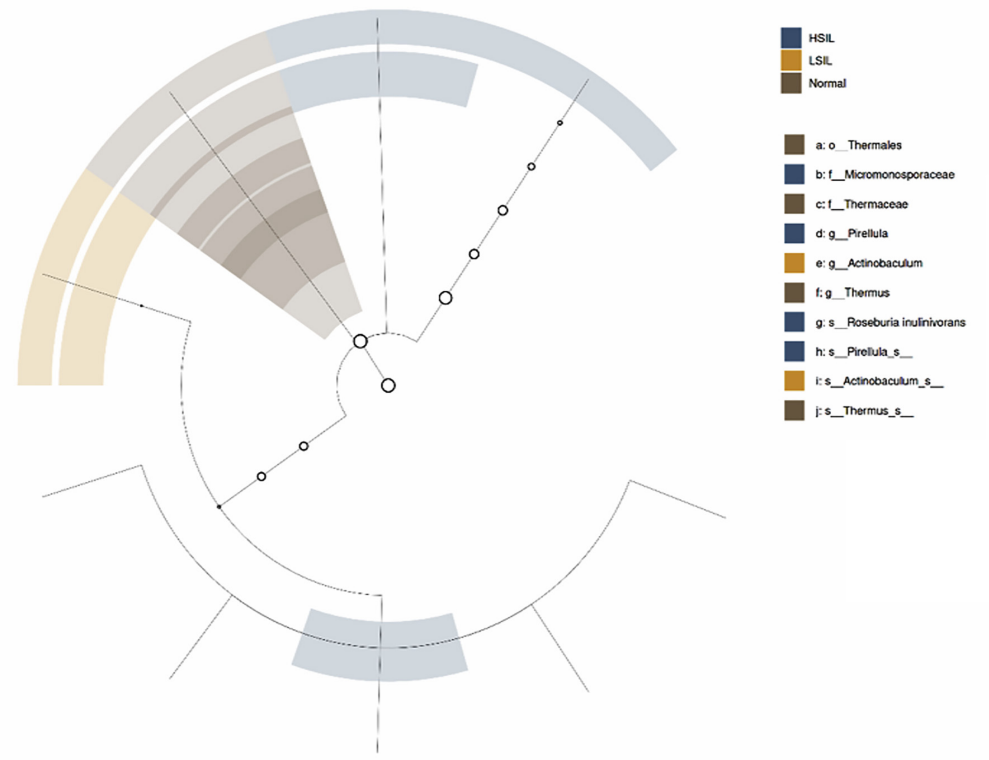


**Figure S1.** Linear discriminant analysis effect cladogram appearance according to groups

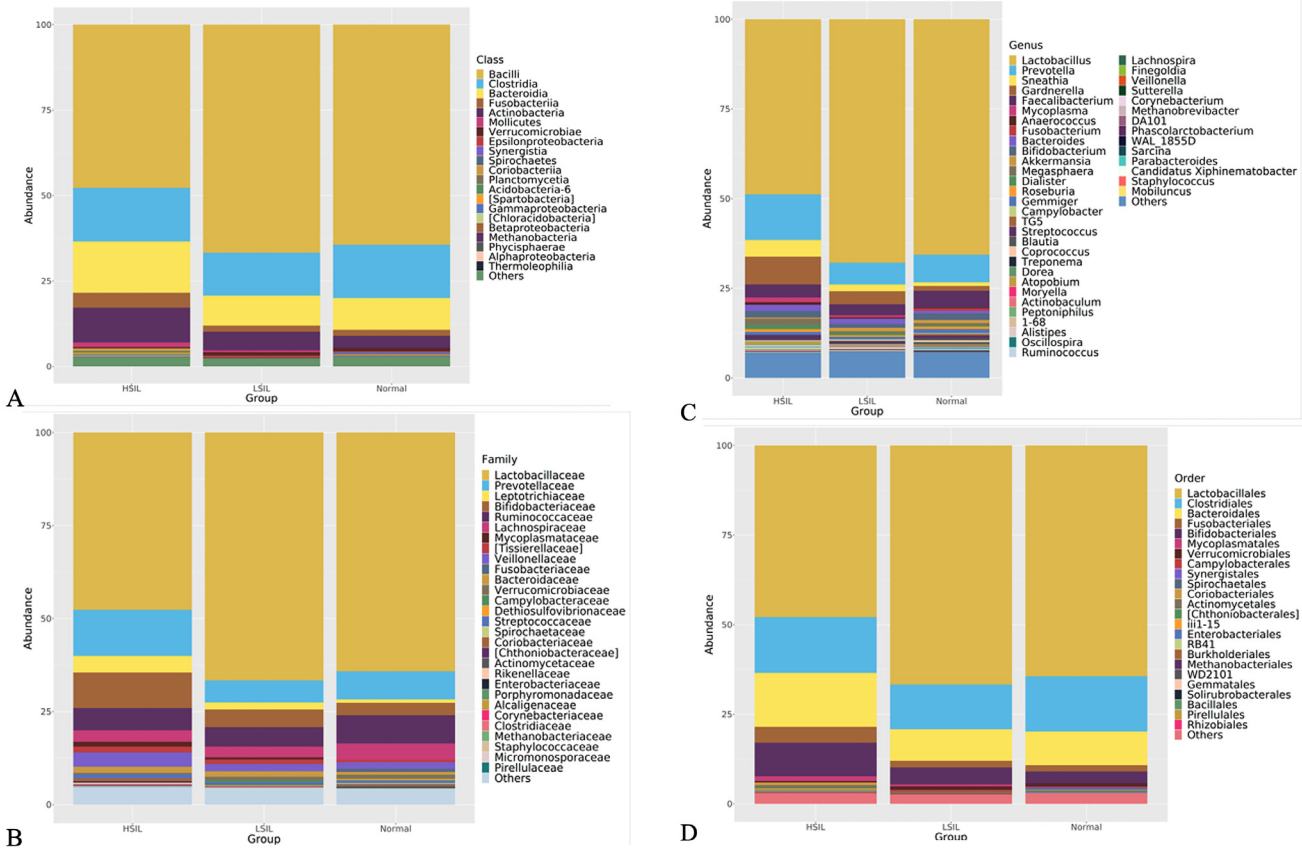


**Figure S2A.** Bacteria abundance distribution at class level; B: Bacteria abundance distribution at family level; C: Bacteria abundance distribution at genus level; D: Bacteria abundance distribution at order level

*Clinical Investigation / Araştırma*

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# The retrospective data analysis of NLRP7 and KHDC3L mutations in Turkish patients with recurrent hydatidiform mole

## *Tekrarlayan mol hidatidiformlu Türk hastalarda NLRP7 ve KHDC3L mutasyon verilerinin retrospektif analizi*

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### Abstract

**Objective:** Recurrent hydatidiform mole (RHM) is a rare disorder which is characterized by the presence of at least two molar pregnancies. The mutations in the *NLRP7* and *KHDC3L* genes are responsible for the majority of recurrent molar pregnancies. This study aimed to demonstrate the diversity and frequency of NLRP7 and KHDC3L gene mutations in our Turkish cohort with recurrent molar pregnancies, and to establish genotype-phenotype correlation.

**Materials and Methods:** It was aimed to represent the detected *NLRP7* and *KHDC3L* gene variants and reproductive history of 32 recurrent mole hydatidiform patients. We analysed the retrospective clinical and sequence data of 32 patients, who were referred to the laboratory for NLRP7 and KHDC3L sequencing.

**Results:** Among the detected 32 patients with recurrent molar pregnancy, 18 of 32 patients had no mutation in these two genes; we found 7 cases of homozygous NLRP7 variant, 1 case of heterozygous NLRP7 variant, 3 cases of homozygous *KHDC3L* gene variant, and 1 case of heterozygous *KHDC3L* gene variant. Among the detected NLRP7 variants, 3 of 11 variants were classified as pathogenic, 7 of 11 variants were classified as likely pathogenic, and 1 of 11 variants was classified as variant of unknown significance (VUS). Among the detected KHDC3L variants, 1 of 4 was classified as pathogenic, 2 of 4 were classified as likely pathogenic, and 1 of 4 was classified as VUS. Seven unpublished *NLRP7* gene variants and two unpublished *KHDC3L* gene variants were first reported in this study.

**Conclusion:** Here we report new RHM patients with NLRP7 and KHDC3L mutations. The current study highlights the importance of defining new cases and novel mutations in the pathogenesis and clinical management of RHM. Understanding genotype-phenotype correlations in RHM patients will also contribute to the selection of treatment methods and patient management.

**Keywords:** NLRP7 protein, KHDC3L protein, recurrent mole hydatidiform, next generation sequencing

### Öz

**Amaç:** Tekrarlayan mol gebeliklerin (RHM) büyük bir kısmından NLRP7 ve KHDC3L genlerinin mutasyonları sorumludur. Bu çalışmada tekrarlayan mol gebelikli Türk kohortumuzdaki NLRP7 ve KHDC3L gen mutasyonlarının çeşitliği ve sıklığının gösterilmesi ve genotip-fenotip korelasyonunun kurulması amaçlandı.

**Gereç ve Yöntemler:** Otuz iki tekrarlayan mol hidatidiform hastanın *NLRP7* ve *KHDC3L* gen varyantları ve üreme geçmişleri sunulmuştur. NLRP7 ve KHDC3L dizilmesi için laboratuvara sevk edilen 32 hastanın retrospektif klinik ve dizi verilerini analiz ettik.

**PRECIS:** Retrospective analysis of NLRP7 and KHDC3L sequence data revealed novel variants. The findings support genotype-phenotype correlations and have important implications for diagnosis, genetic counseling, and clinical management in recurrent molar pregnancies.

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**Bulgular:** Tespit edilen 32 tekrarlayan molar gebelik hastası arasında 32 hastanın 18'inde bu iki gende mutasyon saptanmamış olup, 7 olguda homozigot NLRP7 varyantı, 1 olguda heterozigot NLRP7 varyantı, 3 olguda homozigot *KHDC3L* gen varyantı ve 1 olguda heterozigot *KHDC3L* gen varyantı saptandı. Tespit edilen NLRP7 varyantlarından 11 varyanttan 3'ü patojenik, 11 varyanttan 7'si muhtemel patojenik ve 11 varyanttan 1'i önemi bilinmeyen varyant (VUS) olarak sınıflandırıldı. Tespit edilen KHDC3L varyantlarından 4 varyanttan 1'i patojenik, 4 varyanttan 2'si olası patojenik ve 4 varyanttan 1'i VUS olarak sınıflandırıldı. Bu çalışmada ilk kez 7 adet yayınlanmamış *NLRP7* gen varyantı ve 2 adet yayınlanmamış *KHDC3L* gen varyantı bildirildi.

**Sonuç:** Bu çalışmada NLRP7 ve KHDC3L mutasyonları olan yeni RHM hastalarını bildirilmektedir. Mevcut çalışma, RHM'nin patogenezinde ve klinik yönetiminde yeni olguların ve yeni mutasyonların tanımlanmasının önemini göstermektedir. RHM hastalarında genotip-fenotip korelasyonlarının anlaşılması tedavi yöntemlerinin seçimi ve hasta yönetimine de katkı sağlayacaktır.

**Anahtar Kelimeler:** NLRP7 protein, KHDC3L protein, tekrarlayan mol hidaditiform, yeni-nesil sekanslama

## Introduction

Molar pregnancy or mole hydatidiform is a gestational trophoblastic disease characterized by abnormal embryonic development and excessive proliferation of trophoblasts. Most cases of molar pregnancy are sporadic, occurring in approximately 1 in 600 pregnancies[1,2]. A hydatidiform mole is histopathologically classified into two main categories based on parental origin: complete hydatidiform mole and partial hydatidiform mole. Complete mole hydatidiform is characterized by excessive trophoblastic proliferation and the absence of extra-embryonic membranes. In contrast, partial molar hydatidiform shows mild trophoblastic proliferation and may contain extra-embryonic membranes and embryonic tissue. Most complete mole hydatidiform cases are diploid androgenetic, while most partial mole hydatidiform cases are dispermic triploid. Recurrent mole hydatidiform (RHM) is defined as the occurrence of ≥2 molar pregnancies[1,2]. Although the frequency varies among the different ethnicities, RHM accounts for approximately 1-10% of all molar hydatidiform cases[3]. The most common genetic cause of recurrent molar hydatidiform is a homozygous mutation of the *NLRP7* gene (OMIM 231090), which accounts for approximately 55% of cases[4-6]. The second most common cause is homozygous mutations of the *KHDC3L* gene (OMIM 611687), accounting for 5% of cases. In cases with mutations in *NLRP7* or *KHDC3L*, molar hydatidiform tissues are found to be diploid biparental[6-9]. Both genes regulate gene expression during oocyte and embryo development through genomic imprinting or epigenetic mechanisms. The *NLRP7* and *KHDC3L* genes are maternal-effect genes and components of the subcortical maternal complex, playing roles in the epigenetic reprogramming of the oocyte and the activation of embryonic development[6,10]. Mutations in the *NLRP7* and *KHDC3L* genes contribute to the pathogenesis of molar pregnancy by disrupting cytokine secretion and the implantation process. In addition to playing a role in oocyte and embryo development, *NLRP7* also regulates the release of interleukin-1 beta, contributing to inflammation and immune responses[8]. Mutations in the *NLRP7* or *KHDC3L* genes lead to the inactivation of the maternal allele and the expression of only the paternal allele. Monoallelic paternal expression of these genes causes defective placenta-specific imprinting and recurrent molar pregnancies[10,11]. Several studies have demonstrated that mutations in the *NLRP7* gene negatively impact oocyte quality and lead to arrest in embryonic development. *NLRP7* plays a role in sustaining genomic stability by regulating the alternative splicing of genes involved in homologous recombination repair[12]. Similarly, the *KHDC3L* mutation causes genomic instability in embryonic cells, which can result in increased DNA damage and subsequent embryonic developmental arrest[13]. Mutations in both *NLRP7* and *KHDC3L* disrupt DNA repair mechanisms, thereby contributing to impaired embryonic development[9]. Mutations of the *NLRP7* and *KHDC3L* genes have been reported to be associated with recurrent pregnancy losses and recurrent molar pregnancies.

It is not easy to clinically and histologicallydistinguish recurrent from spontaneous molar pregnancies. Therefore, the diagnosis of RHMs is often delayed. Although the mechanisms leading to molar pregnancy are not fully known, screening for known genetic factors is necessary to establish the genetic diagnosis of recurrent molar pregnancies. Late diagnosis in these patients may lead to recurrent molar pregnancies, unnecessary medications, and in vitro fertilization (IVF) treatments. Early diagnosis of recurrent molar pregnancies is essential for patients to receive appropriate treatment. The aim was to highlight the importance of the genetic diagnosis of RHM in clinical decision-making by reporting new cases. Here, we present the reproductive history and genomic variants, specifically NLPR7 and *KHDC3L* mutations, in Turkish patients with RHMs, including nine previously unpublished variants.

## Materials and Methods

### Patient Selection

A total of 32 patients with recurrent hydatidiform mole who were referred to the Mikrogen Genetic Diagnosis Center for *NLPR7* and *KHD3L* gene sequencing were retrospectively analyzed. Thirty-one patients had at least two molar pregnancies, and one patient had a history of choriocarcinoma. This study was approved by the Yüksek İhtisas University Medical School Ethical Committee (approval no: 295, date: 14.04.2025), and written consent was obtained. This study was conducted in accordance with the Declaration of Helsinki.

### *NLRP7* and *KH3DL* Gene Sequencing

Genomic DNA was isolated from peripheral blood samples using the QIAamp DNA Blood Kit (QIAGEN, Germany). Next-generation sequencing of the *NLRP7* and *KHD3L* genes was

performed on an Illumina MiSeq sequencing platform (Illumina Inc., San Diego, CA, USA) by following the manufacturer's instructions. The exon/exon-intron junctions of *NLRP7* and *KHDC3L* genes were sequenced to obtain a minimum read depth of 20x for >98% of the targeted bases. The Hg19 (GRCh37) sequence was used as a reference genome. FASTQ and VCF files were obtained. Bioinformatic solutions such as NextGENe (Version 2.4.2.3/SoftGenetics LLC-USA), Geneticist Assistant (Version 1.8.1.0/SoftGenetics LLC-USA), and Franklin Genoxx (Genoox, Israel) were used for VCF data analysis. All detected variants were evaluated according to their pathogenicity and classified according to the recommendations of international guidelines. Detected *NLRP7* and *KHDC3L* variants have been classified into five categories based on international standards, which are determined by their pathogenic effects (ACMG 2015): pathogenic, likely pathogenic, variant with unknown clinical significance (VUS), likely benign, and benign. A pathogenic variant is defined as a genetic change for which there is strong and well-established evidence indicating a direct causative role in disease. A likely pathogenic (LP) variant has greater than 90% certainty, of being disease-causing, based on available evidence. Conversely, a likely benign variant also has greater than 90% certainty; however, in this case, the evidence suggests that the variant is not associated with disease. A benign variant is one for which there is conclusive evidence demonstrating that it does not cause disease. A VUS refers to a genetic alteration for which the current evidence is either insufficient or conflicting regarding its role in disease. Identification of a VUS does not confirm or exclude a diagnosis. The variant classification is made according to the mutation type, its functional effect, whether it is defined in relevant databases and literature, and whether it is compatible with the patient's clinical findings.

## Results

A total of 32 patients with RHMs were analyzed for mutations in *NLRP7* and *KHDC3L* genes. 7 of 32 patients (21.8%) had a homozygous *NLRP7* variant, 1 of 32 patients (3.1%) had compound heterozygous *NLRP7* variants, 2 of 32 patients (6.2%) had a heterozygous *NLRP7* variant, 3 of 32 patients (9.3%) had a homozygous *KHDC3L* variant and 1 of 32 patients (%) had a heterozygous *KHDC3L* variant (Figure 1). No mutation was found in 56.2% of patients (18/32). Among the detected *NLRP7* variants, 3 of 11 variants were classified as pathogenic, 7 of 11 variants were classified as LP, and 1 of 11 variants was classified as VUS. Regarding the type of *NLRP7* gene variants, one missense, five frameshift, two nonsense, and three splice variants were reported. Among the detected *KHDC3L* variants, 3 out of 4 were classified as LP, and 1 out of 4 was classified as VUS. Regarding the type of *KHDC3L* gene variants, one missense and three frameshift variants have been reported. Seven unpublished *NLRP7* gene variants and two unpublished *KHDC3L* gene variants were first reported in this study (Table 1).

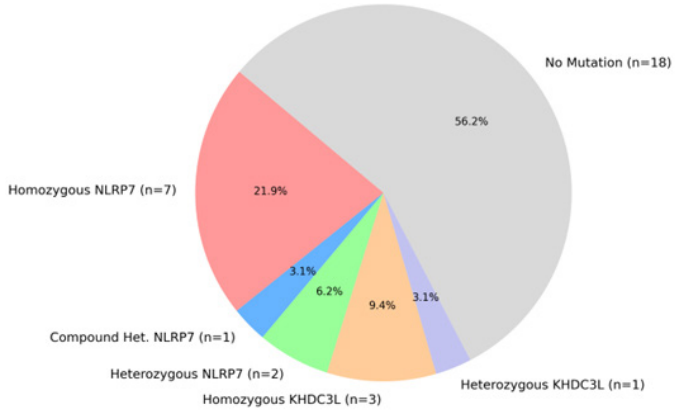


**Figure 1.** Distribution of genetic findings in RHM patients

*RHM: Recurrent hydatidiform mole, NLRP7: NLR family, pyrin domain-containing 7, KHDC3L: KHDC3L like protein, subcortical maternal complex member*

The clinical phenotypes of patients with *NLRP7* or *KHDC3L* gene variants are presented in Table 1. Eighteen of 32 patients had no variant in the *NLRP7* or *KHDC3L* genes. Seventeen of 18 patients (17/18), had at least two molar pregnancies, and one of them had a history of choriocarcinoma.

## Discussion

Here, we have reported the retrospective data of 32 patients with RHM referred to the Mikrogen Genetic Diagnosis Center. Seven patients had a homozygous *NLRP7* variant, one patient had a compound heterozygous *NLRP7* variant, and two had a heterozygous *NLRP7* variant. Individuals with *NLRP7* variants exhibit a broad spectrum of reproductive outcomes, including stillbirth, spontaneous abortion, blighted ovum, partial hydatidiform mole, complete hydatidiform mole, and, in rare instances, live birth. The underlying mechanisms through which specific mutation types in *NLRP7* contribute to the pathogenesis of molar pregnancies remain to be elucidated[14]. Biallelic *NLRP7* variants cause recurrent hydatidiform mole disease. Still, female carriers have an increased risk of reproductive failure, recurrent pregnancy loss, or having offspring with aberrant methylation and imprinting disorders. Limited data are reported regarding the effect of heterozygous *NLRP7* variants on phenotype. These studies suggested that female heterozygous carriers have a history of reproductive failure without molar pregnancy. Hayward et al.[15] demonstrated aberrant methylation in embryonic tissues of *NLRP7* heterozygous carriers, and they stated that the *NLRP7* gene regulates oocyte growth or controls the transduction of signals to initiate imprinting. Some studies have demonstrated that reproductive failure in heterozygous carriers of *NLRP7* variants is consistent with imprinting defects in placental tissues and increased maternal methylated transcripts[16,17]. Soellner et al.[18] reported a case of reproductive failure and fetal

**Table 1.** Clinical and genotypic data of RHM cases with NLRP7 and KHDC3L variants and our cases

| Patient no | Age | Clinical features | Gene | Variant | Zygosity | Variant type | Classification | References |
|---|---|---|---|---|---|---|---|---|
| 1 | 26 | Recurrent mole hydtatiform | *NLRP7* | c.241delA | Homozygous | Frameshift | Likely pathogenic | Recent study |
| 2 | 39 | Recurrent mole hydtatiform | *NLRP7* | c.1557delA | Homozygous | Frameshift | Likely pathogenic | Recent study |
| 3 | 27 | Recurrent mole hydtatiform and have 3 sisters with same condition | *NLRP7* | c.368G>A<br>c.2471+1G>A | Compound heterozygous | Nonsense<br>Splice altering | Likely pathogenic<br>Pathogenic | Recent study<br>Murdoch et al.[20],<br>Kocabey et al.[21] |
| 4 | 28 | Recurrent mole hydtatiform | *NLRP7* | c.368G>A | Homozygous | Nonsense | Likely pathogenic | Recent study |
| 5 | 41 | Recurrent mole hydtatiform | *NLRP7* | c.1374_1375del | Homozygous | Frameshift | Likely pathogenic | Recent study |
| 6 | 29 | Recurrent mole hydtatiform | *NLRP7* | c.2471+1G>A | Homozygous | Splice altering | Pathogenic | Murdoch et al.[20],<br>Kocabey et al.[21] |
| 7 | 38 | Recurrent mole hydtatiform | *NLRP7* | c.994del | Homozygous | Frameshift | Likely pathogenic | Recent study |
| 8 | 39 | Recurrent mole hydtatiform, ectopic pregnancy, IVF failure | *NLRP7* | c.2471+1G>A | Homozygous | Splice altering | Pathogenic | Murdoch et al.[20],<br>Kocabey et al.[21] |
| 9 | 26 | She had an affected daughter with neurological problems and her sister had a homozygous NLRP7 variant | *NLRP7* | c.2063delC | Heterozygous | Frameshift | Likely pathogenic | Wang et al.[5] |
| 10 | 27 | Recurent pregnancy losses and one molar pregnancy | *NLRP7* | c.799C>G | Heterozygous | Missense | VUS | Recent study |
| 11 | 23 | Recurrent mole hydtatiform | *KHDC3L* | c.322_325del | Homozygous | Frameshift | Likely pathogenic | Fallahian et al.[23],<br>Reddy et al.[7],<br>Landolsi et al.[9],<br>Parry et al.[10],<br>Wang et al.[5] |
| 12 | 27 | Recurrent mole hydtatiform | *KHDC3L* | c.322_325del | Homozygous | Frameshift | Likely pathogenic | Fallahian et al.[23],<br>Reddy et al.[7],<br>Landolsi et al.[9],<br>Parry et al.[10],<br>Wang et al.[5] |
| 13 | 26 | Recurrent mole hydtatiform | *KHDC3L* | c.396_397dup | Homozygous | Frameshift | Likely pathogenic | Recent study |
| 14 | 28 | Recurrent pregnancy losses | *KHDC3L* | c.572C>A | Heterozygous | Missense | VUS | Recent study |

NLRP7: NLR family, pyrin domain-containing 7, KHDC3L: KHDC3L like protein, subcortical maternal complex member, VUS: Variant of uncertain significance, RHM: Recurrent hydatidiform mole, IVF: In vitro fertilization

aberrant methylation. They stated that heterozygous variants of the *NLRP7* gene are associated with reproductive failures. The reported index patient had a frameshift mutation in *NLRP7* (NM_001127255.1: c.2010_2011del, p.(Phe671Glnfs*18)), resulting in a stop codon. In silico prediction tools suggested nonsense-mediated mRNA decay as the mechanism for the translated mRNA product. Qian et al.[19] also reported a female patient with a heterozygous *NLRP7* variant (c.295G>T, p.Glu99*) who had one stillbirth and three normal pregnancies. In the current study, we found heterozygous NLPR7 variants

in 2 cases. One of them had no reproductive failures, but she had an affected daughter with neurological problems (epilepsy, hypotonia, cerebellar atrophy), and her sister had a history of recurrent molar pregnancies. Another heterozygous carrier in our study had a missense mutation in the *NLRP7* gene and had a history of recurrent pregnancy losses and one molar pregnancy. Our case is also compatible with previously reported cases of heterozygous *NLRP7* mutations associated with reproductive failure.

The homozygous *NLRP7* variant (c.2471+1G>A) was first reported in a Pakistani patient with spontaneous abortion and complete hydatidiform mole[20]. Kocabey et al.[21] reported homozygous *NLRP7* c.2471+1G>A splice site variant in two Turkish patients with recurrent molar pregnancies. In the current study, we reported two different patients with homozygous *NLRP7* variants and one patient with a heterozygous *NLRP7* variant, all with the c.2471+1G>A mutation. One of the patients with a homozygous variant has a history of recurrent hydatidiform mole and one ectopic pregnancy. Another case with a homozygous variant has a history of recurrent hydatidiform mole (more than two hydatidiform moles). The patient with a heterozygous *NLRP7* variant (c.2471+1G>A) also had a LP heterozygous *NLRP7* variant (c.368G>A). The compound heterozygous patient had recurrent hydatidiform moles, and her three sisters also had recurrent molar pregnancies.

Wang et al.[5] reported a case with a homozygous *NLRP7* c.2147delC variant. The patient had 4 CHM and 2 PTD. Our study reported a patient with a heterozygous *NLRP7* variant (c.2147delC) who had no recurrent molar pregnancy, but she had a sister with a history of recurrent hydatidiform mole. Human epidermal growth factor receptor 2-year-old boy had a heterozygous *NLRP7* variant (c.2147delC) and neurological abnormalities.

We reported three patients with a homozygous *KHDC3L* variant (c.322_325delGACT) and one patient with a heterozygous *KHDC3L* variant (c.572C>A). Biallelic *KHDC3L* variants are associated with early embryonic arrest, recurrent hydatidiform moles, and recurrent pregnancy loss[11,19]. The *KHDC3L* variant (c.322_325delGACT) was reported in several studies[10,13,22,23]. Fatemi et al.[8] reported a large pedigree with a homozygous *KHDC3L* (c.322_325delGACT) variant and a history of recurrent molar pregnancies. Wang et al.[13] reported another patient with four recurrent molar pregnancies (complete hydatidiform mole). Wang et al.[13] reported another patient with four recurrent molar pregnancies who was compound heterozygous for *KHDC3L* variants (c.1A>G and c.322_325delGACT). In the current study, we found a homozygous *KHDC3L* (c.322_325delGACT) variant in two patients with recurrent molar pregnancies. Our patient with a heterozygous *KHDC3L* variant (c.572C>A) had recurrent pregnancy losses but no hydatidiform mole.

Previous studies suggest that patients with biallelic *KHDC3L* mutations may have a more severe phenotype compared to patients with biallelic *NLRP7* mutations. Fatemi et al.[8] reported that patients with a biallelic truncating *KHDC3L* variant, could not have a successful pregnancy and suffer from PL and HM; however, heterozygosity of the same variant does not result in HM and causes recurrent pregnancy losses[9]. Our patient with a heterozygous missense *KHDC3L* mutation had recurrent pregnancy losses but no HM, and the phenotype is consistent with the previously reported heterozygous cases.

Limited studies were reported in Turkish patients with *KHDC3L* and *NLRP7* gene variants. To date, no *KHDC3L* gene variant has been reported in Turkish patients with recurrent hydatidiform mole. Some of the variants detected in the *NLRP7* gene in our study were reported for the first time in Turkish patients. Kocabey et al.[21] reported a homozygous *NLRP7* splice site variant (c.2471+1G>A) and homozygous frameshift variant (c.2571dupC) in three Turkish patients with recurrent molar pregnancies. Balci et al.[24] found a homozygous *NLRP7* variant (c.3024_3025insC) in a Turkish patient with six hydatidiform moles and five missed abortions[24].

The likelihood of a normal pregnancy in women with a history of recurrent molar pregnancies has been reported to be very low[23,25]. Several studies have demonstrated that oocyte donation is the best treatment option for women carrying biallelic *NLRP7* variants; however, some women with these variants have been reported to have live births from their own oocytes, albeit rarely[25,26]. Although oocyte donation is considered the most effective treatment for *NLRP7* homozygous individuals, live birth cannot be achieved in all these patients. The reported live birth from women with biallelic *NLRP7* variants was attributed to different types of mutations. Missense, splice variants, or protein-truncating mutations of the *NLRP7* gene are expected to cause a milder functional effect[5,24,25].

### Study Limitations

The current study has some limitations. Mutations in the *NLRP7* and *KHDC3L* genes are responsible for most RHM cases; however, other genes have been reported in rare cases. We sequenced only the *NLRP7* and *KHDC3L* genes, but sequencing of other responsible genes is necessary to exclude the presence of mutations in *NLRP7-KHDC3L* mutation-negative cases. Another limitation of the current study is the lack of population-based data on the prevalence of *NLRP7* and *KHDC3L* mutations in the Turkish population. As no prior large-scale epidemiological studies exist, a power analysis could not be performed. Additionally, only a few published reports have described these mutations in Turkish patients. Nevertheless, this study includes the largest Turkish cohort to date screened for *NLRP7* and *KHDC3L* mutations, and offers preliminary but valuable data that may guide future studies aiming to establish

national prevalence rates and explore genotype–phenotype correlations. Furthermore, there was no information available on treatment options or live birth rates in cases with mutations. Further studies with large cohorts are needed to understand the etiopathogenesis of RHM and develop effective treatment options.

## Conclusion

Genetic counseling for RHM associated with pathogenic variants in the *NLRP7* and *KHDC3L* genes is a highly complex process that plays a crucial role in the clinical management of patients with RHM. Given the markedly low likelihood of successful pregnancy outcomes in patients with biallelic variants of these genes, it is essential to provide comprehensive counseling regarding reproductive options and the limited probability of achieving a live birth. Although there is no treatment addressing the genetic causes in RHMs with a genetic diagnosis, oocyte donation is the best reproductive method for pregnancy success. Most biallelic variants in *NLRP7* and *KHDC3L* are incompatible with live birth; therefore, studies aimed at identifying clinically relevant genetic variants will aid in the genetic counseling of these patients. Reporting additional cases with confirmed pathogenic variants will not only expand the understanding of genotype-phenotype correlations but also guide patients toward appropriate reproductive strategies, such as oocyte donation. Moreover, such data will help to prevent the patients from unnecessary IVF cycles, medications, and pregnancy expectations.

### Ethics

**Ethics Committee Approval:** This study was approved by the Yüksek İhtisas University Medical School Ethical Committee (approval no: 295, date: 14.04.2025).
**Informed Consent:** Written consent was obtained.

### Footnotes

### Authorship Contributions

Concept: L.Ö., E.Ü., S.A., Design: L.Ö., Data Collection or Processing: L.Ö., E.Ü., S.A., Analysis or Interpretation: L.Ö., E.Ü., S.A., Literature Search: L.Ö., Writing: L.Ö., E.Ü., S.A.
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## References

1. Kronfol NM, Iliya FA, Hajj SN. Recurrent hydatidiform mole: a report of five cases with review of the literature. J Med Liban. 1969;22:507-20.
2. Horn LC, Kowalzik J, Bilek K, Richter CE, Einenkel J. Clinicopathologic characteristics and subsequent pregnancy outcome in 139 complete hydatidiform moles. Eur J Obstet Gynecol Reprod Biol. 2006;128:10-4.
3. Eagles N, Sebire NJ, Short D, Savage PM, Seckl MJ, Fisher RA. Risk of recurrent molar pregnancies following complete and partial hydatidiform moles. Hum Reprod. 2015;30:2055-63.
4. Aguinaga M, Rezaei M, Monroy I, Mechtouf N, Pérez J, Moreno E. The genetics of recurrent hydatidiform moles in Mexico: further evidence of a strong founder effect for one mutation in NLRP7 and its widespread presence. J Assist Reprod Genet. 2021;38:1879-86.
5. Wang CM, Dixon PH, Decordova S, Hodges MD, Sebire NJ, Ozalp S, et al. Identification of 13 novel NLRP7 mutations in 20 families with recurrent hydatidiform mole; missense mutations cluster in the leucine-rich region. J Med Genet. 2009;46:569-75.
6. Bahutair SNM, Dube R, Kuruba MGB, Salama RAA, Patni MMAF, Kar SS, et al. Molecular basis of hydatidiform moles—a systematic review. Int J Mol Sci. 2024;25:8739.
7. Reddy R, Akoury E, Nguyen NMP, Abdul-Rahman OA, Dery C, Gupta N. Report of four new patients with protein-truncating mutations in C6orf221/KHDC3L and colocalization with NLRP7. Eur J Hum Genet. 2013;21:957-64.
8. Fatemi N, Ray PF, Ramezanali F, Shahani T, Amiri-Yekta A, Kherraf ZE, et al. KHDC3L frame-shift mutation causes both recurrent pregnancy loss and hydatidiform mole. Eur J Obstet Gynecol Reprod Biol. 2021;259:100-4.
9. Landolsi H, Rittore C, Philibert L, Hmissa S, Gribaa M, Touitou I, et al. NLRP7 mutation analysis in sporadic hydatidiform moles in Tunisian patients. Arch Pathol Lab Med. 2012;136:646-51.
10. Parry DA, Logan CV, Hayward BE, Shires M, Landolsi H, Diggle C, et al. Mutations causing familial biparental hydatidiform mole implicate C6orf221 as a regulator of genomic imprinting in the oocyte. Am J Hum Genet. 2011;89:451-8.
11. Florea A, Caba L, Grigore AM, Antoci LM, Grigore M, Gramescu M, et al. Hydatidiform mole—Between chromosomal abnormality, uniparental disomy, and monogenic variants: a narrative review. Life. 2023;13:2314.
12. Chen Z, Jiang L, Su M, Zeng O, Luo P, Chu L. NLRP7 maintains genomic stability during early embryogenesis via mediating alternative splicing. Commun Biol. 2025;8:125.
13. Wang X, Song D, Mykytenko D, Kuang Y, Lv Q, Li B, et al. Novel mutations in subcortical maternal complex proteins may cause embryonic developmental arrest. Reprod Biomed Online. 2018;36:698-704.
14. Mehta S, Mahay SB, Satapathy A, Arora K. Decoding the genetics of recurrent molar pregnancy. J Hum Reprod Sci. 2024;17:61-4.
15. Hayward BE, De Vos M, Talati N, Abdollahi MR, Taylor GR, Meyer E, et al. Genetic and epigenetic analysis of recurrent hydatidiform mole. Hum Mutat. 2009;30:E629-39.
16. Sanchez-Delgado M, Martin-Trujillo A, Tayama C, Vidal E, Esteller M, Iglesias-Plataset I, et al. Absence of maternal methylation in biparental hydatidiform moles from women with NLRP7 mutations reveals placenta-specific imprinting. PLoS Genet. 2015;11:e1005644.
17. Kou YC, Shao L, Peng HH, Rosetta R, Gaudio D, Wagner AF, et al. Novel mutations in NLRP7 and imprinting defects in biparental hydatidiform moles. Mol Hum Reprod. 2008;14:33-40.
18. Soellner L, Begemann M, Degenhardt F, Geipel A, Eggermann T, Mangold E. Maternal heterozygous NLRP7 variant results in

reproductive failure and imprinting disturbances in offspring. Eur J Hum Genet. 2017;25:924-9.

19. Qian J, Deveault C, Bagga R, Xie X, Slim R. Women heterozygous for NLRP7 mutations are at risk for reproductive wastage. Hum Mutat. 2007;28:741.
20. Murdoch S, Djuric U, Mazhar B, Seoud M, Khan R, Kuick R, et al. Mutations in NALP7 cause recurrent hydatidiform moles and reproductive wastage. Nat Genet. 2006;38:300-2.
21. Kocabey M, Gulhan I, Koc A, Cankaya T, Karatasli V, Ileri A. High risk of gestational trophoblastic neoplasia development in recurrent hydatidiform moles with NLRP7 pathogenic variations. Balkan J Med Genet. 2023; 25:45-50.
22. Zhang W, Chen Z, Zhang D, Zhao B, Liu L, Xie Z, et al. KHDC3L mutation causes recurrent pregnancy loss by inducing genomic instability. PLoS Biol. 2019;17:e3000468.
23. Fallahian M, Sebire NJ, Savage PM, Seckl MJ, Fisher RA. Mutations in NLRP7 and KHDC3L confer a complete mole phenotype on digynic triploid conceptions. Hum Mutat. 2013;34:301-8.
24. Balci MF, Kaya ÖÖ, Gezici AE, Oral S, Akpak YK, Karaduman AB, et al. Recurrent hydatidiform moles: detection of a new mutation in the NLRP7 gene in the family. Int J Reprod Contracept Obstet Gynecol. 2023;12:2251-4.
25. Akoury E, Gupta N, Bagga R, Brown S, Déry C, Kabra M, et al. Live births in women with recurrent hydatidiform mole and two NLRP7 mutations. Reprod Biomed Online. 2015;31:120-4.
26. Cozette C, Scheffler F, Lombart M, Massardier J, Bolze PA, Hajri T, et al. Pregnancy after oocyte donation in a patient with NLRP7 mutations and recurrent molar pregnancies. J Assist Reprod Genet. 2020;37:2273-7.

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# Risk factors for parametrial invasion in early-stage cervical cancer: Toward less radical surgery

## *Erken evre serviks kanserinde parametrial invazyon risk faktörleri: Daha az radikal cerrahi için ipuçları*

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### Abstract

**Objective:** Radical hysterectomy with parametrectomy remains the standard treatment for early-stage cervical cancer but is associated with significant morbidity. Identifying patients at low risk for parametrial invasion is critical to support less invasive surgical strategies.

**Materials and Methods:** This retrospective study evaluated 177 patients with Federation of Gynecology and Obstetrics 2018 stage IA-IIB cervical cancer who underwent type III radical hysterectomy with lymphadenectomy between 2001 and 2020. Clinical and pathological data were analyzed to identify predictors of parametrial invasion.

**Results:** Parametrial invasion was observed in 40 patients (22.6%). These patients were significantly older (mean age 56.05±11.16 vs. 49.21±10.80 years, p=0.013), and they were more likely to be postmenopausal. Parametrial invasion was associated with larger tumor size (35.10±13.72 mm vs. 24.15±13.50 mm), greater depth of stromal invasion (>10 mm), lymphovascular space invasion (LVSI), and lymph node metastases, (pelvic and paraaortic), all p<0.01. Bivariate logistic regression identified age ≥55 years [odds ratio (OR): 3.302 95% confidence interval (CI): 1.432-7.614, p=0.005], LVSI positivity [OR: 3.952 (95% CI: 1.641-9.518, p=0.002], and stromal invasion depth >10 mm [OR: 5.326 (95% CI: 2.157-13.153, p<0.001] as independent predictors of parametrial invasion.

**Conclusion:** Age ≥55, LVSI, and deep stromal invasion are significant independent risk factors for parametrial invasion. Careful evaluation of these parameters may guide the selection of patients suitable for less radical surgery, potentially reducing morbidity without compromising oncologic outcomes.

**Keywords:** Early-stage cervical cancer, parametrial invasion, radical hysterectomy, lymphovascular space invasion

**PRECIS:** Age over 55, lymphovascular space invasion positivity, and deep stromal invasion are independent risk factors for parametrial invasion in early-stage cervical cancer, supporting careful selection for less radical surgery.

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**Öz**

**Amaç:** Erken evre kanserinde parametrektomiyi de içeren radikal histerektomi standart tedavi olmaya devam etmektedir, ancak önemli morbidite ile ilişkilidir. Parametrial invazyon riski düşük hastaların belirlenmesi, daha az invaziv cerrahi stratejilerini desteklemek açısından önemlidir.

**Gereç ve Yöntemler:** Bu retrospektif çalışmada, Jinekoloji ve Obstetrik Federasyonu 2018'e göre evre IA-IIB serviks kanseri tanısı almış, 2001-2020 yılları arasında tip III radikal histerektomi ve lenfadenektomi uygulanmış 177 hasta değerlendirildi. Klinik ve patolojik veriler analiz edilerek parametrial invazyonu öngören faktörler belirlendi.

**Bulgular:** Parametrial invazyon 40 hastada (%22,6) saptandı. Bu hastalar anlamlı olarak daha yaşlı (ortalama yaş 56,05±11,16'ya karşı 49,21±10,80 yıl, p=0,013) ve çoğunlukla menopozdaki hastalardı. Parametrial invazyon; tümör boyutunun büyüklüğü (35,10±13,72 mm'ye karşı 24,15±13,50 mm), derin stromal invazyon (>10 mm), lenfovasküler alan invazyonu (LVSI) ve lenf nodu metastazı (pelvik ve paraaortik) (p<0,01) ile ilişkili olarak değerlendirildi. Lojistik regresyon analizinde 55 yaş ve üzeri olmak [risk oranı (RO): 3,302 %95 güven aralığı (GA): 1,432-7,614), p=0,005], LVSI pozitifliği [RO: 3,52 (%95 GA: 1,641-9,518), p=0,002] ve stromal invazyon derinliği >10 mm [RO: 5,326 (%95 GA: 2,157-13,153), p<0,001] parametrial invazyonun bağımsız risk faktörleri olarak saptandı.

**Sonuç:** Elli beş yaş ve üzeri olmak, LVSI pozitifliği ve derin stromal invazyon, parametrial invazyon için anlamlı bağımsız risk faktörleridir. Bu parametrelerin dikkatli değerlendirilmesi, daha az radikal cerrahiye uygun hastaların seçimini yönlendirebilir ve onkolojik sonuçlardan ödün vermeden morbiditeyi azaltabilir.

**Anahtar Kelimeler:** Erken evre serviks kanseri, parametrial invazyon, radikal histerektomi, lenfovasküler alan invazyonu

## Introduction

Cervical cancer has one of the highest mortality rates among gynecologic cancers. The Global Cancer Observatory 2020 study[1] estimated 604,127 cases and 341,831 deaths from the disease in 2020 based on the World Health Organization data[2]. The approved treatment for early-stage cervical cancer is radical type 3 hysterectomy, and removal of the parametrium, and lymph node dissection. These treatments, in particular, removal of the parametrium, causes several morbidities like bladder, bowel, and sexual dysfunction due to the extensive dissection of autonomic nerve fibers controlling these organs[3,4].

The shift toward less radical surgery in early-stage cervical cancer began with Dargent's pioneering retrospective analysis in 2000, which demonstrated that patients with tumor size >2 cm and invasion depth >10 mm had higher recurrence after fertility-preserving surgery[5]. Subsequent studies reinforced the idea that a subset of patients had minimal risk of parametrial invasion, particularly those with tumors <2 cm and invasion depth <10 mm. Reported parametrial invasion rates in this group were 1.94%[6], 0.4%[7], and 0.6%[8]. Frumovitz et al.[9] found a 7.7% parametrial invasion rate among 350 patients with stage IA1-IB1 tumors [Federation of Gynecology and Obstetrics (FIGO) 2009 classification] and identified risk factors for parametrial invasion: tumor size >2 cm, high grade, lymphovascular space invasion (LVSI), and pelvic lymph node involvement.

Based on these studies, the European Society of Gynecological Oncology (ESGO) recommended minimally invasive treatment options for stage 1A-1B1 cervical cancer patients in 2018[10]. Later on, a systematic review of 21 studies, reported that stage IA and a small portion of Stage IB1 patients were suitable for minimal invasive surgery and they emphasized potential increased risk of mortality among IB1 patients[11].

Recently, Plante et al.[12] reported their non-inferiority trial -the simple hysterectomy and pelvic node assessment trial- in patients with a tumor size less than 2 cm and cervical invasion depth less than 10 mm that simple hysterectomy did not worsen the 3-year outcomes of early-stage cervical cancer. However, extra-pelvic recurrence and death from cervical cancer were higher in the simple hysterectomy group, although statistically insignificant.

It is well known that parametrial invasion is an important risk factor for the prognosis of cervical cancer[9,13-17]. In this study, we aimed to identify the risk factors affecting parametrial invasion in our prospectively collected data.

## Materials and Methods

This study was approved by the Kanuni Sultan Süleyman Training and Research Hospital Local Ethics Committee (approval number: KAEK/2014/3/10, date: 31.12.20214). Our clinic has a prospectively collected database of all the oncologic patients starting from 1998, in which demographic information, type of surgery, postoperative complications, and postoperative follow-ups are documented. In this study, patients in this database who had cervical cancer stages IA-IIB according to the FIGO 2009 classification were analyzed retrospectively from 2001 to 2020. Only patients operated by Özgür Akbayır, Volkan Ülker, Ceyhun Numanoğlu and Merve Aldıkaçtıoğlu Talmaç were included. Clinical staging was performed by pelvic examination under general anesthesia and magnetic resonance imaging, following the diagnosis from cervical biopsies, endocervical curettage, and cold conization. They all had type III radical hysterectomy, including resection of pelvic and paraaortic lymph nodes and parametria, and most had bilateral salpingo-oophorectomy according to their menopausal status. The patients were analyzed for

their age, menopausal status, parity, body mass index (BMI), pathological type of the tumor, diameter of the tumor, clinical stage of the tumor, grade of the tumor, LVSI, pelvic lymph node metastasis, parametrial invasion, paraaortic lymph node metastasis, and depth of the invasion. The stages were then converted to FIGO 2018 classification when preparing the data.

Patients with concomitant cancer, those who received neo-adjuvant therapy, those without pelvic lymph node dissection, or those with missing data were excluded.

After informed consent was obtained, the standard surgery applied to all patients was type III radical hysterectomy (type C2 according to Querleu-Morrow classification) with pelvic and paraaortic lymph node dissection. Paraaortic lymph node dissection was applied to patients with indications such as palpable lymph nodes or patients with occult disease. In those without indications, inspection of paraaortic lymph nodes was still performed. The largest diameter of the tumor was regarded as the tumor size on the pathologic specimen. The presence of tumoral cells within lymphatic vessels outside the main tumor was defined as LVSI. Lymph node positivity was determined by the pathologist.

### Statistical Analysis

The data were presented as mean ± standard error for normally distributed variables, where normality was checked using the Kolmogorov-Smirnov test. Variables with non-normal distributions were expressed as medians (ranges). All statistical analyses were performed using the IBM SPSS Statistics 22 software (New York, USA). ANOVA was applied for the differences in age and BMI in different stages of the patients and Kruskal-Wallis test was applied for gravida, parity and menopause state. Pearson's chi-square test was applied to evaluate the patient surgical characteristics. For the evaluation of the factors related to parametrial invasion, independent samples t-test or chi-square tests were performed. Receiver operating characteristic (ROC) analysis was performed to define a cut-off for the effect of age on parametrial invasion. Bivariate logistic regression was applied to evaluate the factors associated with parametrial invasion. A *p*-value less than 0.05 was considered statistically significant.

## Results

There were 177 patients included in the study. The mean age of the patients was 50.76±11.22; of these, 57.60% were premenopausal and 42.40% were postmenopausal. Only 3 (1.69%) patients did not have any pregnancies, and the median parity was 3 (0-13). Mean BMI was 27.20±4.98 kg/m$^2$. Details of demographic variables by groups are presented in Table 1a.

The majority of the patients were stage IB2 (39.0%) according to FIGO 2018 classification, followed by stage IB1 (24.3%) and IIB (15.8%) as presented in Table 1b. Squamous cell carcinoma (88.07%) was the most prominent pathology. Among the pathology specimens, 55 were grade I (29.9%), 100 were grade II (56.5%), and 24 (13.6%) were grade III. The average diameter of the tumor was 26.62±13.72 mm, and cervical invasion size was 11.23±7.83 mm. LVSI was positive in 80 (45.2%) patients, pelvic lymph nodes were positive in 49 (27.6%) patients, and paraaortic lymph nodes were positive in 10 (5.6%) patients. All patients had pelvic and paraaortic lymph node dissection, and the median number of pelvic and paraaortic lymph nodes dissected was 20 (7-66) and 3 (0-60), respectively. The number of patients without paraaortic lymph node dissections was 12 (Stage IA1 and IA2).

When patients were grouped according to their stages, there were no differences in age, BMI, gravida, parity, menopausal status, and tumor histology between the groups (Tables 1a and 1b). Histological grades, LVSI, parametrial invasion, pelvic lymph node positivity, and paraaortic lymph node positivity were higher in more advanced stages, as reported in Table 1b. The average age of the patients with parametrial invasion was 56.05±11.16; higher than the average age of patients without parametrial invasion (p=0.013, Table 2). BMI, gravida, and parity of the patients with parametrial invasion were similar to those of patients without invasion (Table 2). Parametrial invasion was more common among menopausal patients (p=0.01, Table 2). To determine a cut-off value for the impact of age on parametrial invasion, we performed a ROC analysis. The area under the curve (AUC) was found to be 68% [AUC: 0.679 (95% confidence interval (CI): 0.579-0.778), p <0.001]. Age was set at 55 with 60.0% sensitivity and 73.7% specificity. For patients with parametrial invasion, mean tumor size was 35.10±13.72 mm, larger than those without invasion whose was 24.15±13.50 mm (p<0.01, Table 3). Moreover, for those whose tumor size was greater than 2 cm, parametrial invasion was prevalent, occurring in 90.00% of cases (n=36) (p<0.001, Table 2). Among those with parametrial invasion, 22.50% of patients were in stage IB, 7.50% were in stage IIA, and 70.00% were in stage IIB (p=0.009, Table 3). Grade I tumor was detected in 12.50%, whereas grades II and III were detected in 87.50% (p=0.006, Table 3). LVSI was positive in 75.00% of the patients with parametrial invasion (p<0.001, Table 3). Pelvic lymph node metastasis was present in 45.00% of the cases (p=0.005, Table 3), and paraaortic lymph node metastasis was present in 15.00% (p=0.004, Table 3) among patients with parametrial invasion. Lastly, average invasion depth was 17.10±7.37 mm (p<0.001, Table 3) for those with parametrial invasion, and for 80.00% of this group, the ratio of

**Table 1a.** Demographic data of the study population

| | | Age | BMI | Gravida | Parity | Menopause |
|---|---|---|---|---|---|---|
| Clinical stage | n (%) Total: 177 | Mean ± SD | Mean ± SD | Median (range) | Median (range) | n (%) |
| Stage IA1 | 8 (4.2) | 49.63±8.60 | 26.38±2.72 | 3 (2-12) | 2 (1-4) | 4 (50.0) |
| Stage IA2 | 4 (2.3) | 54.75±16.70 | 26.25±1.50 | 4 (2-5) | 2 (2-3) | 2 (50.0) |
| Stage IB1 | 43 (24.3) | 49.84±11.43 | 27.30±5.07 | 4 (0-11) | 3 (0-10) | 16 (37.2) |
| Stage IB2 | 69 (39.0) | 49.46±10.54 | 27.00±5.00 | 4 (2-13) | 3 (1-13) | 25 (36.2) |
| Stage IB3 | 17 (9.6) | 50.11±11.93 | 26.18±4.33 | 3 (2-12) | 2 (1-10) | 5 (29.4) |
| Stage IIA1 | 4 (2.3) | 54.25±7.04 | 27.25±6.65 | 4 (2-6) | 4 (2-6) | 1 (25.0) |
| Stage IIA2 | 4 (2.3) | 47.25±13.4 | 23.75±2.06 | 2 (1-5) | 2 (1-5) | 2 (50.0) |
| Stage IIB | 28 (15.8) | 55.50±11.92 | 29.00±5.80 | 4 (0-10) | 3 (0-9) | 19 (67.8) |
| p | | 0.369[a] | 0.456[a] | 0.869[b] | 0.665[b] | 0.161[b] |

BMI: Body mass index, SD: Standard deviation, [a]: ANOVA test, [b]: Kruskal-Wallis test

**Table 1b.** Surgical properties of the study group

| | Pathology | | | Grade | | | LSVI | Parametrial invasion | Pelvic lymph node positivity | Paraaortic lymph node positivity |
|---|---|---|---|---|---|---|---|---|---|---|
| Clinical Stage | SCC | AdCa | Others | I | II | III | n (%) | n (%) | n (%) | n (%) |
| Stage IA1 | 7 | 1 | 0 | 7 | 1 | 0 | 0 | 0 | 0 | 0 |
| Stage IA2 | 3 | 0 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 0 |
| Stage IB1 | 39 | 3 | 1 | 18 | 23 | 2 | 14 (32.6) | 0 | 6 (14.0) | 1 (2.3) |
| Stage IB2 | 60 | 9 | 0 | 17 | 45 | 7 | 28 (41.1) | 7 (10.1) | 19 (27.5) | 0 |
| Stage IB3 | 14 | 1 | 2 | 2 | 9 | 6 | 12 (70.5) | 2 (11.8) | 7 (41.2) | 4 (23.5) |
| Stage IIA1 | 3 | 0 | 1 | 0 | 3 | 1 | 3 (75.0) | 3 (75.0) | 1 (25.0) | 0 |
| Stage IIA2 | 4 | 0 | 0 | 2 | 1 | 1 | 3 (75.0) | 0 | 3 (75.0) | 0 |
| Stage IIB | 25 | 2 | 1 | 4 | 17 | 7 | 20 (71.4) | 28 (100) | 13 (46.4) | 5 (17.9) |
| p | 0.861 | | | **<0.001**[a*] | | | **<0.001**[a*] | **<0.001**[a*] | **0.007**[a*] | **0.001**[a*] |

SCC: Squamous cell carcinoma, AdCa: Adenocarcinoma, [a]: Pearson chi-square test, *: p<0.05

cervical invasion was greater than 10 mm (p<0.001, Table 2). The factors significantly associated with parametrial invasion (age, menopausal status, tumors larger than 2 cm, grade, LVSI positivity, pelvic lymph node positivity, paraaortic lymph node positivity, cervical invasion depth greater than 10 mm) were then analyzed again using the backward regression method. Only age ≥55, LVSI positivity and cervical invasion depth >10 mm were significantly associated with parametrial invasion p=0.007, p=0.011 and p=0.011, respectively; Table 3). Bivariate logistic regression analysis was performed with these three independent factors, and all three were found to be statistically significant (Table 4). Age ≥55 increased the odds of parametrial invasion 3.3-fold [odds ratio (OR): 3.302 (95% CI: 1.432–7.614, p=0.005)], LVSI positivity 4-fold [OR: 3.952 (95% CI: 1.641-9.518, p=0.002)], and cervical invasion more than 10 mm by 5.3-fold [OR: 5.326 (95% CI: 2.157-13.153, p<0.001)] (Table 4).

A subgroup analysis of our cohort (tumor size <2 cm and cervical invasion <10 mm) identified 4 patients with parametrial invasion among 62 patients (6.45%). Within these patients, three were older than 55; one had LVSI; and one had both LVSI and pelvic lymph node positivity. Among patients younger than 55 years who had no LVSI, tumor size <2 cm, and stromal invasion <10 mm, only 1 of 49 (2.0%) had parametrial involvement.

Table 5 summarizes the distribution of key pathological variables according to age groups. Parametrial invasion was observed in 25 of 65 patients (38.46%) aged 55 years or older, compared to 15 of 112 patients (13.39%) under the age of 55.

**Table 2.** Factors related with parametrial invasion (n=177)

| | Parametrial invasion | | |
|---|---|---|---|
| | Absent (n=137) | Presence (n=40) | |
| Parameters | n (%) | n (%) | p |
| Age (mean ± SD) | 49.21±10.80 | 56.05±11.16 | **0.013**[a*] |
| <55 | 97 (70.8) | 15 (37.5) | **<0.001**[b*] |
| ≥55 | 40 (29.2) | 25 (62.5) | |
| Menopausal state | | | **0.010**[b*] |
| Premenopausal | 86 (62.8) | 16 (40.0) | |
| Postmenopausal | 51 (37.2) | 24 (60.0) | |
| Gravida (median, range) | 4 (0-13) | 4 (0-12) | 0.943[b] |
| Parity (median, range) | 3 (0-13) | 3 (0-10) | 0.923[b] |
| BMI (mean ± SD) | 26.85±4.78 | 28.36±5.50 | 0.089[a] |
| <30 | 100 (73.0) | 26 (65.0) | 0.326[b] |
| ≥30 | 37 (27.0) | 14 (35.9) | |
| Pathology | | | 0.269[b] |
| SCC | 122 (89.0) | 33 (82.5) | |
| Others | 15 (11.0) | 7 (17.5) | |
| Tumor maximum dimension (mm) (mean ± SD) | 24.15±13.50 | 35.10±13.72 | **<0.001**[a*] |
| ≤20 mm | 61 (44.5) | 4 (10.0) | **<0.001**[b*] |
| >20 mm | 76 (55.5) | 36 (90.0) | |
| Clinical stage | | | **<0.001**[b*] |
| Stage IA1-2 | 12 (8.8) | 0 (0.0) | |
| Stage IB1-3 | 120 (87.5) | 9 (22.5) | |
| Stage IIA1-2 | 5 (3.7) | 3 (7.5) | |
| Stage IIB | 0 (0.0) | 28 (70.0) | |
| Grade | | | **0.006**[b*] |
| I | 48 (35.0) | 5 (12.5) | |
| II-III | 89 (65.0) | 35 (87.5) | |
| LVSI | | | **<0.001**[b*] |
| None | 87 (63.5) | 10 (25.0) | |
| Present | 50 (36.5) | 30 (75.0) | |
| Pelvic lymph node positivity | | | **0.005**[b*] |
| None | 106 (77.3) | 22 (55.0) | |
| Present | 31 (22.7) | 18 (45.0) | |
| Paraortic lymph node positivity | | | **0.004**[b*] |
| None | 133 (97.1) | 34 (85.0) | |
| Present | 4 (2.9) | 6 (15.0) | |
| Invasion deepness (mm) (mean ± SD) | 9.52±7.12 | 17.10±7.37 | **<0.001**[a*] |
| ≤10 mm | 92 (67.2) | 8 (20.0) | **<0.001**[b*] |
| >10 mm | 45 (32.8) | 32 (80.0) | |

[a]: Independent Samples t-test, [b]: Chi-square tests (pearson chi-square, continuity correction, Fisher's exact test), [*]: p<0.05, SD: Standard deviation

**Table 3.** The factors related with parametrial invasion

| Factors | Unstandardized coefficients | | Standardized coefficients | | | 95% CI | |
|---|---|---|---|---|---|---|---|
| | B | S.E. | Beta | t | p | Lower | Upper |
| Constant | -0.192 | 0.128 | | -1.499 | 0.136 | -0.445 | 0.061 |
| Menopausal status | -0.145 | 0.106 | -0.171 | -1.364 | 0.174 | -0.354 | 0.065 |
| LVSI positivity | 0.176 | 0.068 | 0.209 | 2.577 | 0.011 | 0.041 | 0.310 |
| Pelvic lymph node positivity | -0.011 | 0.080 | -0.012 | -0.139 | 0.890 | -0.168 | 0.146 |
| Paraaortic lymph node positivity | 0.204 | 0.134 | 0.113 | 1.525 | 0.129 | -0.060 | 0.469 |
| Age ≥55 | 0.298 | 0.109 | 0.343 | 2.738 | 0.007 | 0.083 | 0.513 |
| Tumor size >2 cm | 0.040 | 0.076 | 0.046 | 0.526 | 0.599 | -0.110 | 0.190 |
| Cervical invasion >10 mm | 0.196 | 0.076 | 0.231 | 2.579 | 0.011 | 0.046 | 0.345 |
| BMI | 0.064 | 0.064 | 0.069 | 1.003 | 0.317 | -0.062 | 0.190 |
| Grade | 0.053 | 0.065 | 0.058 | 0.819 | 0.414 | -0.075 | 0.181 |

LVSI: Lymphovascular space invasion, BMI: Body mass index, S.E.: Standard error, Method: Backward regression, Model1: $R^2$ = 0.279; p<0.001, CI: Confidence interval

**Table 4.** Independent parameters associated with parametrial invasion (Logistic regression analysis results)

| Factors | Reference group | B | S.E. | Wald | df | p | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|
| LVSI | No | 1.374 | 0.448 | 9.389 | 1 | 0.002 | 3.952 | 1.641-9.518 |
| Depth of invasion | ≤10 mm | 1.673 | 0.461 | 13.152 | 1 | <0.001 | 5.326 | 2.157-13.153 |
| Age | <55 | 1.194 | 0.426 | 7.849 | 1 | 0.005 | 3.302 | 1.432-7.614 |
| Constant | - | -3.508 | 0.514 | 46.545 | 1 | <0.001 | 0.030 | - |

LVSI: Lymphovascular space invasion, S.E.: Standard error, Method: Forward stepwise, Model2: $X^2$ = 47.143; p<0.001, CI: Confidence interval

**Table 5.** Stratified analysis of patients according to age

| | Parametrial invasion in those <55 years (n=112) | | | Parametrial invasion in those ≥55 years (n=65) | | |
|---|---|---|---|---|---|---|
| | Absent (n=97) | Present (n=15) | | Absent (n=40) | Present (n=25) | |
| Parameters | n (%) | n (%) | p | n (%) | n (%) | p |
| Age (mean ± SD) | 43.67±6.51 | 43.73±6.44 | 0.972[a] | 62.65±6.37 | 63.44±5.15 | 0.604[a] |
| **Menopausal state** | | | 0.801[b] | | | 0.281[b] |
| Premenopausal | 86 (88.7) | 14 (93.3) | | 0 (0.0) | 2 (8.0) | |
| Postmenopausal | 11 (11.3) | 1 (6.7) | | 40 (100.0) | 23 (92.0) | |
| Gravida (median, range) | 3 (0-13) | 3 (0-8) | 0.479[c] | 5 (2-13) | 5 (0-12) | 0.531[c] |
| **Parity (median, range)** | 2 (0-13) | 2 (0-6) | 0.343[c] | 4 (1-13) | 3 (0-10) | 0.465[c] |
| BMI (mean ± SD) | 26.48±4.77 | 25.40±4.13 | 0.407[a] | 27.75±4.74 | 30.16±5.52 | 0.066[a] |
| <30 | 73 (75.3) | 12 (80.0) | 0.940[b] | 23 (63.9) | 13 (54.2) | 0.451[b] |
| ≥30 | 24 (24.7) | 3 (20.0) | | 13 (36.1) | 11 (45.8) | |
| **Pathology** | | | 0.600[b] | | | 0.743[b] |
| SCC | 86 (88.7) | 12 (80.0) | | 36 (90.0) | 21 (84.0) | |
| Others | 11 (11.3) | 3 (20.0) | | 4 (10.0) | 4 (16.0) | |

**Table 5.** Continued

| | Parametrial invasion in those <55 years (n=112) | | | Parametrial invasion in those ≥55 years (n=65) | | |
|---|---|---|---|---|---|---|
| | Absent (n=97) | Present (n=15) | | Absent (n=40) | Present (n=25) | |
| Parameters | n (%) | n (%) | p | n (%) | n (%) | p |
| **Tumor max dimension (mm) (mean ± SD)** | 23.65±12.39 | 37.60±11.30 | **<0.001**[a*] | 25.38±15.97 | 33.60±10.65 | **0.026**[a*] |
| ≤20 mm | 43 (44.3) | 1 (6.7) | **0.005**[b*] | 18 (45.0) | 3 (12.0) | **0.012**[b*] |
| >20 mm | 54 (55.7) | 14 (93.3) | | 22 (55.0) | 22 (88.0) | |
| **Clinical stage** | | | **<0.001**[b*] | | | **<0.001**[b*] |
| Stage IA1-2 | 8 (8.2) | 0 (0.0) | | 4 (10.0) | 0 (0.0) | |
| Stage IB1-3 | 86 (88.7) | 4 (26.7) | | 34 (85.0) | 5 (20.0) | |
| Stage IIA1-2 | 3 (3.1) | 1 (6.7) | | 2 (5.0) | 2 (8.0) | |
| Stage IIB | 0 (0.0) | 10 (66.6) | | 0 (0.0) | 18 (72.0) | |
| **Grade** | | | 0.070[b] | | | 0.159[b] |
| I | 36 (37.1) | 2 (13.3) | | 12 (30.0) | 3 (12.0) | |
| II-III | 61 (62.9) | 13 (86.7) | | 28 (70.0) | 22 (88.0) | |
| **LVSI** | | | **<0.001**[b*] | | | **0.017**[b*] |
| None | 61 (63.5) | 2 (13.3) | | 25 (62.5) | 8 (32.0) | |
| Present | 35 (36.5) | 13 (86.7) | | 15 (37.5) | 17 (68.0) | |
| **Pelvic lymph node positivity** | | | **0.010**[b*] | | | 0.153[b] |
| None | 74 (76.3) | 6 (40.0) | | 32 (80.0) | 16 (64.0) | |
| Present | 23 (23.7) | 9 (60.0) | | 8 (20.0) | 9 (36.0) | |
| **Paraortic lymph node positivity** | | | **0.037**[b*] | | | 0.308[b] |
| None | 94 (97.0) | 12 (80.0) | | 39 (97.5) | 22 (88.0) | |
| Present | 3 (3.0) | 3 (20.0) | | 1 (2.5) | 3 (12.0) | |
| **Invasion deepness (mm) (mean ± SD)** | 8.95±6.70 | 16.47±7.58 | **<0.001**[a*] | 10.90±7.98 | 17.48±7.38 | **0.001**[a*] |
| ≤10 mm | 70 (72.2) | 3 (20.0) | **<0.001**[b*] | 22 (55.0) | 5 (20.0) | **0.005**[b*] |
| >10 mm | 27 (27.8) | 12 (80.0) | | 18 (45.0) | 20 (80.0) | |

[a]: Independent Samples t-test, [b]: Chi-square tests (pearson chi-square, continuity correction, Fisher's exact test), [c]: Mann-Whitney U test, [*]: p<0.05
SD: Standard deviation

## Discussion

In this study, we aimed to identify the risk factors associated with parametrial invasion in early-stage cervical cancer patients who underwent radical hysterectomy. Our findings indicate that age ≥55 years, LVSI positivity, and cervical invasion depth >10 mm are significant independent risk factors for parametrial invasion.

Our results suggested that the incidence of parametrial involvement in early-stage cervical cancer is relatively low, at 6%, particularly in tumors <2 cm with a cervical invasion depth <10 mm. This finding is in line with the literature[6,7,9,18,19]. The ESGO guidelines have already proposed less radical surgical options for carefully selected cases with early-stage disease[10]. Recently, a systematic review found that while less radical surgery is feasible for stage 1A and some stage 1B1 patients, it may be associated with an increased risk of recurrence in patients with larger tumors[11]. We aimed to examine the risk factors that would aid in better patient selection for future studies, especially in this IB1 group.

Our study revealed that patients with parametrial invasion were significantly older than those without invasion, and menopausal status was significantly associated with higher parametrial involvement. After the logistic regression analysis, determined with ROC analysis, we showed that age ≥55 years increases the odds of parametrial invasion by 3.3-fold. Most studies on risk factors affecting parametrial invasion did not show age differences in parametrial invasion[20-22]. A study of a Taiwanese cohort demonstrated that the average age of patients with parametrial invasion was around 56 years, which supported our findings[16]. A French cohort suggested that age over 65 is an independent risk factor for parametrial invasion[13]. Taskum et al.[23] found that older age was associated with LVSI positivity, however, was not an independent risk factor.

Postmenopausal women have been shown to be more susceptible to HPV positivity[24]. This is often attributed to viral reactivation during menopause and changes in sexual behavior, such as, new partner acquisition[25]. This susceptibility is compounded by age-related immunologic changes, including impaired immune surveillance and hormonal alterations, which may contribute to a more aggressive course of cervical cancer in older women compared to their younger counterparts[26]. Supporting this, studies have demonstrated more severe cytological abnormalities in postmenopausal women, along with a reduced number of transformation zone cells in cervical cytology samples - potentially limiting early detection[27].

The average age of menopause in Türkiye is around 45-49[28,29], and in France it is 52[30]. Additionally, several studies have reported that women are less likely to continue routine gynecologic screenings after menopause[31]. This decline in participation can lead to delayed diagnosis, allowing for disease progression before clinical detection. As a result, age over 55 may emerge as an independent risk factor for parametrial invasion— not solely due to chronological age, but as a surrogate marker for physiological changes and decreased screening participation. These combined factors likely increase the probability of detecting cervical cancer at a more advanced pathological stage, contributing to the higher rates of parametrial involvement observed in older women.

The most critical factors associated with parametrial invasion were LVSI and depth of cervical invasion. In our cohort, tumor size was significantly larger in patients with parametrial invasion, and among those with tumors >2 cm, there was a 90% prevalence of parametrial involvement. However, this was not a significant factor for determining parametrial invasion in the bivariate logistic regression model. Previous studies have consistently demonstrated that larger tumors pose a higher risk for parametrial invasion, supporting the need for careful patient selection when considering fertility-sparing or less radical surgeries[6,19].

LVSI positivity was observed in 75% of the patients with parametrial invasion, and increased the odds of parametrial invasion fourfold in bivariate logistic analysis. LVSI has been recognized as a key predictor of lymphatic spread and worse prognosis in cervical cancer, often guiding decisions regarding adjuvant treatment[12]. Similarly, a cervical invasion depth >10 mm was observed in 80% of cases with parametrial invasion, which increased the odds by 5.3-fold, emphasizing its importance in surgical planning. This finding aligns with prior reports indicating that deep stromal invasion is associated with an increased risk of parametrial invasion[14,16,21,22,32].

Additionally, we found a significant association between parametrial invasion and pelvic lymph node metastasis, as well as paraaortic lymph node metastasis. Lymph node metastasis is a well-established factor influencing the prognosis and treatment strategy for cervical cancer, often necessitating adjuvant chemoradiotherapy to reduce recurrence risk[21].

### Study Limitations

Our study has some limitations, including its retrospective nature and single-center design, which may limit generalizability. Additionally, although we performed bivariate analysis to control for confounders, the possibility of selection bias remains. Future prospective studies and clinical trials are needed to validate our findings and refine patient selection criteria for less radical surgeries.

## Conclusion

In conclusion, our study highlights that age ≥55 years, LVSI positivity, and cervical invasion depth >10 mm are the most significant predictors of parametrial invasion in early-stage cervical cancer. These factors should be carefully considered when selecting candidates for less radical surgical approaches to optimize oncologic outcomes while minimizing morbidity.

### Ethics

**Ethics Committee Approval:** This study was approved by the Kanuni Sultan Süleyman Training and Research Hospital Local Ethics Committee (approval number: KAEK/2014/3/10, date: 31.12.20214).

**Informed Consent:** Retrospective study.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: S.G.Ç., M.A.T., C.N., V.Ü., Ö.A., Concept: S.G.Ç., A.A., C.N., V.Ü., Ö.A., Design: S.G.Ç., M.A.T., A.A., C.N., V.Ü., Ö.A., Data Collection or Processing: M.B., M.A.T., A.A., Analysis or Interpretation: S.G.Ç., E.A., E.U.B.Ö., Literature Search: S.G.Ç., M.B., E.A., E.U.B.Ö., Writing: S.G.Ç., C.N., V.Ü., Ö.A.

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## References

1. Singh D, Vignat J, Lorenzoni V, Eslahi M, Ginsburg O, Lauby-Secretan B et al. Global estimates of incidence and mortality of cervical cancer

in 2020: a baseline analysis of the WHO Global Cervical Cancer Elimination Initiative. Lancet Glob Health. 2023;11:e197-206.

2. Arbyn M, Weiderpass E, Bruni L, de Sanjose S, Saraiya M, Ferlay J, et al. Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. Lancet Glob Health. 2020;8:e191-203.
3. Jackson KS, Naik R. Pelvic floor dysfunction and radical hysterectomy. Int J Gynecol Cancer. 2006;16:354-63.
4. Trimbos JB, Franchi M, Zanaboni F, Velden J, Vergote I. 'State of the art' of radical hysterectomy; current practice in European oncology centres. Eur J Cancer. 2004;40:375-8.
5. Dargent D, Martin X, Sacchetoni A, Mathevet P. Laparoscopic vaginal radical trachelectomy: a treatment to preserve the fertility of cervical carcinoma patients. Cancer. 2000;88:1877-82.
6. Stegeman M, Louwen M, van der Velden J, ten Kate FJ, den Bakker MA, Burger CW, et al. The incidence of parametrial tumor involvement in select patients with early cervix cancer is too low to justify parametrectomy. Gynecol Oncol. 2007;105:475-80.
7. Wright JD, Grigsby PW, Brooks R, Powell MA, Gibb RK, Gao F, et al. Utility of parametrectomy for early stage cervical cancer treated with radical hysterectomy. Cancer. 2007;110:1281-6.
8. Kinney WK, Hodge DO, Egorshin EV, Ballard DJ, Podratz KC. Identification of a low-risk subset of patients with stage IB invasive squamous cancer of the cervix possibly suited to less radical surgical treatment. Gynecol Oncol. 1995;57:3-6.
9. Frumovitz M, Sun CC, Schmeler KM, Deavers MT, Dos Reis R, Levenback CF, et al. Parametrial involvement in radical hysterectomy specimens for women with early-stage cervical cancer. Obstet Gynecol. 2009;114:93-9.
10. Cibula D, Potter R, Planchamp F, Avall-Lundqvist E, Fischerova D, Haie Meder C, et al. The European Society of Gynaecological Oncology/ European Society for Radiotherapy and Oncology/European Society of Pathology guidelines for the management of patients with cervical cancer. Radiother Oncol. 2018;127:404-16.
11. Wu J, Logue T, Kaplan SJ, Melamed A, Tergas AI, Khoury-Collado F, et al. Less radical surgery for early-stage cervical cancer: a systematic review. Am J Obstet Gynecol. 2021;224:348-58.e5.
12. Plante M, Kwon JS, Ferguson S, Samouelian V, Ferron G, Maulard, A et al. Simple versus radical hysterectomy in women with low-risk cervical cancer. N Engl J Med. 2024;390:819-29.
13. Dabi Y, Willecocq C, Ballester M, Carcopino X, Bendifallah S, Ouldamer L, et al. Identification of a low risk population for parametrial invasion in patients with early-stage cervical cancer. J Transl Med. 2018;16:163.
14. Boyraz G, Basaran D, Salman MC, Ozgul N, Yuce K. Clinical and pathological characteristics related to parametrial involvement in clinical early-stage cervical cancer. Ginekol Pol. 2016;87:417-21.
15. He F, Du J, Chen X, He L. Assessment of parametrial involvement in early stages cervical cancer with preoperative magnetic resonance imaging. Int J Gynecol Cancer. 2018;28:1758-65.
16. Hsu HC, Tai YJ, Chen YL, Chiang YC, Chen CA, Cheng WF. Factors predicting parametrial invasion in patients with early-stage cervical carcinomas. PLoS One. 2018;13:e0204950.
17. Jiamset I, Hanprasertpong J. Risk factors for parametrial involvement in early-stage cervical cancer and identification of patients suitable for less radical surgery. Oncol Res Treat. 2016;39:432-8.
18. Schmeler KM, Frumovitz M, Ramirez PT. Conservative management of early stage cervical cancer: is there a role for less radical surgery? Gynecol Oncol. 2011;120:321-5.
19. Steed H, Capstick V, Schepansky A, Honore L, Hiltz M, Faught W. Early cervical cancer and parametrial involvement: is it significant? Gynecol Oncol. 2006;103:53-7.
20. Li C, Yang S, Hua K. Nomogram predicting parametrial involvement based on the radical hysterectomy specimens in the early-stage cervical cancer. Front Surg. 2021;8:759026.
21. Ma C, Zhang Y, Li R, Mao H, Liu P. Risk of parametrial invasion in women with early stage cervical cancer: a meta-analysis. Arch Gynecol Obstet. 2018;297:573-80.
22. Vanichtantikul A, Tantbirojn P, Manchana T. Parametrial involvement in women with low-risk, early-stage cervical cancer. Eur J Cancer Care (Engl). 2017;26.
23. Taskum I, Bademkiran MH, Cetin F, Sucu S, Yergin E, Balat O, et al. A novel predictive model of lymphovascular space invasion in early-stage endometrial cancer. Turk J Obstet Gynecol. 2024;21:37-42.
24. Gonzalez P, Hildesheim A, Rodriguez AC, Schiffman M, Porras C, Wacholder S, et al. Behavioral/lifestyle and immunologic factors associated with HPV infection among women older than 45 years. Cancer Epidemiol Biomarkers Prev. 2010;19:3044-54.
25. Gravitt PE, Rositch AF, Silver MI, Marks MA, Chang K, Burke AE, et al. A cohort effect of the sexual revolution may be masking an increase in human papillomavirus detection at menopause in the United States. J Infect Dis. 2013;207:272-80.
26. Rodriguez-Garcia M, Patel MV, Shen Z, Wira CR. The impact of aging on innate and adaptive immunity in the human female genital tract. Aging Cell. 2021;20:e13361.
27. Campaner AB, Fernandes GL. Discussion on cervical cytology in postmenopausal women. Minerva Obstet Gynecol. 2024;76:532-9.
28. Ozdemir O, Col M. The age at menopause and associated factors at the health center area in Ankara, Turkey. Maturitas. 2004;49:211-9.
29. Pirincci E, Oguzoncul AF, Tasdemir R. Age at the onset of menopause and its influencing factors in Turkish women in a rural area. J Women Aging. 2016;28:238-46.
30. Cassou B, Mandereau L, Aegerter P, Touranchet A, Derriennic F. Work-related factors associated with age at natural menopause in a generation of French gainfully employed women. Am J Epidemiol. 2007;166:429-38.
31. Celentano DD, Klassen AC, Weisman CS, Rosenshein NB. Cervical cancer screening practices among older women: results from the Maryland cervical cancer case-control study. J Clin Epidemiol. 1988;41:531-41.
32. Baiocchi G, de Brot L, Faloppa CC, Mantoan H, Duque MR, Badiglian-Filho L, et al. Is parametrectomy always necessary in early-stage cervical cancer? Gynecol Oncol. 2017;146:16-9.

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# Potential biomarkers for predicting the efficacy of a pembrolizumab-containing regimen in advanced cervical cancer: A real-world analysis

## *İleri evre serviks kanserinde pembrolizumab içeren bir rejimin etkinliğini tahmin etmek için olası biyobelirteçler: Gerçek dünya analizi*

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**Abstract**

**Objective:** Prognostic biomarkers in patients with advanced cervical cancer treated with immune checkpoint inhibitors remain unclear. An evaluation of combined positive score (CPS) and tumor proportion score (TPS), and a comparison of their usefulness with inflammatory biomarkers in real-world data could be informative.

**Materials and Methods:** We analyzed 28 patients who were treated with the KEYNOTE-826 regimen between November 2022 and June 2024. The complete cohort (group 1), patients with no prior chemotherapy (group 2), and treatment-naïve (group 3) were evaluated as follows: 1) CPS, TPS, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and hemoglobin, albumin, lymphocyte, and platelets (HALP score) in peripheral blood samples were obtained prior to initial treatment and KEYNOTE-826 regimen, and receiver operating curve analysis was used to compare them. The optimal cut-off values that showed the highest level of discrimination for progression-free survival were identified.

**Results:** The areas under the curve (AUC) for progression-free survival in group 2 were measured for CPS, TPS, NLR, PLR, and HALP scores before the KEYNOTE-826 regimen. The AUC values for these scores were 0.644, 0.662, 0.852, 0.667, and 0.700, respectively. The lower NLR (≤5.52) group had a significantly longer median survival than the higher NLR (>5.52) group ($p<0.001$), with median survivals of 14.0 vs. 7.6 months, respectively. In group 3, CPS and TPS were highest at 0.700 for predicting progression-free survival, compared to NLR, PLR, and HALP score. CPS and TPS appear positively correlated with progression-free survival.

**Conclusion:** CPS and TPS showed a modest correlation with progression-free survival and NLR prior to immunotherapy demonstrated the best treatment efficacy for advanced cervical cancer.

**Keywords:** Cervical cancer, biomarker, combined positive score, tumor proportion score, neutrophil-to-lymphocyte ratio, immunotherapy, pembrolizumab

**PRECIS:** Combined positive score showed a modest correlation with survival, and neutrophil-to-lymphocyte ratio was shown to be the most predictive biomarker for advanced cervical cancer chemotherapy, including immunotherapy.

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## Öz

**Amaç:** İmmün kontrol noktası inhibitörleri ile tedavi edilen ileri evre serviks kanseri olan hastalarda prognostik biyobelirteçler hala belirsizliğini korumaktadır. Kombine pozitif skor (CPS) ve tümör oranı skoru (TPS) değerlendirmesi ve bunların gerçek dünya verilerindeki enflamatuvar biyobelirteçlerle yararlılıklarının karşılaştırılması bilgilendirici olabilir.

**Gereç ve Yöntemler:** Kasım 2022 ile Haziran 2024 arasında KEYNOTE-826 rejimi ile tedavi edilen 28 hastayı analiz ettik. Tam kohort (grup 1), daha önce kemoterapi almamış hastalar (grup 2) ve tedavi görmemiş hastalar (grup 3) aşağıdaki şekilde değerlendirildi: 1) Başlangıç tedavisi ve KEYNOTE-826 rejiminden önce periferik kan örneklerinde CPS, TPS, nötrofil-lenfosit oranı (NLR), trombosit-lenfosit oranı (PLR) ve hemoglobin, albümin, lenfosit ve trombosit değerleri (HALP skoru) elde edildi ve bunları karşılaştırmak için alıcı çalışma karakteristiği eğrisi analizi kullanıldı. Progresyon içermeyen sağkalım için en yüksek ayrım seviyesini gösteren optimum kesme değerleri belirlendi.

**Bulgular:** Grup 2'de progresyon içermeyen sağkalım için eğri altında kalan alanlar (AUC), KEYNOTE-826 rejiminden önce CPS, TPS, NLR, PLR ve HALP skorları için ölçüldü. Bu puanlar için AUC değerleri sırasıyla 0,644, 0,662, 0,852, 0,667 ve 0,700 idi. Düşük NLR (≤5,52) grubu, yüksek NLR (>5,52) grubundan önemli ölçüde daha uzun bir medyan sağkalıma sahipti (p<0,001), medyan sağkalımlar sırasıyla 14,0'a karşı 7,6 ay idi. Grup 3'te, CPS ve TPS, 0,700 değerinde NLR, PLR ve HALP puanına kıyasla progresyonsuz sağkalımı tahmin etmede en yüksek güce sahipti. CPS ve TPS, progresyonsuz sağkalımla pozitif olarak ilişkili görünmektedir.

**Sonuç:** CPS ve TPS, progresyonsuz sağkalımla ılımlı bir korelasyon gösterdi ve immünoterapiden önceki NLR, ileri servikal kanser için en iyi tedavi etkinliğini gösterdi.


**Anahtar Kelimeler:** Servikal kanser, biyobelirteç, kombine pozitif skor, tümör oranı skoru, nötrofil-lenfosit oranı, immünoterapi, pembrolizumab

## Introduction

Despite the widespread implementation of screening programs and introduction of the human papillomavirus vaccine, cervical cancer remains the fourth most commonly diagnosed cancer and the fourth leading cause of mortality in women[1,2]. Patients with advanced cervical cancer may benefit from the monoclonal antibody pembrolizumab (Pem), which targets the programmed death 1 (PD-1) pathway[3,4]. The PD-1 to programmed death-ligand 1 (PD-L1) signaling pathway is essential for maintaining immune homeostasis[5]. The binding of PD-L1 to PD-1 inhibits T-cell proliferation and cytokine production via the T-cell receptor, preventing excessive immune responses[6]. PD-L1 is not only expressed on tumor cells but also on tumor-infiltrating immune cells. Anti-PD-1/L1 therapy is mainly used to target the negative signals mediated by PD-L1; thus, PD-L1 expression in the tumor microenvironment is the most studied biomarker[7]. The combined positive score (CPS), tumor proportion score (TPS), and PD-L1 immunohistochemistry (IHC) assays are important methods for evaluating PD-L1 expression in patients with cancer.

In recurrent or metastatic cervical cancer, Pem plus chemotherapy in a previous phase 3 KEYNOTE-826 study (with or without bevacizumab; Bev) was shown to prolong progression-free survival (PFS) and overall survival (OS) compared to chemotherapy alone[3,4]. Regarding the role of PD-L1 therapy for CPS, the hazard ratio (HR) for PFS compared with the chemotherapy group was 0.62 [95% confidence interval (CI), 0.50-0.77, p<0.001] for patients with PD-L1 CPS ≥1; a conclusion was drawn that it was effective for all patients with CPS ≥1. On the other hand, immune-related events occurred in 34.5% of the Pem group, and grade 3–5 adverse events occurred in 12.1% of the group; including two patients (0.7%) who died from immune-mediated encephalitis and pancreatitis[4]. Biomarkers that can reliably guide the decision-making process for treatment strategies and be highly predictive of responses to immune checkpoint inhibitors (ICI) therapy are required to improve the treatment outcomes of these patients.

Biomarkers in cancer treatment are essential for enabling individualized treatment and predicting patient treatment responses. Important criteria include predictive ability, reliability, clinical usefulness, diversity of data sources, and non-invasive measurement methods[8-11]. Biomarkers that fulfill these criteria will enable the development of more effective treatment strategies. Many studies have evaluated PD-L1 expression in tumor cells as a predictive biomarker of ICI[7]. The PD-L1 expression rate is determined by TPS for non-small cell lung cancer and by CPS for patients with head and neck cancer, esophageal cancer, breast cancer, and cervical cancer[3,12-15].

Recent studies addressing gynecological cancers have reported that inflammatory biomarkers, including the neutrophil-to-lymphocyte ratio (NLR), are significantly associated with clinical prognosis[16-18]. Peripheral neutrophil counts assessed by NLR are directly related to intratumoral neutrophil infiltration and have been shown to impair antitumor immune responses[19,20]. In theory, neutrophilia indicates a response to systemic inflammation, and lymphocytopenia reflects a decrease in cell-mediated immunity[21]. Our recent publications report that the peripheral blood NLR score, sampled prior to Pem inclusion in the regimen, is a significant predictor for the prognosis of regimens containing Pem for endometrial cancer[17,18].

In the present study, we aimed to evaluate the possibility of using CPS and TPS as prognostic biomarkers in data from real-world settings, and to compare their usefulness with inflammatory biomarkers in advanced cervical cancer.

## Materials and Methods

### Patient Population

We performed a retrospective review of a clinical database to identify cases of advanced or recurrent cervical cancer in patients who received the KEYNOTE-826 treatment protocol, consisting of Pem, chemotherapy, and Pem ± Bev, followed by Pem ± Bev as maintenance therapy, between November 2022 and June 2024. The Institutional Review Board at Kagoshima University Graduate School of Medical Sciences granted approval for the study protocol (approval number: 230081, date: 19.09.2023). The 2018 FIGO staging system was used to classify the disease, and clinical data were collected by reviewing inpatient medical records. Pathological information was obtained from biopsies performed at our outpatient clinic on patients who did not undergo surgery or from uterine specimens that were surgically removed from patients receiving primary surgical treatment. PD-L1 expression in formalin-fixed tumor samples was evaluated at a central laboratory in our institute using a commercially available PD-L1 IHC 22C3 pharm Dx assay (Dako, Carpinteria, California, U.S.A.).

A study flowchart is shown in Figure 1. The study included 32 patients with endometrial cancer who had progressed to stages III-IV or recurred and who received ICI. Finally, after excluding four cases due to insufficient data, 28 cases formed group 1, the complete cohort of patients; patients with no prior chemotherapy formed group 2; and treatment-naïve patients formed group 3. Patients were evaluated as follows. Group 2 had the most similar inclusion criteria to the previous KEYNOTE-826 study and included patients who had undergone concurrent chemoradiotherapy.

First, the predictive prognostic biomarkers, including CPS, TPS, and inflammatory biomarkers, along with NLR, PLR, and hemoglobin, albumin, lymphocyte, and platelets (HALP) scores in peripheral blood samples, were compared using receiver operating curve (ROC) analysis. Pre-treatment values of neutrophil, hemoglobin, platelet, and albumin counts were obtained immediately prior to undergoing the KEYNOTE-826 regimen. CPS was calculated as (number of PD-L1 positive tumor cells + number of PD-L1 positive immune cells)/total number of viable tumor cells × 100. TPS was calculated as (number of PD-L1 positive tumor cells/total number of viable

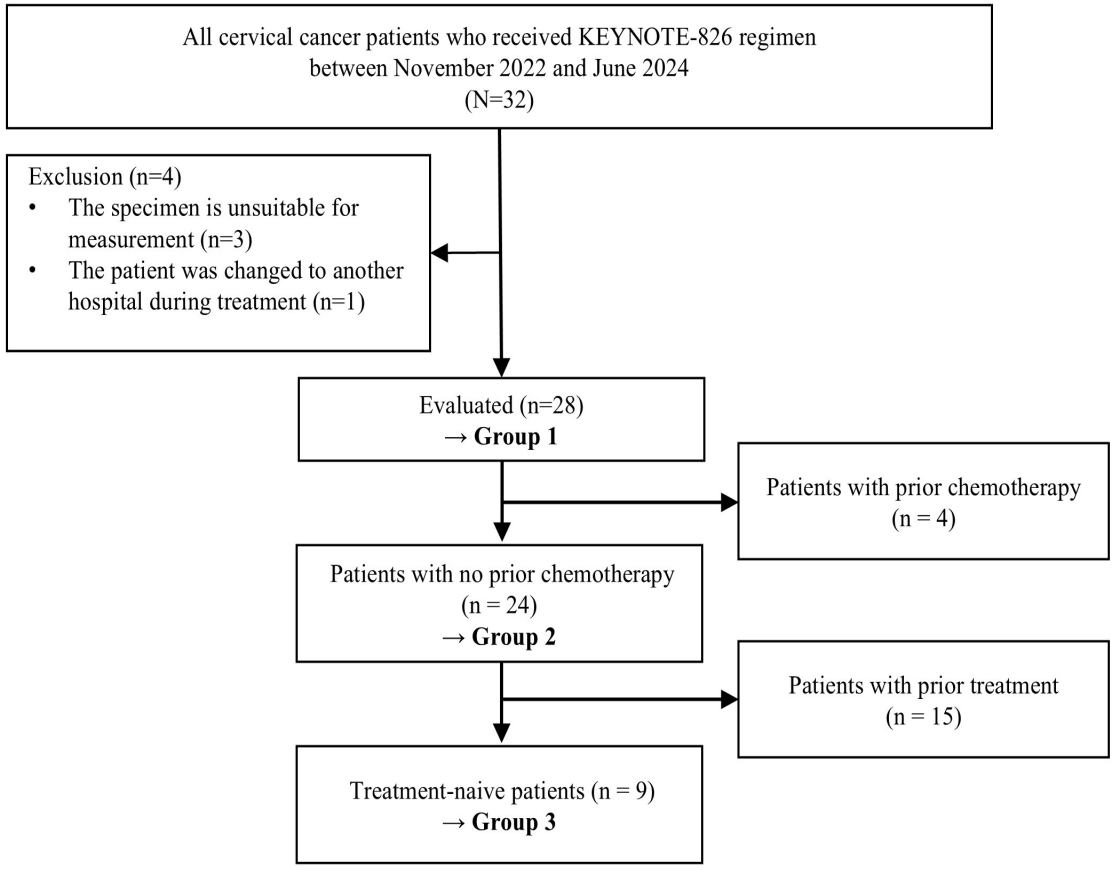


**Figure 1.** Flowchart summarizing the study. The KEYNOTE-826 regimen was defined as Pem plus chemotherapy ± Bev followed by maintenance therapy with Pem ± Bev

*Pem: Pembrolizumab, Bev: Bevacizumab*

tumor cells) × 100. NLR, PLR, HALP scores were defined as follows: neutrophil [L]/lymphocytes [L], platelets [L]/lymphocytes [L], and hemoglobin [g/L] × albumin [g/L] × lymphocytes [L]/platelets [L]. The usefulness of each prediction parameter in identifying overall response (OR), disease control (DC), and a progression-free (PF) period of 8 months or more was evaluated, and their potential as surrogates for clinical benefit was assessed. Second, the Youden index was employed to identify the optimal cut-off values for the predictor that demonstrated the highest level of discrimination for PF.

All participants were admitted to and provided care at the Kagoshima University Hospital. All patients who had previously undergone chemotherapy had fully recovered from any bone marrow suppression caused by the treatment, and none of them were administered immunosuppressive drugs, including steroids, that might influence the complete blood count. PFS was described as the time span from the initiation of the treatment plan to the confirmation of tumor advancement. The proportion of patients who achieve either a partial response (PR) or a complete response (CR) is typically defined as OR. DC was achieved with PR, CR, and stable disease. The KEYNOTE-826 regimen was defined as at least one course of chemotherapy plus Pem ± Bev, followed by Pem ± Bev, as maintenance therapy.

### Statistical Analysis

The threshold for statistical significance was established at $p<0.05$. The Kaplan-Meier method was employed to generate survival curves, and the log-rank test was used to compare PFS across the groups. All statistical analyses were performed on a personal computer using a statistical software package (SPSS for Windows, v.29; SPSS Inc., Chicago, IL, USA).

## Results

Table 1 shows the characteristics of group 1 patients enrolled in the KEYNOTE-826 regimen receiving Pem for advanced or recurrent cervical cancer. The median age of participants was 51.5 years, with a median follow-up time of 9.5 months (range, 1-25 months); and 19 (67.9%) cases were recurrent. All patients were initially treated with chemotherapy with paclitaxel plus carboplatin (TC) along with bevacizumab (Bev), and continued with the KEYNOTE-826 regimen for at least six courses. However, two patients were excluded from completing the regimen due to disease progression, and one patient did not receive the sixth course because of complications from COVID-19.

Of the four patients with prior chemotherapy regimens, two had undergone only one regimen, one had undergone two regimens, and one had undergone four regimens; all patients underwent the TC ± Bev regimen. The two cases with CPS of only 1.0 progressed within 0 and 1 month, respectively, and both died of the disease. Three out of the four cases with zero TPS had recurrence at 0, 8, and 11 months, and disease death at 1, 12, and 22 months. The remaining patient survived 11 months.

### Potential as a Biomarker of CPS, TPS, and Exploratory Research for NLR, PLR, and HALP Score for the KEYNOTE-826 Regimen

The median values for the predictive biomarkers are shown in Table 2. In group 3, the CPS and the NLRs prior to initial treatment and prior to the KEYNOTE-826 regimen appear to be higher than those for groups 1 and 2.

The areas under the ROC curve for predictive biomarkers for the KEYNOTE-826 regimen in all groups are shown in Table 3. Among the inflammatory biomarkers (NLR, PLR, and HALP score) measured prior to the KEYNOTE-826 regimen NLR was the highest predictor of OR, DC, and PF. The CPS and TPS had only modest OR, DC, and PF prediction accuracy in groups 1 and 2, but they were higher for predicting PF than for NLR, PLR, and the HALP score in group 3. In all groups, CPS and TPS were consistently higher in the order of PF, DC, and OR levels.

Major analysis results for groups 1-3 in advanced cervical cancer treated with the KEYNOTE-826 regimen are shown in Figures 2-4. In group 1, the area under the curve (AUC) and scatter diagrams between CPS, TPS, and NLR for PF and PFS of NLR using the Kaplan-Meier method is shown in Figure 2. The AUC in group 1 of PF included CPS, TPS NLR, PLR, and HALP scores measured prior to the KEYNOTE-826 regimen; results were 0.636, 0.636, 0.826, 0.674, and 0.686, respectively. Scatter diagrams of CPS and TPS for PFS revealed a mild positive correlation. The lower NLR ($NLR \leq 5.525$) group had a significantly longer PFS than the higher NLR ($5.525 < NLR$) group ($p<0.001$, median survival: 13.6 months vs. 7.8 months; Figure 2E); furthermore, NLR and PFS were each negatively correlated (Figure 2F).

In group 2 of PF, the areas under the curve included CPS, TPS, and NLR; PLR, and HALP scores measured prior to the KEYNOTE-826 regimen; results were 0.644, 0.662, 0.852, 0.667, and 0.700, respectively. The group with a lower NLR ($NLR \leq 5.525$) exhibited a notably longer survival compared to the group with a higher NLR ($5.525 < NLR$) ($p<0.001$, median survival: 14 months vs. 7.6 months; Figure 3E). Scatter diagrams of the CPS and TPS for PFS revealed a mild positive correlation; and the NLR for PFS was negatively correlated (Figure 3F).

In group 3, CPS and TPS were the most predictive biomarkers for PF, with an area under the ROC curve of 0.700 for both. The higher CPS ($20.0 \leq CPS$) group tended to have longer PFS than the lower CPS ($CPS < 20$) group ($p=0.210$, median survival: 3 months vs. not statistically reached; Figure 4B). Similarly, the higher TPS ($25.0 \leq TPS$) group tended to have longer PFS than the lower TPS ($TPS < 25$) group ($p=0.210$, median survival: 3.5 months compared to a median survival that was not statistically reached). The scatter diagrams of CPS and TPS for PFS seem to be positively correlated (Figure 4C, D).

**Table 1.** Baseline characteristic of 28 patients in advanced cervical cancer

| Case | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 45 | 52 | 41 | 56 | 38 | 48 | 41 | 51 | 50 | 46 | 57 | 65 | 64 | 57 |
| Histological type | Gastric | SCC | SCC | SCC | SCC | SCC | SCC | Gastric | SCC | Adeno | SCC | Others | SCC | Others |
| FIGO stage (FIGO 2013) | IVB | IB3 | IIB | IIIC2 | IIIC1 | IVA | IVB | IIIC1 | IIA2 | IB3 | IIIC1 | IVB | IB2 | IVB |
| Timing of TC plus Pem ± Bev administration | Initial | Rec | Rec | Rec | Rec | Rec | Initial | Rec | Rec | Rec | Rec | Initial | Rec | Initial |
| Sites of recurrence | Pelvic+Distant | Distant | Distant | Distant | Pelvic+Distant | Distant | Pelvic+Distant | Pelvic+Distant | Pelvic | Pelvic+Distant | Pelvic | Pelvic+Distant | Distant | Pelvic+Distant |
| TC plus Pem + Bev | 6 | 5 | 8 | 6 | 6 | 6 | 6 | 6 | 4 | 9 | 6 | 6 | 6 | 1 |
| TC plus Pem | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Pem + Bev | 4 | 7 | 10 | 14 | 10 | 5 | 9 | 19 | 5 | 0 | 16 | 17 | 5 | 0 |
| Pem | 4 | 3 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Number of prior chemotherapy | 0 | 0 | 2 | 0 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Prior RT or CCRT | Nil | CCRT | CCRT | CCRT | Nil | CCRT | Nil | Nil | Nil | CCRT | Nil | Nil | Nil | Nil |
| Treatment delay | Nil | Once | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Dose reduction of TC | 1 level | Nil | 1 level | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil |
| Best overall response | PR | CR | SD | CR | PR | PD | PR | CR | CR | PR | PR | CR | SD | PD |
| Prognosis | DOD | AWD | DOD | NED | DOD | DOD | DOD | NED | AWD | DOD | NED | NED | DOD | DOD |
| Specimen of CPS and TPS | Bx | Resected uterus | Resected uterus | Bx | Bx | Bx | Bx | Resected uterus | Resected uterus | Resected uterus | Bx | Bx | Resected uterus | Bx |
| Timinig of examination | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial | Initial |
| CPS value | 10 | 10 | 20 | 10 | 10 | 80 | 30 | 30 | 30 | 1 | 100 | 40 | 15 | 1 |
| TPS value | 10 | 0 | 10 | 5 | 5 | 70 | 30 | 1 | 20 | 0 | 100 | 40 | 10 | 0 |
| Pre-initial treatment | | | | | | | | | | | | | | |
| NLR | 2.11 | 1.74 | 2.91 | 4.02 | 5.02 | 7.43 | 11.01 | 1.26 | 6.75 | 2.30 | 2.31 | 4.42 | 3.53 | 8.78 |
| PLR | 206.6 | 134.1 | 21.5 | 203.9 | 321.6 | 34.3 | 546.6 | 133.1 | 216.2 | 164.0 | 220.3 | 298.2 | 267.6 | 366.1 |
| HALP | 0.24 | 0.24 | 1.91 | 0.22 | 0.13 | 1.25 | 0.07 | 0.40 | 0.04 | 0.31 | 0.14 | 0.15 | 0.17 | 0.06 |
| Pre-TC plus Pem ± Bev | | | | | | | | | | | | | | |
| NLR | 1.13 | 3.42 | 2.02 | 1.56 | 5.09 | 7.43 | 11.01 | 1.78 | 10.38 | 5.69 | 2.99 | 2.90 | 5.36 | 10.37 |
| PLR | 121.2 | 330.3 | 213.9 | 248.4 | 199.3 | 343.3 | 546.6 | 170.6 | 521.8 | 313.9 | 205.8 | 160.1 | 560.0 | 342.4 |
| HALP | 0.41 | 0.15 | 0.25 | 0.24 | 0.22 | 0.11 | 0.06 | 0.29 | 0.08 | 0.12 | 0.20 | 0.28 | 0.08 | 0.05 |

**Table 1.** Continued

| Case | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age | 46 | 43 | 50 | 55 | 54 | 44 | 51 | 60 | 64 | 60 | 65 | 68 | 56 |
| Histological type | SCC | Adeno | SCC | SCC | SCC | SCC | Others | Adeno | SCC | SCC | Adeno | Others | Others |
| FIGO stage (FIGO 2013) | IVB | IVB | IIIC1 | IIIC1 | IVB | IB | IVB | IIIB | IIIB | IIIC1p | IVB | IVB | IVB |
| Timing of TC plus Pem ± Bev administration | Initial | Initial | Rec | Rec | Initial | Rec | Rec | Rec | Rec | Rec | Initial | Rec | Initial |
| Sites of recurrence | Distant | Pelvic+Distant | Pelvic+Distant | Distant | Pelvic+Distant | Pelvic | Pelvic | Distant | Distant | Distant | Distant | Pelvic+Distant | Distant |
| TC plus Pem + Bev | 6 | 4 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 1 | 6 | 6 |
| TC plus Pem | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 |
| Pem + Bev | 14 | 0 | 1 | 7 | 6 | 2 | 0 | 6 | 7 | 0 | 0 | 4 | 7 |
| Pem | 0 | 0 | 11 | 0 | 0 | 2 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Number of prior chemotherapy | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Prior RT or CCRT | Nil | Nil | Nil | Nil | Nil | RT | Nil | CCRT | CCRT | CCRT | Nil | CCRT | Nil |
| Treatment delay | Once | Nil | Nil | Nil | Nil | Nil | Once | Nil | Nil | Once | Once | Nil | Nil |
| Dose reduction of TC | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | Nil | 1 level | 1 level | Nil | Nil |
| Best overall response | PR | PD | SD | SD | PR | CR | CR | CR | PR | PR | SD | PR | CR |
| Prognosis | NED | DOD | NED | NED | NED | NED | NED | NED | NED | NED | AWD | NED | NED |
| Specimen of CPS and TPS | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx | Bx |
| Timinig of examination | Initial | Initial | Initial | Initial | Initial | Initial | Rec | Rec | Initial | Initial | Initial | Rec | Initial |
| CPS value | 5 | 10 | 5 | 60 | 40 | 100 | 10 | 5 | 30 | 10 | 10 | 5 | 20 |
| TPS value | 1 | 1 | 0 | 60 | 20 | 100 | 20 | 20 | 40 | 20 | 20 | 10 | 30 |
| Pre-initial treatment | | | | | | | | | | | | | |
| NLR | 4.89 | 3.49 | 4.33 | 6.48 | 21.27 | 3.81 | 3.40 | 3.02 | 6.48 | 1.81 | 5.03 | 2.69 | 6.08 |
| PLR | 249.1 | 254.9 | 358.1 | 388.0 | 788.9 | 159.0 | 199.2 | 121.3 | 208.5 | 100.5 | 234.0 | 174.8 | 309.4 |
| HALP | 0.20 | 0.21 | 0.13 | 0.13 | 0.06 | 0.36 | 0.22 | 0.47 | 0.25 | 0.55 | 0.24 | 0.28 | 0.10 |
| Pre-TC plus Pem ± Bev | | | | | | | | | | | | | |
| NLR | 4.83 | 6.07 | 2.71 | 5.32 | 26.60 | 3.81 | 11.33 | 3.56 | 2.25 | 4.05 | 4.19 | 4.62 | 1.02 |
| PLR | 296.6 | 223.3 | 290.0 | 335.6 | 695.6 | 159.0 | 384.6 | 206.0 | 193.2 | 169.8 | 175.2 | 201.4 | 174.8 |
| HALP | 0.15 | 0.23 | 0.13 | 0.16 | 0.07 | 0.35 | 0.11 | 0.25 | 0.40 | 0.30 | 0.28 | 0.09 | 0.21 |

SCC: Squamous cell carcinoma, Adeno: Adenocarcinoma, FIGO: International Federation of Gynecology and Obstetrics, TC: Paclitaxel plus carboplatin, Pem: Pembrolizumab, Bev: Bevacizumab, RT: Radiothearapy, CCRT: Concurrent chemoradiotherapy, CR: Complete response, PR: Partial response, SD: Stable disease, PD: Progressive disease, DOD: Dead of disease, AWD: Alive with disease, NED: No evidence of disease, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, HALP score: hemoglobin, albumin, lymphocyte, and platelet score, Rec: Recurrence

**Table 2.** Median values for each predictive biomarkers

| | | | Measurement periods | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Prior to initial treatment | | | Prior to KEYNOTE-826 regimen | | |
| | CPS | TPS | NLR | PLR | HALP score | NLR | PLR | HALP score |
| Group 1 (range) | 12.5 (1.0-100) | 20 (0-100) | 3.91(1.26-21.27) | 214.9 (21.5-788.9) | 0.220 (0.04-1.91) | 4.12 (1.02-26.60) | 235.9 (121.2-695.6) | 0.185 (0.050-0.041) |
| Group 2 (range) | 12.5 (1.0-100) | 20.0 (0-100) | 4.18 (1.74-21.30) | 218.3 (34.3-788.9) | 0.215 (0.04-1.25) | 4.12 (1.02-26.60) | 251.4 (121.23-695.6) | 0.165 (0.05-0.41) |
| Group 3 (range) | 30.0 (1.0-100) | 20 (0-100) | 5.03 (2.31-21.27) | 254.9 (34.3-788.9) | 0.150 (0.06-1.25) | 6.07 (2.90-26.60) | 296.6 (160.1-695.6) | 0.150 (34.33-788.89) |

NLR, PLR, and HALP scores were sampled in peripheral blood. CPS: Combined positive score, TPS: Tumor proportion score, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, HALP score: Hemoglobin, albumin, lymphocyte, and platelet score

**Table 3.** Area under the ROC curve and cut-off value in predictive biomarkers

| | | Area under the ROC curve | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Measurement periods | | | | | | |
| | | Prior to initial treatment | | | Prior to KEYNOTE-826 regimen | | | | |
| | | CPS | TPS | NLR | PLR | HALP score | NLR | PLR | HALP score |
| Group 1 | | | | | | | | | |
| | Overall response | 0.583 | 0.602 | 0.534 | 0.526 | 0.508 | 0.620 | 0.703 | 0.638 |
| | Disease control | 0.580 | 0.633 | 0.760 | 0.533 | 0.513 | **0.804** | 0.707 | 0.727 |
| | Progression-free (*estimated cut-off value) | 0.636 | 0.636 | 0.689 | 0.492 | 0.534 | **0.826 (5.52)** | 0.674 | 0.686 |
| Group 2 | | | | | | | | | |
| | Overall response | 0.579 | 0.632 | 0.539 | 0.593 | 0.554 | 0.664 | 0.614 | 0.621 |
| | Disease control | 0.579 | 0.635 | 0.730 | 0.508 | 0.516 | **0.857** | 0.698 | 0.714 |
| | Progression-free (*estimated cut-off value) | 0.644 | 0.662 | 0.657 | 0.546 | 0.505 | **0.852 (5.52)** | 0.667 | 0.700 (0.12) |
| Group 3 | | | | | | | | | |
| | Overall response | 0.500 | 0.500 | 0.700 | **0.850** | 0.725 | 0.600 | 0.650 | 0.575 |
| | Disease control | 0.639 | 0.694 | 0.500 | 0.611 | 0.639 | 0.667 | 0.611 | 0.667 |
| | Progression-free (*estimated cut-off value) | 0.700 (20.0) | 0.700 (25.0) | 0.500 | 0.700 | 0.775 | 0.550 | 0.550 | 0.525 |

*The estimated cut-off value is only shown for progression-free of ≥0.700. Bold indicates ≥0.8, and underlined indicates ≥0.7. NLR, PLR, and HALP scores were measured from peripheral blood samples. ROC: Receiver operating characteristic, CPS: Combined positive score, TPS: Tumor proportion score, NLR: Neutrophil to lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, HALP score: Hemoglobin, albumin, lymphocyte, and platelet score

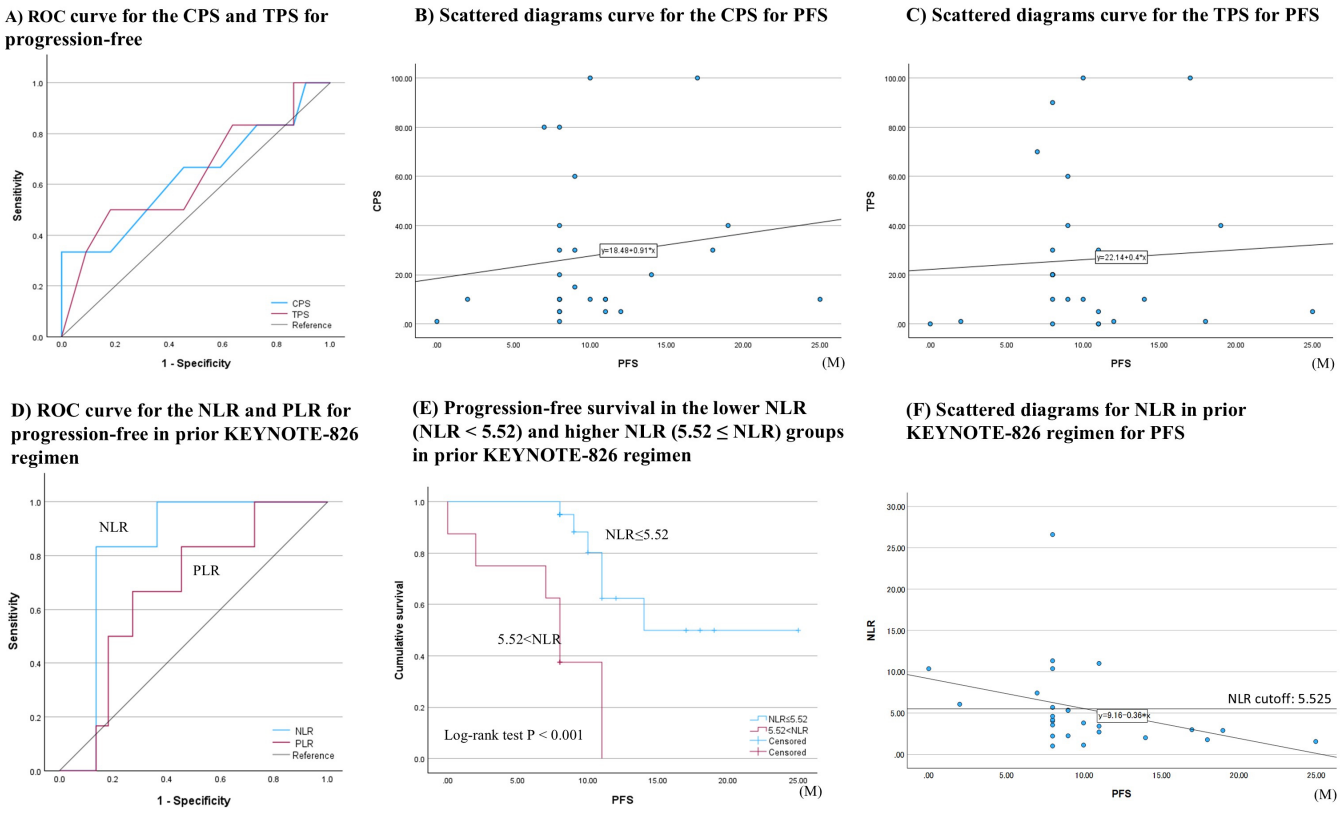


**Figure 2.** Major analysis results of group 1 in advanced cervical cancer treated with the KEYNOTE-826 regimen. A) ROC curve for CPS and TPS for progression-free. The areas under the curve were 0.636 and 0.636, respectively. Scatter diagrams for CPS and TPS for PFS are shown in (B) and (C). In both cases, a mild positive correlation seems to be observed. (D) ROC curve for NLR and PLR for progression-free in the Prior to KEYNOTE-826 regimen. The AUC was 0.826 and 0.674, and the cut-off value for NLR was 5.52. (E) PFS in the lower NLR (NLR <5.52) and higher NLR (5.52 ≤NLR) groups in the Prior KEYNOTE-826 regimen. The lower NLR (NLR ≤5.52) group had a significantly longer PFS than the higher NLR (5.52< NLR) group (p<0.001, median survival: 13.6 M vs. 7.8 months), (F) Scattered diagram for NLR in the Prior to the KEYNOTE-826 regimen for PFS. A negative correlation was observed between the NLR and PFS

*ROC: Receiver operating characteristic, CPS: Combined positive score, TPS: Tumor proportion score, PFS: Progression-free survival, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, AUC: Area under the curve*

## Discussion

In our study, CPS and TPS showed a modest positive correlation with PF, but they could not be established as absolute biomarkers in patients receiving the KEYNOTE-826 regimen. In contrast, inflammatory biomarkers, measured using the NLR in peripheral blood samples immediately prior to the KEYNOTE-826 regimen, were the most predictive of treatment efficacy for advanced cervical cancer.

The complex and individual interactions between host factors, which indicate dysfunctional immune responses, and tumor factors, which contribute to aggressive malignancies, are crucial[22,23]. There is increasing evidence that both neutrophils and lymphocytes, components of the immune system, are involved in tumor progression and prognosis[24]. The presence of neutrophils in peripheral blood indicates inflammation, and lymphocytes are important indicators of immune status. In healthy human participants, the mean value and corresponding 95% reference interval for the inflammatory biomarkers NLR and PLR were 1.76 (0.83-3.92) and 120 (61-239), respectively[25]; both values were clearly high in our study population, as shown in Table 2, especially in patients in group 3. In group 3, the median NLR was remarkably high, and in such a population with inherently poor prognosis, NLR may be less useful as a biomarker of treatment efficacy[22,24]. However, it is also known that an increase in baseline NLR does not necessarily prevent long-term survival, and such an increase alone does not seem to have prognostic significance sufficient to warrant discontinuation of ICI[22]. In patients with an extremely high NLR, it is necessary to predict treatment efficacy using multiple biomarkers such as CPS and TPS.

The role of biomarkers in the KEYNOTE-826 regimen for CPS has not been fully analyzed. Concerns exist regarding using PD-L1 IHC as a prognostic biomarker for anti-PD-1 or PD-L1

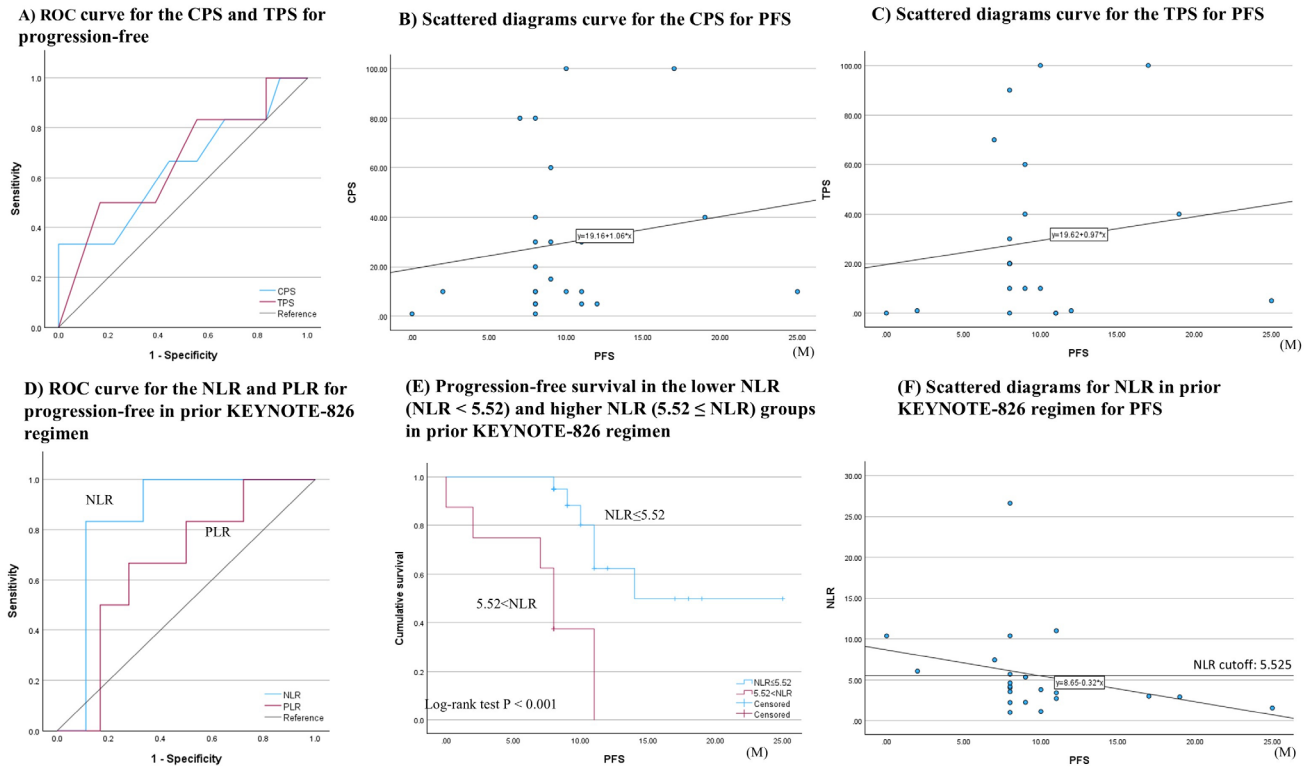


**Figure 3.** Major analysis results of group 2 in advanced cervical cancer treated with the KEYNOTE-826 regimen. A) ROC curve for CPS and TPS for progression-free. The areas under the curves are 0.644 and 0.662, respectively. Scatter diagrams for CPS and TPS for PFS are shown in (B) and (C). In both the cases, a mild positive correlation was observed. (D) ROC curve for NLR and PLR for progression-free in the Prior to the KEYNOTE-826 regimen. The AUC was 0.852 and 0.667, and the cut-off value for the NLR was 5.52. (E) PFS in the lower NLR (NLR <5.52) and higher NLR (5.52≤ NLR) groups in the Prior to the KEYNOTE-826 regimen. The lower NLR (NLR ≤5.52) group had a significantly longer PFS than the higher NLR (5.52< NLR) group (p<0.001, median survival: 14 months vs. 7.6 months). (F) Scatter diagrams of NLR in the Prior to the KEYNOTE-826 regimen for PFS. A negative correlation was observed between the NLR and PFS

*ROC: Receiver operating characteristic, CPS: Combined positive score, TPS: Tumor proportion score, PFS: Progression-free survival, NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, AUC: Area under the curve*

therapy, 1) localized PD-L1 expression may be underestimated in small biopsy specimens, 2) the expression of PDL1 in multiple tumor lesions may change depending on the time course and anatomical site, and 3) there is a possibility that the expression of PDL1 may change over time due to anticancer treatment after biopsy. Considering these factors, CPS or TPS obtained from the target lesion immediately before administration may have greater potential for improving the accuracy of biomarkers; in group 3 of this study, the AUC for PFS was slightly higher than that in the other groups. However, 89% of patients had multiple lesions of stage IV; therefore, it does not necessarily reflect the tumor environment in all areas. Therefore, in the real world, patients eligible for the KEYNOTE-826 regimen may not necessarily be suitable for biomarker evaluation of the local environment using CPS or TPS.

In the previous KEYNOTE-826 study, the effect of Pem on OS and PFS increased with CPS ≥1; however, there was no further significant increase in the CPS ≥10 group. Nevertheless, the results from group 3, which was treatment-naïve, may show further therapeutic benefit with a CPS ≥10. In contrast, the HRs for OS and PFS in the subgroup with CPS <1 (11% of the study population) were approximately 1 compared to conventional chemotherapy, but the 95% CIs for estimates, were wide and overlapped with those for the entire population[26]. Non-squamous tumors are more likely to be PD-L1 negative; however, the KEYNOTE-826 study suggested that non-squamous histology may still be beneficial[26]. Therefore, we did not restrict the application of the KEYNOTE-826 regimen for CPS or TPS, and administered the regimen to patients with CPS <1. Although we did not identify any cases with CPS <1 in our study, of the four cases with TPS <1, one was disease-free.

Identification of the optimal measurement period for biomarker testing is also an important factor. In particular, CPS and TPS in group 3 patients were measured immediately prior

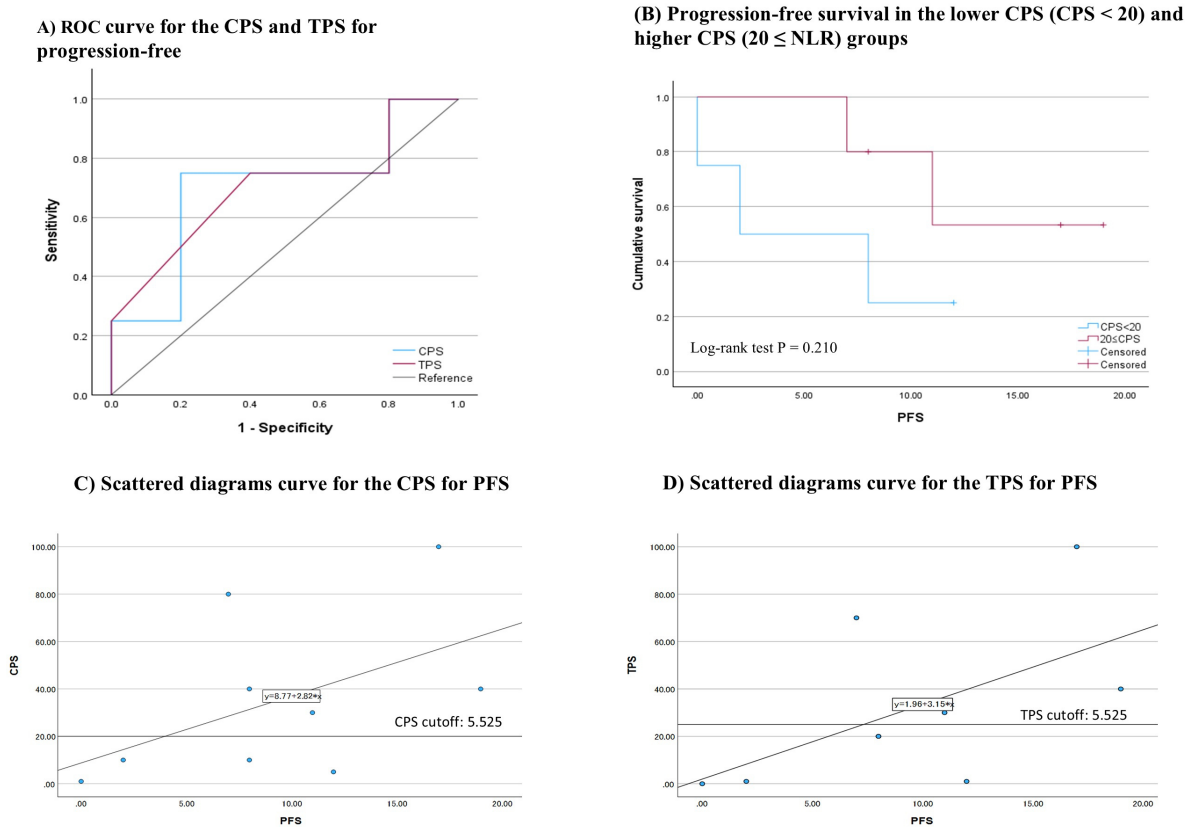


**Figure 4.** Major analysis results of group 3 in advanced cervical cancer treated with the KEYNOTE-826 regimen. A) ROC curve for CPS and TPS for progression-free. The AUC was 0.700 and 0.700, and the cut-off values for NLR were 20 and 25, respectively. The higher CPS (20≤ CPS) group tended to have longer PFS than the lower CPS (CPS <20) group (p=0.210, median survival: 3 months vs. statistically not reached). Scatter diagrams for CPS and TPS for PFS are shown in (C) and (D). A positive correlation seems to be observed in both cases

*ROC: Receiver operating characteristic, CPS: Combined positive score, TPS: Tumor proportion score, PFS: Progression-free survival, AUC: Area under the curve*

to administering the KEYNOTE-826 regimen, which may be one of the reasons why they were the most useful candidate biomarkers. when considering the measurement period after the initial treatment, we did not find any useful correlation with inflammatory biomarkers, including NLR. These results are also consistent with a similar study that we previously reported regarding regimens containing Pem for endometrial cancer[17].

### Study Limitations

This study has the following limitations: First, the sample size was relatively small; and the study was conducted with a retrospective design at a single facility. Second, the limited observation period made it difficult to evaluate long-term prognoses; therefore, an analysis of OS was not performed. We do, however, believe that there would be no significant new findings in the interpretation of biomarkers through further long-term observations when retrospectively exploring treatment outcomes.

## Conclusion

CPS and TPS from tissue samples taken directly from isolated target regions immediately prior to ICI use have the potential to become useful prognostic biomarkers. However, in the real world, patients eligible for the KEYNOTE-826 regimen already have systemic diseases, and tissue samples may not always be available. The usefulness of inflammatory biomarkers, such as NLR, which are easily measured, inexpensive, and minimally invasive, may also be significant. Our study suggests that further investigation is warranted into the utility of inflammatory indicators as prognostic biomarkers, for regimens containing ICI for cervical cancer.

### Ethics

**Ethics Committee Approval:** The Institutional Review Board at Kagoshima University Graduate School of Medical Sciences granted approval for the study protocol (approval number: 230081, date: 19.09.2023).

**Informed Consent:** Retrospective study.

## Footnotes

### Authorship Contributions

Surgical and Medical Practices: S.Y., I.K., S.T., A.T., H.K., Concept: S.Y., A.T., Design: S.Y., Data Collection or Processing: I.K., Analysis or Interpretation: S.Y., I.K., Literature Search: S.T., A.T., H.K., Writing: S.Y.

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

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## References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71:209-49.
2. Masucci GV, Cesano A, Hawtin R, Janetzki S, Zhang J, Kirsch I, et al. Validation of biomarkers to predict response to immunotherapy in cancer: volume I - pre-analytical and analytical validation. J Immunother Cancer. 2016;4:76.
3. Colombo N, Dubot C, Lorusso D, Caceres MV, Hasegawa K, Shapira-Frommer R, et al. Pembrolizumab for persistent, recurrent, or metastatic cervical cancer. N Engl J Med. 2021;385:1856-67.
4. Monk BJ, Colombo N, Tewari KS, Dubot C, Caceres MV, Hasegawa K, et al. First-line pembrolizumab + chemotherapy versus placebo + chemotherapy for persistent, recurrent, or metastatic cervical cancer: final overall survival results of KEYNOTE-826. J Clin Oncol. 2023;41:5505-11.
5. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med. 2002;8:793-800.
6. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. J Exp Med. 2000;192:1027-34.
7. Shen H, Yang ES, Conry M, Fiveash J, Contreras C, Bonner JA, et al. Predictive biomarkers for immune checkpoint blockade and opportunities for combination therapies. Genes Dis. 2019;6:232-46.
8. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer. 2016;16:275-87.
9. Duffy MJ, Harbeck N, Nap M, Molina R, Nicolini A, Senkus E, et al. Clinical use of biomarkers in breast cancer: updated guidelines from the European Group on Tumor Markers (EGTM). Eur J Cancer. 2017;75:284-98.
10. Twomey JD, Brahme NN, Zhang B. Drug-biomarker co-development in oncology - 20 years and counting. Drug Resist Updat. 2017;30:48-62.
11. Duffy MJ, Crown J. Use of circulating tumour DNA (ctDNA) for measurement of therapy predictive biomarkers in patients with cancer. J Pers Med. 2022;12.
12. Herbst RS, Baas P, Kim DW, Felip E, Perez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387:1540-50.
13. Mehra R, Seiwert TY, Gupta S, Weiss J, Gluck I, Eder JP, et al. Efficacy and safety of pembrolizumab in recurrent/metastatic head and neck squamous cell carcinoma: pooled analyses after long-term follow-up in KEYNOTE-012. Br J Cancer. 2018;119:153-9.
14. Kojima T, Shah MA, Muro K, Francois E, Adenis A, Hsu CH, et al. Randomized phase III KEYNOTE-181 study of pembrolizumab versus chemotherapy in advanced esophageal cancer. J Clin Oncol. 2020;38:4138-48.
15. Schmid P, Cortes J, Dent R, McArthur H, Pusztai L, Kummel S, et al. Overall survival with pembrolizumab in early-stage triple-negative breast cancer. N Engl J Med. 2024;391:1981-91.
16. Zhou Q, Hong L, Zuo MZ, He Z. Prognostic significance of neutrophil to lymphocyte ratio in ovarian cancer: evidence from 4,910 patients. Oncotarget. 2017;8:68938-49.
17. Yanazume S, Nagata C, Kobayashi Y, Fukuda M, Mizuno M, Togami S, et al. Potential prognostic predictors of treatment with immune checkpoint inhibitors for advanced endometrial cancer. Jpn J Clin Oncol. 2025;55:29-35.
18. Yanazume S, Kobayashi Y, Kirita Y, Kitazono I, Nagata C, Kozai A, et al. A potential inflammatory biomarker for advanced endometrial cancer treated with lenvatinib plus pembrolizumab. J Obstet Gynaecol Res. 2025;51:e16182.
19. Moses K, Brandau S. Human neutrophils: their role in cancer and relation to myeloid-derived suppressor cells. Semin Immunol. 2016;28:187-96.
20. Dumitru CA, Lang S, Brandau S. Modulation of neutrophil granulocytes in the tumor microenvironment: mechanisms and consequences for tumor progression. Semin Cancer Biol. 2013;23:141-8.
21. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140:883-99.
22. Bartlett EK, Flynn JR, Panageas KS, Ferraro RA, Sta Cruz JM, Postow MA, et al. High neutrophil-to-lymphocyte ratio (NLR) is associated with treatment failure and death in patients who have melanoma treated with PD-1 inhibitor monotherapy. Cancer. 2020;126:76-85.
23. Marrero I, Ware R, Kumar V. Type II NKT cells in inflammation, autoimmunity, microbial immunity, and cancer. Front Immunol. 2015;6:316.
24. Li Y, Zhang Z, Hu Y, Yan X, Song Q, Wang G, et al. Pretreatment neutrophil-to-lymphocyte ratio (NLR) may predict the outcomes of advanced non-small-cell lung cancer (NSCLC) patients treated with immune checkpoint inhibitors (ICIs). Front Oncol. 2020;10:654.
25. Fest J, Ruiter R, Ikram MA, Voortman T, van Eijck CHJ, Stricker BH. Reference values for white blood-cell-based inflammatory markers in the Rotterdam study: a population-based prospective cohort study. Sci Rep. 2018;8:10566.
26. Tewari KS, Colombo N, Monk BJ, Dubot C, Caceres MV, Hasegawa K, et al. Pembrolizumab or placebo plus chemotherapy with or without bevacizumab for persistent, recurrent, or metastatic cervical cancer: subgroup analyses from the KEYNOTE-826 randomized clinical trial. JAMA Oncol. 2024;10:185-92.

*Clinical Investigation / Araştırma*

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# Effectiveness of pelvic floor muscle training in managing urinary incontinence in pregnant women with and without gestational diabetes mellitus

## *Gestasyonel diyabetli ve diyabetsiz gebe kadınlarda idrar kaçırmayı yönetmede pelvik taban kas eğitiminin etkinliği*

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### Abstract

**Objective:** Urinary incontinence (UI) is a common issue during pregnancy. Pelvic floor muscle training (PFMT) may offer an effective solution for managing this condition. This study aimed to evaluate the effect of PFMT on reducing UI symptoms in pregnant women.

**Materials and Methods:** This study was conducted on 40 UI pregnant women with gestational diabetes mellitus (GDM) and 40 UI pregnant women without GDM. The participants in the experimental group were treated for 10 weeks in the third trimester, whereas the control groups received an educational pamphlet. Quality of life and UI severity were assessed using questionnaires, and pelvic floor muscle performance was measured through ultrasound-based bladder base displacement. Assessments were performed before treatment, after 10 weeks, and 2 weeks postpartum.

**Results:** In the non-diabetic group, significant reductions in UI symptoms were observed at the end of the third trimester and 2 weeks postpartum [adjusted difference -7.56, 95% confidence interval (CI) -10.62 to -4.49, p<0.001]. However, in the diabetic group, a reduction was noted, but it was not statistically significant. Additionally, the intervention positively impacted quality of life in the non-diabetic group (adjusted difference 30.8, 95% CI 17.6 to 44.1, p<0.001) but not in the diabetic group. Notably, no significant improvement in pelvic floor muscle performance was observed in either group.

**Conclusion:** This study suggests that PFMT can be more effective than routine pamphlets in reducing UI symptoms and improving the quality of life in pregnant women, both with and without GDM. Further research is needed to explore effects on pelvic floor muscle performance.

**Keywords:** Urinary incontinence, pregnant women, pelvic floor muscle training, ultrasonography, quality of life

### Öz

**Amaç:** İdrar kaçırma (UI) gebelikte sık görülen bir sorundur. Pelvik taban kas eğitimi (PFMT), bu durumu yönetmek için etkili bir çözüm sunabilir. Bu çalışma, PFMT'nin gebe kadınlarda UI semptomlarını azaltmadaki etkisini değerlendirmeyi amaçlamıştır.

**Gereç ve Yöntemler:** Bu çalışma, gestasyonel diyabetli (GDM) 40 UI gebe kadın ve GDM'siz 40 UI gebe kadın üzerinde yürütülmüştür. Deney grubundaki katılımcılar üçüncü trimesterde 10 hafta tedavi edilirken, kontrol gruplarına eğitim broşürü verilmiştir. Yaşam kalitesi ve UI şiddeti anketler kullanılarak değerlendirilmiş ve pelvik taban kas performansı ultrason tabanlı mesane tabanı yer değiştirmesi yoluyla ölçülmüştür. Değerlendirmeler tedaviden önce, 10 hafta sonra ve doğumdan 2 hafta sonra yapılmıştır.

**PRECIS:** We concluded that pelvic floor muscle training effectively reduced urinary incontinence and improved quality of life in pregnant women without gestational diabetes mellitus (GDM), with no impact on pelvic floor muscle performance and limited significant effect in women with GDM.

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**Bulgular:** Diyabetik olmayan grupta, üçüncü trimesterin sonunda ve doğumdan 2 hafta sonra UI semptomlarında önemli azalmalar gözlemlendi [ayarlanmış fark -7,56, %95 güven aralığı (GA) -10,62 ila -4,49, p<0,001]. Diyabetik grupta bir azalma kaydedildi, ancak istatistiksel olarak anlamlı değildi. Ek olarak, müdahale diyabetik olmayan grupta yaşam kalitesini olumlu yönde etkiledi (ayarlanmış fark 30,8, %95 GA 17,6 ila 44,1, p<0,001) ancak diyabetik grupta etkilemedi. Özellikle, her iki grupta da pelvik taban kas performansında önemli bir iyileşme gözlenmedi.

**Sonuç:** Bu çalışma, PFMT'nin hem GDM'li hem de GDM'siz gebe kadınlarda UI semptomlarını azaltmada ve yaşam kalitesini iyileştirmede rutin broşürlerden daha etkili olabileceğini öne sürmektedir. Pelvik taban kas performansı üzerindeki etkileri araştırmak için daha fazla araştırmaya ihtiyaç vardır.

**Anahtar Kelimeler:** İdrar kaçırma, hamile kadınlar, pelvik taban kas eğitimi, ultrasonografi, yaşam kalitesi

## Introduction

Urinary incontinence (UI) is recognized by the World Health Organization as one of the ten major health issues affecting women, particularly during the perinatal period[1]. Women with a history of UI during pregnancy or postpartum are at higher risk of developing the condition in the future, with studies indicating that pregnant women are 3.3 times more likely to experience UI than nulliparous women[2,3]. This risk remains elevated: 2.5 times higher one year after childbirth[4]. The prevalence of UI during pregnancy varies widely, ranging from 10.4% to 71.11%, with the highest rates observed in the third trimester and postpartum period[2].

Gestational diabetes mellitus (GDM), a common metabolic disorder characterized by impaired glucose tolerance during pregnancy, has been identified as a contributing factor to pregnancy-related UI through its impact on pelvic floor muscles (PFMs)[5,6]. Although UI is not critical, it significantly affects quality of life (QoL) by limiting physical activity, disrupting social interactions, and causing emotional distress[7]. Despite its impact, many women normalize the condition, delaying treatment and failing to recognize early symptoms[8].

Pelvic floor muscle training (PFMT) is a non-invasive, cost-effective approach to managing UI[9]. Research has shown that antenatal PFMT can reduce the risk of UI during late pregnancy and the mid-postnatal period[10]. However, its long-term effects, cost-effectiveness, and impact on QoL remain uncertain[11]. Moreover, limited data exist regarding the role of PFMT in women with GDM despite their increased vulnerability to UI[6]. This study addresses the gap by evaluating a structured PFMT program for pregnant women with and without GDM, focusing on UI status, quality of life, and bladder base displacement during late pregnancy. The findings aim to provide practical and effective strategies for managing pregnancy-related UI and improving QoL for this population.

## Materials and Methods

### Study Design

This single-center, parallel, randomized controlled trial was conducted in 2023. The study was jointly conducted by the Shahid Sadoughi University Faculty of Medicine, Department of Obstetrics and Gynecology, and the Shahid Beheshti University, School of Physical Therapy and Rehabilitation. Ethical approval for this study was obtained from the Iranian Registry of Clinical Trials (IRCT) (approval number: IR.SBMU.RETECH.REC.1400.611, date: 28.11.2021). The registration numbers of these clinical trials are IRCT20200825048523N2 and IRCT20200825048523N3.

### Participants

A total of 100 pregnant women at 24 weeks of gestation were recruited. However, twenty participants withdrew, resulting in a final sample of 80 women. Participants were categorized into four groups: two with GDM and two without. This study aimed to evaluate the effectiveness of PFMT in managing pregnancy-related UI.

Randomization was performed using balanced block randomization with sealed envelope concealment, ensuring equal allocation within each category. Participants were sequentially assigned based on the generated randomization sequence. The study followed a single-blind design, with the principal investigator blinded to group assignments during assessments; while an independent researcher conducted the data analysis.

Participants were eligible if they were primiparous women aged 24-30 years, with singleton pregnancies between 24 and 28 weeks of gestation, diagnosed with GDM, and experiencing pregnancy-specific UI. Exclusion criteria included a history of diabetes or UI before pregnancy, orthopedic surgery, mental or personality disorders, or conditions limiting physical activity, such as threatened miscarriage or placenta previa.

### Intervention Protocol

The PFMT protocol used in this study was based on the methodology described by Bø[12]. All participants engaged in a structured home-based exercise program after receiving standardized training from a certified physiotherapist. The training session, lasting 20 minutes, was conducted individually and face-to-face at the Shahid Sadoughi Hospital training center in Yazd.

The session began with theoretical education on the anatomy and function of the urinary system, the pathophysiology and risk factors of pregnancy-related urinary incontinence, associated symptoms and complications, and available treatment strategies. This was followed by practical instruction on PFMT techniques using educational pamphlets, visual aids, and hands-on demonstrations.

The PFMT protocol consisted of three sets of ten slow contractions, each held for 6-8 seconds, followed by a rest period equal to the contraction duration. Additionally, participants performed three to four rapid contractions in each

supine, sitting, and standing position, with exercises scheduled at least three times per day. The contractions were executed with controlled breathing, mimicking the action of interrupting urinary flow while ensuring the relaxation of surrounding pelvic muscles.

Participants continued this exercise regimen for ten consecutive weeks. They were instructed to schedule their sessions based on personal convenience. To support adherence, each participant received an instructional pamphlet on PFMT and a self-report checklist to record the number of contractions and total daily repetitions.

To ensure compliance and address potential concerns, the investigator maintained biweekly remote follow-ups via social media and phone calls. This remote approach was adopted due to the Coronavirus disease-2019 (COVID-19) pandemic to minimize infection risk while maintaining effective monitoring of the intervention.

Participants in the control group did not receive any training. They received an educational pamphlet and were instructed to refrain from any additional treatments for UI during the 10-week study period while maintaining their usual daily activities.

### Outcomes Evaluation

The outcomes were assessed at three time points: baseline, 10 weeks post-intervention, and 2 weeks postpartum. The UI status was evaluated using the International Consultation on Incontinence Questionnaire Short Form (ICI-Q-SF) (Cronbach's $\alpha$=0.81)[13], which was localized and validated in Persian[14]. Healing was defined as a significant decrease in the ICI-Q-SF score, according to the guidelines for the diagnosis and treatment of urological diseases[15]. Additionally, the Incontinence Quality of Life Questionnaire (I-QoL) (Cronbach's $\alpha$=0.963) was used to assess the quality of life[16], using the Persian version of the tool for this study[17]. The examiner remained blinded to group assignments during the evaluation process.

### Measurement of Bladder Base Displacement

The bladder base displacement was measured by ultrasound examination. The participants performed the contraction of PFMs. Before the examination for the training and education of PFM contractions, a perineometer with a latex cover was used. To achieve maximal contraction of PFMs: Lie on the back, bend knees 90 degrees, and contract the vagina and anus forcefully, avoiding any movement of the pelvis and buttocks. The ultrasound transducer was placed in the suprapubic area and tilted posteriorly (15-30°) to visualize the lower posterior bladder. At rest, a marker was positioned on the bladder base. Participants were then instructed to perform a voluntary PFM contraction. When the contraction was visible on the ultrasound screen, an image was captured. The indicator was placed at the point of maximum displacement, and bladder base movement was measured in millimeters[18,19]. Ultrasound examination was performed on a diagnostic ultrasound imaging device with B-mode technology and a 3.5-5 MHz convex array transducer (Resona 6, Mindray Co., China) by the same physician. Three measurements were taken.

### Statistical Analysis

In this study, quantitative variables were presented as a mean, while qualitative variables were expressed as a frequency. The mean values of quantitative outcomes after the intervention were compared between the control and intervention groups, stratified by diabetes status, using analysis of covariance (ANCOVA). Additionally, partial eta squared ($\eta p^2$) was reported for ANCOVA, where values of 0.01-0.06, 0.06-0.14, and >0.14 indicated small, moderate, and large effect sizes, respectively[20]. Data analysis was conducted using SPSS software, version 20, with a significance level set at 0.05. Graphs were generated using GraphPad Prism, version 8.0.1.

The participant flow diagram for non-diabetic women is presented in Figure 1. Flow diagram of pregnant women without diabetes. A total of 50 individuals were screened, and 44 were selected through convenience sampling. The first participant was randomized in October 2021 and the last in June 2023. Follow-up data were available for 40 participants who were included in the intention-to-treat analysis.

Similarly, the participant flow diagram for women with diabetes is shown in Figure 2. Flow diagram of pregnant women with diabetes. Out of 50 screened individuals, 46 were selected, with randomization occurring between October 2021 and June 2023. Follow-up data for 40 participants were available for inclusion in the intention-to-treat analysis.

## Results

The baseline characteristics of participants:

Three patients in each group were lost to follow-up. A total of 20 patients in the training groups and 20 patients in the control group were included in the analysis. The baseline characteristics of these participants are listed in Table 1.

### Comparison of UI Status Before and After Training

The effects of PFM training on UI were explored (Table 2). In women without GDM, after controlling for baseline scores at the beginning of the third trimester, the mean total ICI-Q-UI SF score in the intervention group at the end of the third trimester was significantly lower by 6.38 points [95% confidence interval (CI): 4.82, 7.93] than in the control group ($\eta^2$p=0.651, p<0.001, F=69.15, df=1, 37). The partial eta squared value of 0.651 indicates a large effect size. Furthermore, two weeks postpartum, the mean total ICI-Q-UI SF score in the intervention group was 7.56 points significantly lower (95% CI: 4.49, 10.62) than in the control group ($\eta^2$p=0.403, p<0.001, F=24.98, df=1, 37).

In women with GDM the mean total ICI-Q-UI SF score in the intervention group was lower than in the control group at the end of the third trimester, although this difference was not statistically significant ($\eta^2$p=0.094, p=0.058, F=3.83, df=1, 37). Two weeks postpartum, the mean total ICI-Q-UI SF score in

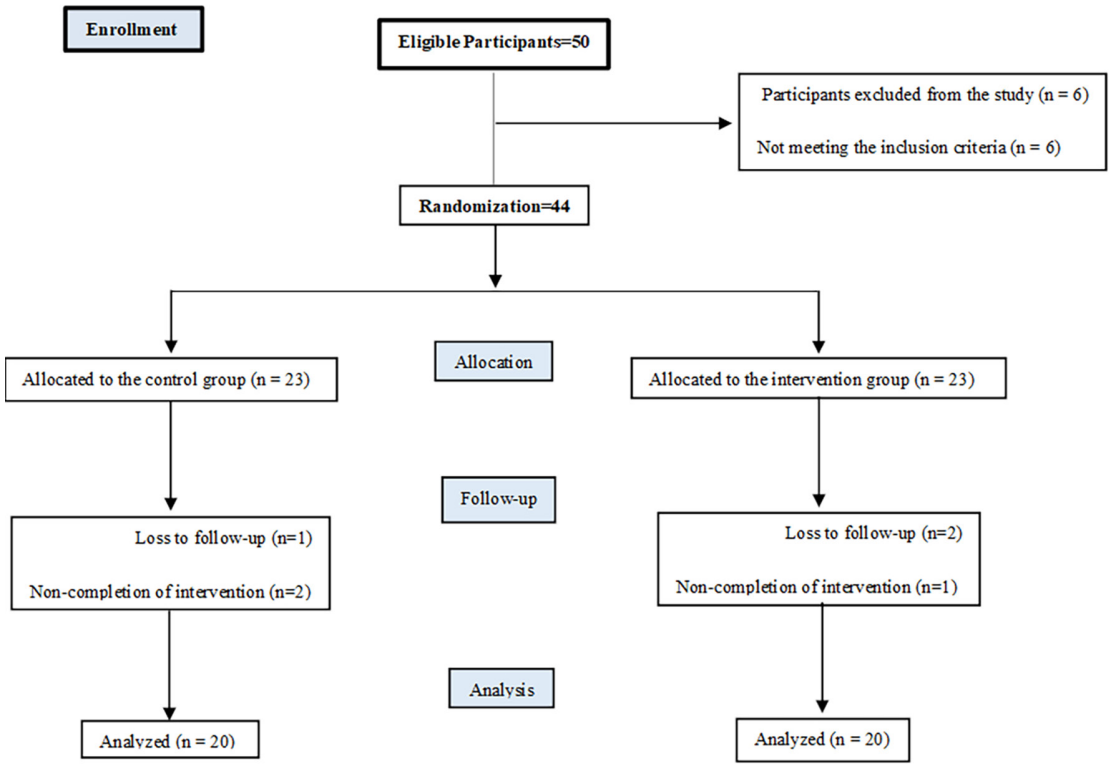


**Figure 1.** Flow diagram of pregnant women without diabetes

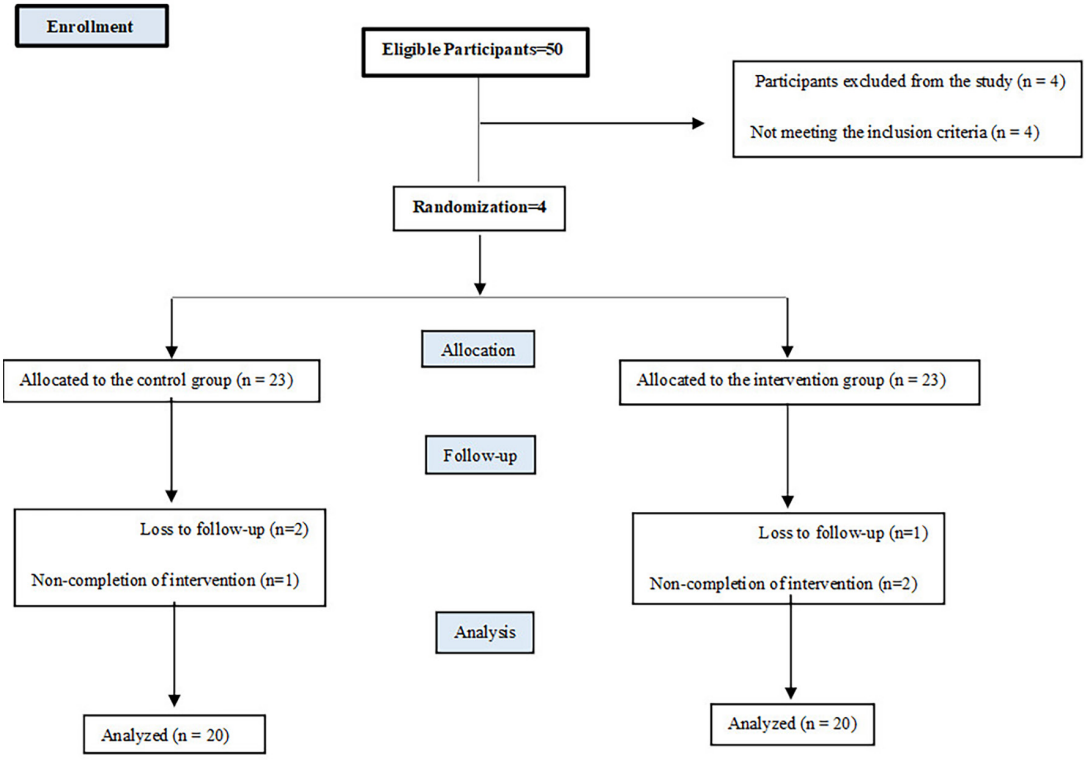


**Figure 2.** Flow diagram of pregnant women with diabetes

the intervention group was significantly lower by 4.62 points (95% CI: 1.21, 8.04) than in the control group ($\eta p^2$=0.169, p<0.001, F=7.51, df=1, 37). The partial eta squared value of 0.169 indicates a large effect size (Figure 3). The training did not cause any side effects.

### Comparison of Quality of Life Before and After Training

The effects of the training on QoL were further evaluated. As shown in Table 3, in non-diabetic women, after controlling for the initial scores at the beginning of the third trimester, the mean total I-QoL score in the intervention group at the end of the third trimester was significantly 18.7 points higher (95% CI: 12.0 to 25.4) than in the control group [p<0.001, $\eta p^2$=0.464, F (1, 37)=31.98]. The partial eta-squared value of 0.464 indicates a large effect size. Furthermore, two weeks postpartum, the mean total I-QoL score in the intervention group was 30.8 points significantly higher (95% CI: 17.6 to 44.1) than in the control group [p<0.001, $\eta p^2$=0.376, F (1, 37)=22.31].

In diabetic women, at the end of the third trimester, there was no statistically significant difference between the mean total I-QoL scores of the intervention and control groups [p=0.690, $\eta p^2$=0.004, F (1, 37)=0.16]. However, two weeks postpartum, the mean total I-QoL score of the intervention group was 15.4 points, significantly higher points (95% CI: 1.4 to 29.4) than in the control group [p=0.032, $\eta p^2$=0.119, F (1, 37)=4.99]. The partial eta-squared value of 0.119 indicates a large effect size (Figure 4).

**Table 1.** Baseline characteristics of participants

| | Total | Control group (n=20) | Intervention group (n=20) |
|---|---|---|---|
| Non-diabetic | | | |
| Age (year) | 26.3±3.5 | 25.6±3.7 | 27.0±3.3 |
| Weight (kg) | 72.4±12.1 | 74.2±11.9 | 70.6±12.3 |
| Height (cm) | 161.4±5.1 | 161.8±4.7 | 160.8±5.6 |
| Body mass index (kg/m$^2$) | 27.7±3.7 | 28.2±3.8 | 27.2±3.6 |
| Diabetic | | | |
| Age (year) | 26.3±3.5 | 25.8±3.7 | 27.0±3.3 |
| Weight (kg) | 71.3±13.9 | 72.5±14.3 | 70.0±13.8 |
| Height (cm) | 162.1±5.6 | 161.7±5.7 | 162.5±5.6 |
| Body mass index (kg/m$^2$) | 27.0±4.6 | 27.7±5.1 | 26.4±4.0 |

Quantitative data aligned to a normal distribution were presented as mean ± standard deviation

**Table 2.** Comparison of mean total of ICIQ-UI SF score between the control and intervention groups, categorized by diabetes status

| | | | | ANCOVA results | | |
|---|---|---|---|---|---|---|
| | Control group (n=20) | Intervention group (n=20) | Adjusted mean differences[a] (CI 95%) | F (1, 37) | p | $\eta p^2$ |
| Non-diabetic | | | | | | |
| Early third trimester | 9.85 (3.03) | 10.90 (3.31) | | | | |
| Late third trimester | 13.05 (3.30) | 7.55 (3.79) | -6.38 (-7.93, -4.82) | 69.15 | <0.001 | 0.651 |
| Two weeks postpartum | 10.25 (6.70) | 3.60 (3.66) | -7.56 (-10.62, -4.49) | 24.98 | <0.001 | 0.403 |
| Diabetic | | | | | | |
| Early third trimester | 12.25 (3.42) | 12.75 (3.31) | | | | |
| Late third trimester | 14.55 (3.95) | 13.20 (4.19) | -1.78 (-3.63, 0.06) | 3.83 | 0.058 | 0.094 |
| Two weeks postpartum | 12.70 (6.10) | 8.65 (6.92) | -4.62 (-8.04, -1.21) | 7.51 | 0.009 | 0.169 |

CI: Confidence interval, ANCOVA: Analysis of covariance, ICIQ-UI SF: International consultation on incontinence questionnaire-urinary incontinence short form values are presented as "(standard deviation) mean". [a]: Adjusted for pre-intervention values. Partial eta squared ($\eta^2 p$) values of 0.01-0.06, 0.06-0.14, and >0.14 indicate small, moderate, and large effect sizes, respectively

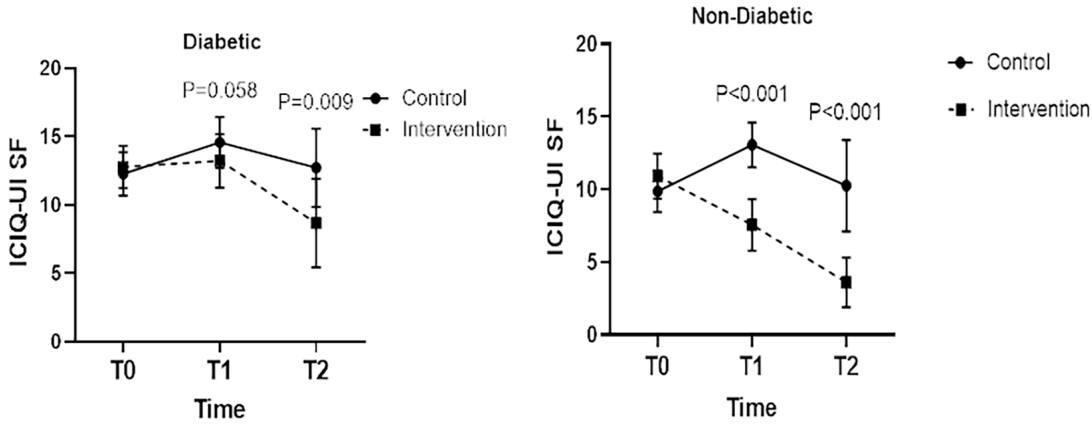


**Figure 3.** a) Comparison of mean total of ICIQ-UI SF score between the control and intervention groups, categorized by diabetes status

*T0: Beginning of the third trimester, T1: End of the third trimester, T2: Two weeks postpartum. The values are presented as "mean with 95% confidence interval". P-values are based on ANCOVA*

**Table 3.** Comparison of mean total I-QoL score between the control and intervention groups, categorized by diabetes status

| | | | | ANCOVA results | | |
|---|---|---|---|---|---|---|
| | Control group (n=20) | Intervention group (n=20) | Adjusted mean differences[a] (CI 95%) | F (1, 37) | p | $\eta p^2$ |
| Non-diabetic | | | | | | |
| Early third trimester | 38.0 (17.6) | 36.5 (17.3) | | | | |
| Late third trimester | 28.0 (17.2) | 45.5 (18.0) | 18.7 (12.0, 25.4) | 31.98 | <0.001 | 0.464 |
| Two weeks postpartum | 34.8 (17.09) | 64.5 (19.0) | 30.8 (17.6, 44.1) | 22.31 | <0.001 | 0.376 |
| Diabetic | | | | | | |
| Early third trimester | 41.7 (21.9) | 33.2 (15.3) | | | | |
| Late third trimester | 36.0 (21.9) | 30.8 (15.2) | 1.5 (-6.1, 9.1) | 0.16 | 0.690 | 0.004 |
| Two weeks postpartum | 45.2 (28.8) | 52.7 (26.1) | 15.4 (1.4, 29.4) | 4.99 | 0.032 | 0.119 |

CI: Confidence interval; ANCOVA: Analysis of covariance; I-QoL: Incontinence quality of life. Values are presented as "(standard deviation) mean". [a]: Adjusted for pre-intervention values. Partial eta-squared ($\eta^2 p$) values of 0.01-0.06, 0.06-0.14, and >0.14 represent small, medium, and large effect sizes, respectively

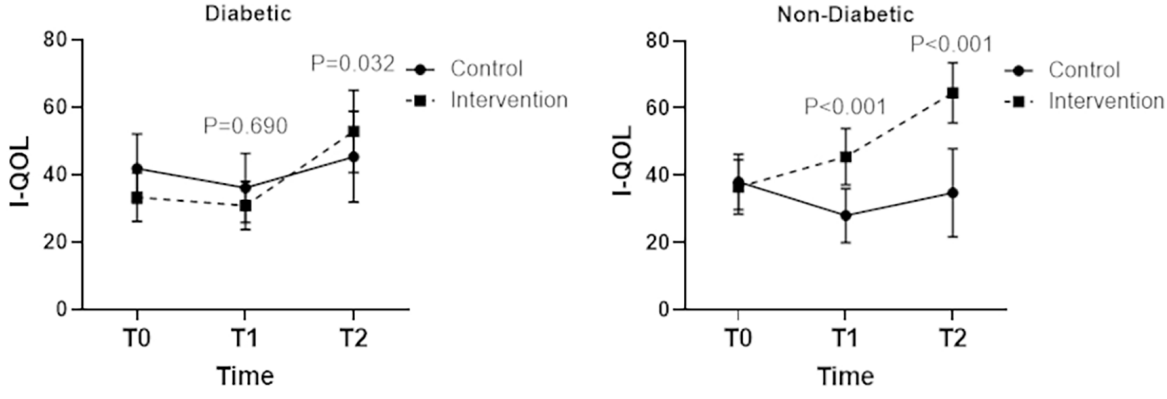


**Figure 4.** Comparison of mean total I-QoL score between the control and intervention groups, categorized by diabetes status

*I-QoL: Incontinence quality of life, T0: Beginning of the third trimester; T1: End of the third trimester; T2: Two weeks postpartum. The values are presented as "mean with 95% confidence interval" P-values are based on ANCOVA*

### Comparison of Bladder Base Displacement Before and After Training

In non-diabetic women, at the end of the third trimester, there was no statistically significant difference in the mean bladder base displacement between the control and intervention groups after adjusting for the initial third trimester values [p=0.300, $\eta p^2$=0.029, F (1, 37)=1.10]. A similar finding was observed two weeks postpartum [p=0.307, $\eta p^2$=0.028, F (1, 37)=1.07] (Figure 5).

In diabetic women at the end of the third trimester, no statistically significant difference in the mean bladder base displacement was found between the control and intervention groups after adjusting for the initial third trimester values [p=0.514, $\eta p^2$=0.012, F (1, 37)=0.43]. A similar result was observed two weeks postpartum [p=0.643, $\eta p^2$=0.006, F (1, 37)=0.22] (Table 4).

## Discussion

The demographic and clinical characteristics were comparable in the intervention and control groups, creating a balanced study population, which is crucial for comparing these groups. PFMT is a common conservative strategy for preventing UI with promising effects. However, the therapeutic potential of PFMT on UI under the pathological condition of GDM remains unclear. This study showed that 10 weeks of PFMT significantly alleviated UI and improved the QoL in non-diabetic pregnant women during the third trimester and postpartum. In pregnant women with GDM, no immediate effect was observed, but the intervention led to significant improvements in I-QoL and ICI-Q-UI SF scores two weeks postpartum. Additionally, the intervention did not lead to significant changes in bladder base displacement in either non-diabetic or diabetic women, both at the end of the third trimester and two weeks postpartum. PFMT

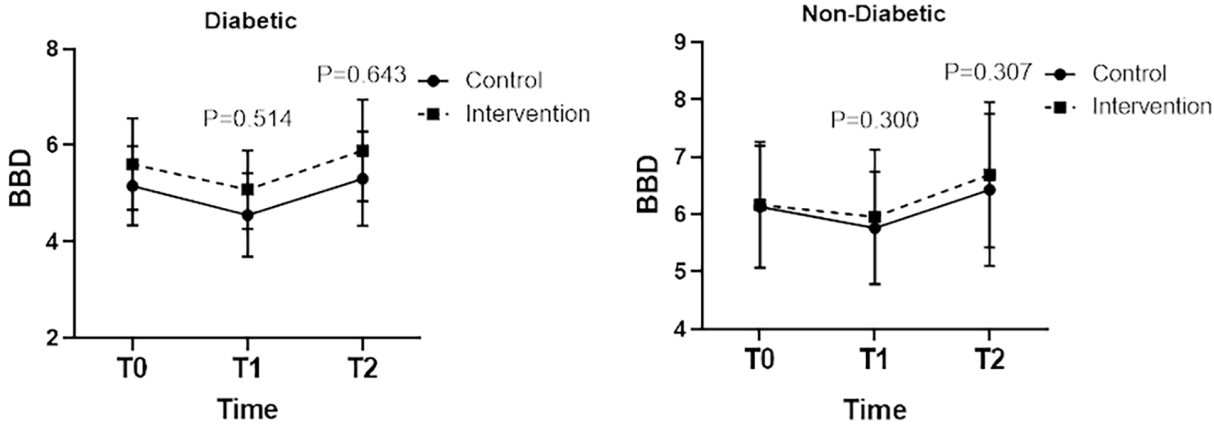


**Figure 5.** Comparison of mean bladder neck displacement between the control and intervention groups, categorized by diabetes status

*BBD: Bladder base displacement, T0: Beginning of the third trimester; T1: End of the third trimester; T2: Two weeks postpartum. The values are presented as "mean with 95% confidence interval". P-values are based on ANCOVA*

**Table 4.** Comparison of mean bladder neck displacement between the control and intervention groups, categorized by diabetes status

| | | | | ANCOVA results | | |
|---|---|---|---|---|---|---|
| | Control group (n=20) | Intervention group (n=20) | Adjusted mean differences[a] (CI 95%) | F (1, 37) | p | $\eta p^2$ |
| Non-diabetic | | | | | | |
| Early third trimester | 6.13 (2.27) | 6.16 (2.34) | | | | |
| Late third trimester | 5.76 (2.10) | 5.96 (2.50) | 0.16 (-0.15, 0.47) | 1.10 | 0.300 | 0.029 |
| Two weeks postpartum | 6.42 (2.84) | 6.69 (2.70) | 0.22 (-0.21, 0.66) | 1.07 | 0.307 | 0.028 |
| Diabetic | | | | | | |
| Early third trimester | 5.16 (1.76) | 5.60 (2.02) | | | | |
| Late third trimester | 4.55 (1.85) | 5.07 (1.74) | 0.12 (-0.25, 0.49) | 0.43 | 0.514 | 0.012 |
| Two weeks postpartum | 5.30 (2.09) | 5.89 (2.25) | 0.09 (-0.29, 0.46) | 0.22 | 0.643 | 0.006 |

CI: Confidence interval; ANCOVA: Analysis of covariance; BBD: Bladder base displacement. Values are presented as "(standard deviation) mean". [a]: Adjusted for the initial third trimester values. Partial eta-squared ($\eta^2 p$) values of 0.01-0.06, 0.06-0.14, and >0.14 represent small, medium, and large effect sizes, respectively

is a repetitive exercise designed to enhance urethral support by strengthening the PFMs[21]. One review summarized that pregnant women who participated in antenatal PFMT had a lower risk of UI in late pregnancy and the early postnatal period compared to those who received standard care[10]. However, evidence regarding the effectiveness of PFMT for treating pregnancy-related UI remains debated. Some earlier studies have found that 12 weeks of PFMT reduced the prevalence of pregnancy-related UI in late pregnancy and/or 3 to 6 months postpartum[22]. Conversely, some studies have shown that PFMT is not effective in treating pregnancy-related UI in late pregnancy, and the later postpartum period[23]. In this study, the QoL and ICI-Q-SF scores improved in non-diabetic pregnant women, in pregnant women with GDM at 2 weeks postpartum. The overall healing was higher in the training group than in the control group after 10 weeks, at 2 weeks postpartum in non-diabetic pregnant women. These results show that consistent PFMT exercises are effective in reducing UI in participants without GDM and with GDM 2 weeks postpartum, aligning with previous research findings[24]. These results may be attributed to a combination of physiological and metabolic factors specific to diabetic mothers. Since UI is not life-threatening, QoL has become a key measure for assessing the effectiveness of intervention strategies. A review concluded that PFMT can significantly enhance the QoL for women suffering from UI[25]. This study found that the total I-QoL score was significantly higher in the training group compared to the control group, both 10 weeks later and 2 weeks postpartum. These results highlight the positive impact of the training on enhancing the QoL for incontinent women without GDM, aligning with previous studies[26]. Despite the observed improvements in UI and QoL in pregnant women, there were no significant changes in bladder base displacement at the end of pregnancy and two weeks postpartum. Several physiological and anatomical factors may explain this discrepancy[27]. During pregnancy, the growing uterus exerts mechanical pressure on the pelvic organs, including the bladder, which could result in displacement. This pressure may counteract the effects of PFMT on structural changes like bladder base displacement, even if it improves muscle function and reduces UI symptoms[9]. Furthermore, hormonal changes during pregnancy, such as elevated levels of progesterone and relaxin, lead to tissue softening, which may reduce the effectiveness of PFMT in producing structural changes in the pelvic floor[28]. Additionally, childbirth, especially vaginal delivery, can lead to further weakening of the PFMs, making it more challenging to achieve immediate structural changes in bladder base displacement. However, PFMT can still improve functional aspects, such as muscle strength and UI symptoms, without significantly altering the anatomical position of the bladder[29,30].

### Study Limitations

Despite the positive outcomes, there are some limitations to this study. One issue was low and inconsistent participation, as well as a lack of control over the regularity of daily training. A training diary would have been beneficial to track adherence. Additionally, the control group received only a pamphlet, with no direct instructions, which may not fully simulate the reality of daily exercise. Given the challenges of implementing clinic-based programs more than three times a week during the COVID-19 pandemic, participants were asked to continue with the exercises they had learned independently. Another limitation is the high number of participants lost to follow-up, which affected the final analysis, as well as the timing of the follow-up assessments. Ideally, this study would have been extended over a longer period to assess long-term effects.

## Conclusion

In conclusion, PFMT effectively improves UI symptoms and QoL in pregnant women, particularly in those without GDM. While no immediate effects were observed in the GDM group, significant improvements were noted postpartum. However, PFMT did not lead to structural changes in bladder base displacement. Given these findings, further long-term studies are required to explore the mechanisms of PFMT in pregnancy and optimize its implementation for different populations.

### Ethics

**Ethics Committee Approval:** Ethical approval for this study was obtained from the Iranian Registry of Clinical Trials (IRCT) (approval number: IR.SBMU.RETECH.REC.1400.611, date: 28.11.2021).
**Informed Consent:** Retrospective study.

### Footnotes

### Authorship Contributions

Surgical and Medical Practices: S.M.H., Design: P.G.H., F.D.M., Data Collection or Processing: S.M.H., A.J., Analysis or Interpretation: A.A.B., Literature Search: S.M.H., A.J., Writing: P.G.H., S.M.H., F.D.M.
**Conflict of Interest:** No conflict of interest was declared by the authors.
**Financial Disclosure:** The authors declared that this study received no financial support.

## References

1. Agarwal BK, Agarwal N. Urinary incontinence: prevalence, risk factors, impact on quality of life and treatment seeking behaviour among middle aged women. Int Surg J. 2017;4:1953-8.
2. Nygaard CC, Schreiner L, Morsch TP, Saadi RP, Figueiredo MF, Padoin AV. Urinary incontinence and quality of life in female patients with obesity. Rev Bras Ginecol Obstet. 2018;40:534-9.

3. Hansen BB, Svare J, Viktrup L, Jørgensen T, Lose G. Urinary incontinence during pregnancy and 1 year after delivery in primiparous women compared with a control group of nulliparous women. Neurourol Urodyn. 2012;31:475-80.
4. Chen L, Liu S, Zhang D, Cai W. Sitting on the fence: A qualitative study of perceptions regarding pelvic floor muscle training among pregnant women with urinary incontinence. Int J Nurs Sci. 2023;10:540-8.
5. Gu ZJ, Song QJ, Gu WQ, Zhang GP, Su Y, Tang Y, et al. New approaches in the diagnosis and prognosis of gestational diabetes mellitus. Eur Rev Med Pharmacol Sci. 2023;27:10583-94.
6. França DCH, França EL, Sobrevia L, Barbosa AMP, Honorio-França AC, Rudge MVC. Integration of nutrigenomics, melatonin, serotonin and inflammatory cytokines in the pathophysiology of pregnancy-specific urinary incontinence in women with gestational diabetes mellitus. Biochim Biophys Acta Mol Basis Dis. 2023;1869:166737.
7. Sangsawang B. Risk factors for the development of stress urinary incontinence during pregnancy in primigravidae: a review of the literature. Eur J Obstet Gynecol Reprod Biol. 2014;178:27-34.
8. Moossdorff-Steinhauser HFA, Berghmans BCM, Spaanderman MEA, Bols EMJ. Urinary incontinence during pregnancy: prevalence, experience of bother, beliefs, and help-seeking behavior. Int Urogynecol J. 2021;32:695-701.
9. Soave I, Scarani S, Mallozzi M, Nobili F, Marci R, Caserta D. Pelvic floor muscle training for prevention and treatment of urinary incontinence during pregnancy and after childbirth and its effect on urinary system and supportive structures assessed by objective measurement techniques. Arch Gynecol Obstet. 2019;299:609-23.
10. Woodley SJ, Lawrenson P, Boyle R, Cody JD, Mørkved S, Kernohan A, et al. Pelvic floor muscle training for preventing and treating urinary and faecal incontinence in antenatal and postnatal women. Cochrane Database Syst Rev. 2020;5:CD007471.
11. Dumoulin C, Morin M, Danieli C, Cacciari L, Mayrand MH, Tousignant M, et al. Group-based vs individual pelvic floor muscle training to treat urinary incontinence in older women: a randomized clinical trial. JAMA Intern Med. 2020;180:1284-93.
12. Bø K. Pelvic floor muscle exercise for the treatment of stress urinary incontinence: An exercise physiology perspective. International Urogynecology Journal. 1995;6:282-91.
13. Avery K, Donovan J, Peters TJ, Shaw C, Gotoh M, Abrams P. ICIQ: a brief and robust measure for evaluating the symptoms and impact of urinary incontinence. Neurourol Urodyn. 2004;23:322-30.
14. Hajebrahimi S, Nourizadeh D, Hamedani R, Pezeshki MZ. Validity and reliability of the international consultation on incontinence questionnaire-urinary incontinence short form and its correlation with urodynamic findings. Urol J. 2012;9:685-90.
15. Huang H. Chinese Guidelines for Diagnosis and Treatment of Urology and Andrology Diseases (2022 edition) - Urinary Incontinence. 2022.
16. Wagner TH, Patrick DL, Bavendam TG, Martin ML, Buesching DP. Quality of life of persons with urinary incontinence: development of a new measure. Urology. 1996;47:67-71; discussion 71-2.
17. Nojomi M, Baharvand P, Moradi Lakeh M, Patrick DL. Incontinence quality of life questionnaire (I-QOL): translation and validation study of the Iranian version. Int Urogynecol J Pelvic Floor Dysfunct. 2009;20:575-9.
18. Dehghan Manshadi F, Parnianpour M, Ghanbari Z, Sarrafzadeh J, Kazemnejad A. An ultrasonic investigation of stability of pelvic floor in women with and without urinary stress incontinence. The Iranian Journal of Obstetrics, Gynecology and Infertility. 2012;15:16-25.
19. Ghadiri Harati P, Hosseini SM, Javaheri A, Dehghan Manshadi F, Akbarzadeh Baghban A. The Reliability of the Rehabilitative Ultrasound Imaging Method for Assessing Pelvic Floor Muscles Function in Pregnant Women. Caspian Journal of Neurological Sciences 2024;10:132-8.
20. Cohen J. Statistical Power Analysis for the Behavioral Sciences. L. Erlbaum Associates; 1988.
21. Cho ST, Kim KH. Pelvic floor muscle exercise and training for coping with urinary incontinence. J Exerc Rehabil. 2021;17(6):379-87.
22. Johannessen HH, Frøshaug BE, Lysåker PJG, Salvesen KÅ, Lukasse M, Mørkved S, et al. Regular antenatal exercise including pelvic floor muscle training reduces urinary incontinence 3 months postpartum-follow up of a randomized controlled trial. Acta Obstet Gynecol Scand. 2021;100:294-301.
23. Fritel X, de Tayrac R, Bader G, Savary D, Gueye A, Deffieux X, et al. Preventing urinary incontinence with supervised prenatal pelvic floor exercises: a randomized controlled trial. Obstet Gynecol. 2015;126:370-7.
24. Stafne SN, Salvesen KÅ, Romundstad PR, Torjusen IH, Mørkved S. Does regular exercise including pelvic floor muscle training prevent urinary and anal incontinence during pregnancy? A randomised controlled trial. BJOG. 2012;119:1270-80.
25. Radzimińska A, Strączyńska A, Weber-Rajek M, Styczyńska H, Strojek K, Piekorz Z. The impact of pelvic floor muscle training on the quality of life of women with urinary incontinence: a systematic literature review. Clin Interv Aging. 2018;13:957-65.
26. Cruz CdS, Riesco MLG, Zanetti MRD. Supervised pelvic floor muscle training to treat urinary incontinence during pregnancy: a randomized controlled trial. Neurourology and Urodynamics, 2014.
27. Wisser J. The effect of pregnancy on the pelvic floor. Childbirth Trauma. 2017:43-56.
28. Kamisan Atan I, Zhang W, Shek KL, Dietz HP. Does pregnancy affect pelvic floor functional anatomy? A retrospective study. Eur J Obstet Gynecol Reprod Biol. 2021;259:26-31.
29. Pires TF, Pires PM, Costa R, Viana R. Effects of pelvic floor muscle training in pregnant women. Porto Biomed J. 2020;5:e077.
30. Romeikienė KE, Bartkevičienė D. Pelvic-floor dysfunction prevention in prepartum and postpartum periods. Medicina (Kaunas). 2021;57:387.

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# Protective effect of N-acetylcysteine in doxorubicin-induced primary ovarian failure in female rats

## *Dişi sıçanlarda doksorubisin kaynaklı primer yumurtalık yetmezliğinde N-asetilsisteinin koruyucu etkisi*

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### Abstract

**Objective:** N-acetylcysteine (NAC), an aminothiol compound, eliminates free radicals and enhances glutathione (GSH) synthesis, thereby strengthening intracellular antioxidant defenses. Although its protective effects against ovarian injury have been reported, its efficacy in doxorubicin (DOX)-induced ovarian failure has not been demonstrated. This study aimed to investigate whether NAC exerts a protective role against DOX-induced ovarian toxicity in female rats.

**Materials and Methods:** Twenty-one adult female rats were randomly assigned to three groups: Control, DOX (10 mg/kg, i.p., single dose), and DOX+NAC (150 mg/kg, i.p., for 5 days; DOX administered on day 3, one hour after NAC). Serum and tissue oxidative stress parameters, histopathological changes, proliferating cell nuclear antigen (PCNA) immunoreactivity, and TUNEL assay were evaluated.

**Results:** DOX significantly reduced serum anti-Müllerian hormone (AMH) (6.75 → 5.31 ng/mL; p<0.001) and GSH (422.64 → 280.98 mg/L; p<0.001), while increasing tumor necrosis factor alpha (TNF-α) (175.87 → 260.77 ng/L; p<0.001) and total oxidant status (TOS) (7.18 → 11.84 U/mL; p=0.002). NAC treatment reversed these alterations, namely: AMH (6.51 ng/mL; p=0.004), GSH (363.86 mg/L; p=0.018), TNF-α (184.55 ng/L; p<0.001), TOS (7.88 U/mL; p=0.003). In ovarian tissue, DOX reduced GSH (123.63 → 80.64 mg/L; p=0.001) and total antioxidant status (14.88 → 10.57 U/mL; p<0.001), while elevating TOS (7.14 → 12.64 U/mL; p<0.001) and caspase-3 (2.06 → 3.14 ng/mL; p<0.001). NAC significantly improved all these parameters (p≤0.005). Histologically, DOX caused edema, hemorrhage, infiltration, and a reduction in the percentage of healthy follicles, whereas NAC markedly ameliorated these alterations. Furthermore, NAC enhanced PCNA expression and reduced TUNEL-positive granulosa cells, supporting its anti-apoptotic effect.

**Conclusion:** NAC preserved ovarian reserve and follicular integrity by suppressing oxidative stress, inflammation, and apoptosis induced by DOX. These findings highlight NAC as a promising protective agent against chemotherapy-induced ovarian toxicity.

**Keywords:** Doxorubicin, infertility, N-acetylcysteine, ovarian function, primary ovarian failure

**PRECIS:** N-acetylcysteine (NAC), a potent antioxidant and glutathione precursor, demonstrated significant protection against doxorubicin (DOX)-induced ovarian toxicity in a rat model. NAC preserved ovarian reserve and follicular integrity by attenuating oxidative stress, inflammation, and apoptosis, thereby restoring serum anti-Müllerian hormone and glutathione levels and reducing tumor necrosis factor alpha, total oxidant status, and caspase-3 activity. Histopathological and immunohistochemical analyses further confirmed enhanced follicular survival, reduced granulosa cell apoptosis, and increased proliferative capacity. These findings provide the first comprehensive evidence that NAC mitigates DOX-related ovarian failure, underscoring its translational potential as an adjuvant for fertility preservation in patients undergoing chemotherapy.

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## Öz

**Amaç:** N-asetilsistein (NAC), serbest radikalleri temizleyen ve glutatyon (GSH) sentezini artırarak antioksidan savunmayı güçlendiren bir aminotiyol türevidir. Yumurtalık hasarına karşı koruyucu etkileri bildirilmiş olsa da, doksorubisin (DOX) kaynaklı over yetmezliğinde etkinliği gösterilmemiştir. Bu çalışmada, NAC'nin DOX ile oluşturulan over hasarına karşı koruyucu etkisi araştırılması hedeflenmiştir.

**Gereç ve Yöntemler:** Yirmi bir erişkin dişi sıçan üç gruba ayrıldı: Kontrol, DOX (10 mg/kg, i.p., tek doz) ve DOX+NAC (150 mg/kg, i.p., 5 gün; 3. gün DOX, NAC'den 1 saat sonra). Serum ve doku oksidatif stres parametreleri, histopatolojik değişiklikler, çoğalan hücre nükleer antijeni (PCNA) immünoreaktivitesi ve TUNEL apoptoz analizi yapıldı.

**Bulgular:** DOX, serum anti-Müllerian hormone (AMH) (6,75 → 5,31 ng/mL; p<0,001) ve GSH'yi (422,64 → 280,98 mg/L; p<0,001) azaltırken; tümör nekroz faktörü alfayı (TNF-α) (175,87 → 260,77 ng/L; p<0,001) ve toplam oksidan kapasitesini (TOS) (7,18 → 11,84 U/mL; p=0,002) artırdı. NAC tedavisi bu değişiklikleri düzeltti: AMH (6,51 ng/mL; p=0,004), GSH (363,86 mg/L; p=0,018), TNF-α (184,55 ng/L; p<0,001), TOS (7,88 U/mL; p=0,003). Doku analizinde DOX, GSH'yi (123,63 → 80,64 mg/L; p=0,001) ve TOS'unu (14,88 → 10,57 U/mL; p<0,001) düşürürken; TOS (7,14 → 12,64 U/mL; p<0,001) ve kaspaz-3'ü (2,06 → 3,14 ng/mL; p<0,001) yükseltti. NAC tüm parametrelerde anlamlı iyileşme sağladı (p≤0,005). Histolojik olarak DOX, ödem, hemoraji ve enflamasyonu artırıp sağlıklı folikül oranını azaltırken; NAC bu bulguları düzeltti. Ayrıca PCNA'yı artırıp TUNEL pozitif hücreleri azalttı.

**Sonuç:** NAC, DOX'in neden olduğu oksidatif stres, enflamasyon ve apoptozu baskılayarak over rezervi ve folikül sağlığını koruyarak; kemoterapiye bağlı over toksisitesine karşı güçlü bir koruyucu ajan potansiyeli sunmuştur.


**Anahtar Kelimeler:** Doksorubisin, kısırlık, N-asetilsistein, yumurtalık fonksiyonu, primer yumurtalık yetmezliği

## Introduction

Ovarian toxicity and infertility are major side effects of cancer treatment among pre-pubertal, adolescent and young adult female cancer patients. The ovary consists of follicles at various stages of development, with the follicles serving as the basic functional unit. There are a limited number of primordial follicles that form immediately after birth, and these follicles remain in a dormant state, representing the ovarian reserve, an indicator of fertility potential. With advancing age, primordial follicles are activated in regular waves and develop into growing follicles that include primary, secondary and antral stages to support hormone secretion, oocyte maturation and ovulation from birth until menopause, when the pool of primordial follicles is depleted[1].

Doxorubicin (DOX) is an antineoplastic drug used to treat various tumors, including leukaemia, lymphomas and soft tissue sarcomas[2]. However, the use of DOX may cause loss of primordial follicles and ovarian function, which may lead to depletion of ovarian reserve and consequently premature ovarian failure[1]. The toxic effects of DOX on the ovary have been associated with decreased antioxidant capacity and increased production of reactive oxygen species (ROS), mitochondrial damage, and inflammatory response, leading to cell apoptosis[3,4]. Administration of natural compounds with antioxidant properties may be a potential strategy to prevent or mitigate DOX-induced ovarian damage[5,6].

The use of antioxidants is steadily increasing as they retain important cytoprotective potential. One effective antioxidant compound is N-acetylcysteine (NAC). It is one of the oldest and most potent mucolytics, the preferred antidote in paracetamol poisoning, and an aminothiol compound[7]. The well-known antioxidant properties of NAC are attributed to its ability to scavenge free radicals and restore intracellular antioxidant defense by increasing glutathione (GSH) production through deacetylation to cysteine, the building block and rate-limiting step in GSH synthesis[8]. In terms of female reproduction, NAC has protective activity against ovarian damage caused by ischemia/reperfusion injury[9]. At the same time, NAC showed protective activity by decreasing the production of ROS and increasing GSH production in cisplatin-induced primary ovarian failure[10]. In gamma radiation-induced ovarian failure, NAC normalized anti-Müllerian hormone (AMH) levels and improved the histopathological and ultrastructural changes induced by γ radiation[11]. However, to the best of our knowledge, there is no study in the literature showing that NAC can protect ovarian function impaired by DOX. Therefore, this study was performed to investigate the protective efficacy of NAC, a GSH precursor, in ovarian failure by focusing on DOX-induced oxidative stress, apoptotic cascade, and inflammation in female rats induced by DOX.

We hypothesized that NAC would attenuate DOX-induced ovarian toxicity by reducing oxidative stress, inflammation, and apoptosis. The primary endpoint was preservation of healthy follicles and serum AMH levels, and secondary endpoints included histopathological injury scores, proliferating cell nuclear antigen (PCNA), TUNEL, and serum/tissue biomarkers [GSH, total antioxidant status (TAS), total oxidant status (TOS), tumor necrosis factor alpha (TNF-α), and caspase-3].

## Materials and Methods

### Ethical Approval

The current study followed the "Principles of laboratory animal care" (NIH publication no: 86-23, revised 1985) and, as well as the Aksaray University Experimental Animals Ethics Committee, and it was carried out in compliance with ethical standards (approval number: 14, date: 19.02.2024).

### Animals and Study Groups

In a five-day study period, twenty-one adult female rats of the same age were randomly assigned to three groups (n=7 per group).

**Control Group:** Received no drug treatment, with saline administered intraperitoneally (i.p.) as a placebo.
**DOX Group:** Received a single dose of DOX (10 mg/kg, i.p.) on the third day of the study.
**DOX+NAC Group:** NAC (150 mg/kg, i.p.) was administered daily for five days. On the third day, DOX (10 mg/kg, i.p.) was injected one hour after NAC administration.
Since there are also studies in the literature in which the prophylactic drug alone was not administered in this model, only the NAC group was not included in this project[12,13].
It is observed that the estrous cycles of female rodents were not evaluated before starting the study. For this reason, healthy female rats of the same age were used in our project, and their cyclic status was ignored[12-14].
**Determination of the Dose of DOX:** The DOX dose was set at 10 mg/kg single dose based on the findings of a previous study[5]. There is evidence that this dosage schedule results in ovarian failure.
Determination of NAC Dose: The dose of NAC was set at 150 mg/kg based on the findings of a previous study[10].
**Sample Collection:** 24 hours after the last pharmacological drug administration*, 5 mL of blood was collected from all rats under anaesthesia. The serum was separated and stored at -80 °C until further study. Subsequently, they were euthanised by cervical dislocation and ovarian tissues were collected. One ovary of each rat was stored for biochemical analyses and the other for histopathological analyses[5].

### Outcome Measures

#### Histomorphometric and Histopathogical Analysis

At the end of the experimental period, ovarian tissue (left ovary per animal) was fixed in 10% formalin for 48 h at room temperature. Samples were then washed in tap water, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin wax by standard histological method. Tissue blocks were sectioned at 5 µm thickness on a rotary microtome (Leica RM2245, Leica Biosystems, Germany) for histomorphometric, immunohistochemical, and apoptotic examinations.
Serial sections were stained with hematoxylin and eosin (H&E) to examine the histopathological changes in the ovarian tissue and to determine the ovarian reserve. Hemorrhage, edema, and infiltration histopathological findings were evaluated and scored for each criterion using a scale ranging from 0 to 3 (0; none, 1; mild, 2; moderate, and 3; severe)[15]. To determine ovarian follicular activity, follicles in each tissue were classified and recorded as primordial, primary, secondary, antral, and atretic. Healthy follicles (%) was calculated as previously described, (primordial+primary+secondary+antral follicles/total number of follicles x 100)[16].

#### Detection of PCNA Immunoreactivity

Immunohistochemical anti-PCNA immunoreactivity was evaluated by the indirect immunohistochemical method[17]. Tissue sections were deparaffinised in xylene, hydrated in graded ethanol, and washed with distilled water. Slides were washed in phosphate buffered saline, and boiled in citrate buffer using a microwave oven for antigen retrieval. To block non-specific binding, the slides were incubated with goat serum for 30 min. Then, slides were incubated with anti-PCNA primary antibody in humidified chamber for 1 hour. Subsequently, slides were incubated with biotinylated secondary antibody and streptavidin peroxidase for 30 min at room temperature. 3,3'-diaminobenzidine (DAB) was used as a chromogen to visualize nuclear PCNA immunoreactivity (brown staining). Counterstain was performed using Mayer's haematoxylin. For PCNA scoring, granulosa cells in follicles, were evaluated according to their DAB-positive staining in three distinct fields from each ovarian section. The number of granulosa cells staining PCNA-positive for each ovarian tissue was calculated as a percentage[18].

#### Detection of Follicular Apoptosis

The TUNEL staining method (Merck Millipore, Apoptag® Peroxidase *In Situ* Apoptosis Detection Kit, Darmstadt, Germany) was used to determine follicular apoptosis. The staining protocol was applied according to the manufacturer's instructions. Mayer's hematoxylin was used for counterstaining. From each ovarian section, TUNEL-positive granulosa cells and all granulosa cells, in the follicles were counted in 5 randomly selected high-power fields at 200x magnification. The percentage of TUNEL-positive granulosa cells (%) was determined by the formula, (number of TUNEL-positive granulosa cells / number of all granulosa cells) x 100[1].

#### Biochemical Analysis

To evaluate oxidative stress, inflammation, and ovarian function, several biochemical parameters were quantified both in serum and ovarian tissue. Specifically, TNF-α (BTLAB; Cat. No. E0764Ra), AMH Cat. No. E0456Ra, TAS Cat. No. E1710Ra, TOS Cat. No. E1512Ra, GSH Cat. No. EA0113Ra were measured in serum, while TNF-α Cat. No. E0764Ra, TAS Cat. No. E1710Ra, TOS Cat. No. E1512Ra, GSH Cat. No. EA0113Ra, and caspase-3 (BTLAB; Cat. No. E1648Ra) were analyzed in ovarian tissue. All analyses were performed using commercially available enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions. The sandwich ELISA method was applied for TAS and TOS. The standard curve range of these kits is 0.02-60 U/mL, and their sensitivity is 0.013 U/mL. The samples were incubated at 37 °C for 60 minutes, washed five times, and then the substrate solutions were incubated in the dark at 37 °C for 10 minutes before being read at 450 nm. For GSH analysis, a competitive ELISA method was used. The standard curve range for this kit is 20-1600 mg/L, and its sensitivity is 10.25 mg/L. The sample and biotinylated antigen were incubated at 37 °C for 60 minutes, followed by avidin-HRP incubation at 37 °C for

60 minutes. After the washing steps, the substrate solutions were incubated in the dark at 37 °C for 10 minutes, and the absorbance was measured at 450 nm.

### Statistical Analysis

Data were analyzed with SPSS (Version 23.0). The conformity of the data with normal distribution was evaluated with the Kolmogorov-Smirnov test. Comparisons of the groups were carried out using the Kruskal-Wallis test and the Mann-Whitney U test. Data were presented as mean ± standard deviation. Values of $p<0.05$ are statistically significant.

## Results

### Histopathological and Histomorphometric Findings

H&E-stained ovarian sections and histopathology scores of the groups are presented in Figure 1. It was observed that the ovarian tissue in the control group exhibited normal histological structure. The control group ovarian tissue cortex included primordial, primary, secondary, and antral follicles belonging to different stages of follicle development, and the stroma between them. The medulla structure of the control group exhibited normal loose connective tissue. In the DOX group, in addition to hemorrhage in the cortex and medulla, vascular congestion, edema, and infiltration were observed

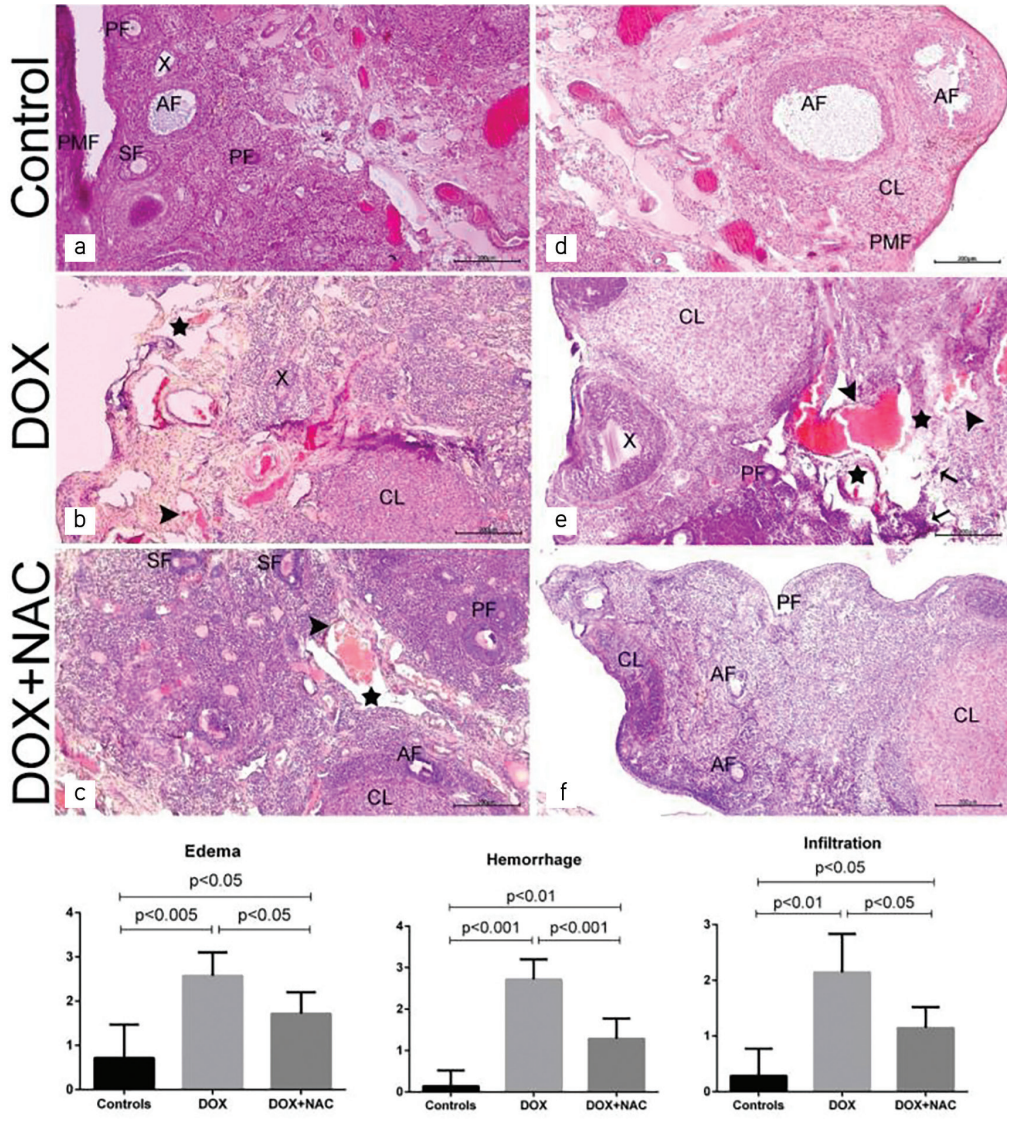


**Figure 1.** Effect of NAC-treatment on DOX-induced histopathological changes in ovarian tissue. Representative images of **(a)** control, **(b)** DOX, **(c)** DOX+NAC groups and the histopathological changes regarding **(d)** edema, **(e)** hemorrhage, **(f)** infiltration levels

*PMF: Primordial follicle, PF: Primary follicle, SF: Secondary follicle, AF: Antral follicle, X: Atretic follicle, CL: Corpus luteum, Star: Edema, Arrowhead: Hemorrhage, Arrow: Infiltration, Dye: Hematoxylin-eosin, 100X, DOX: Doxorubicin, NAC: N-acetylcysteine*

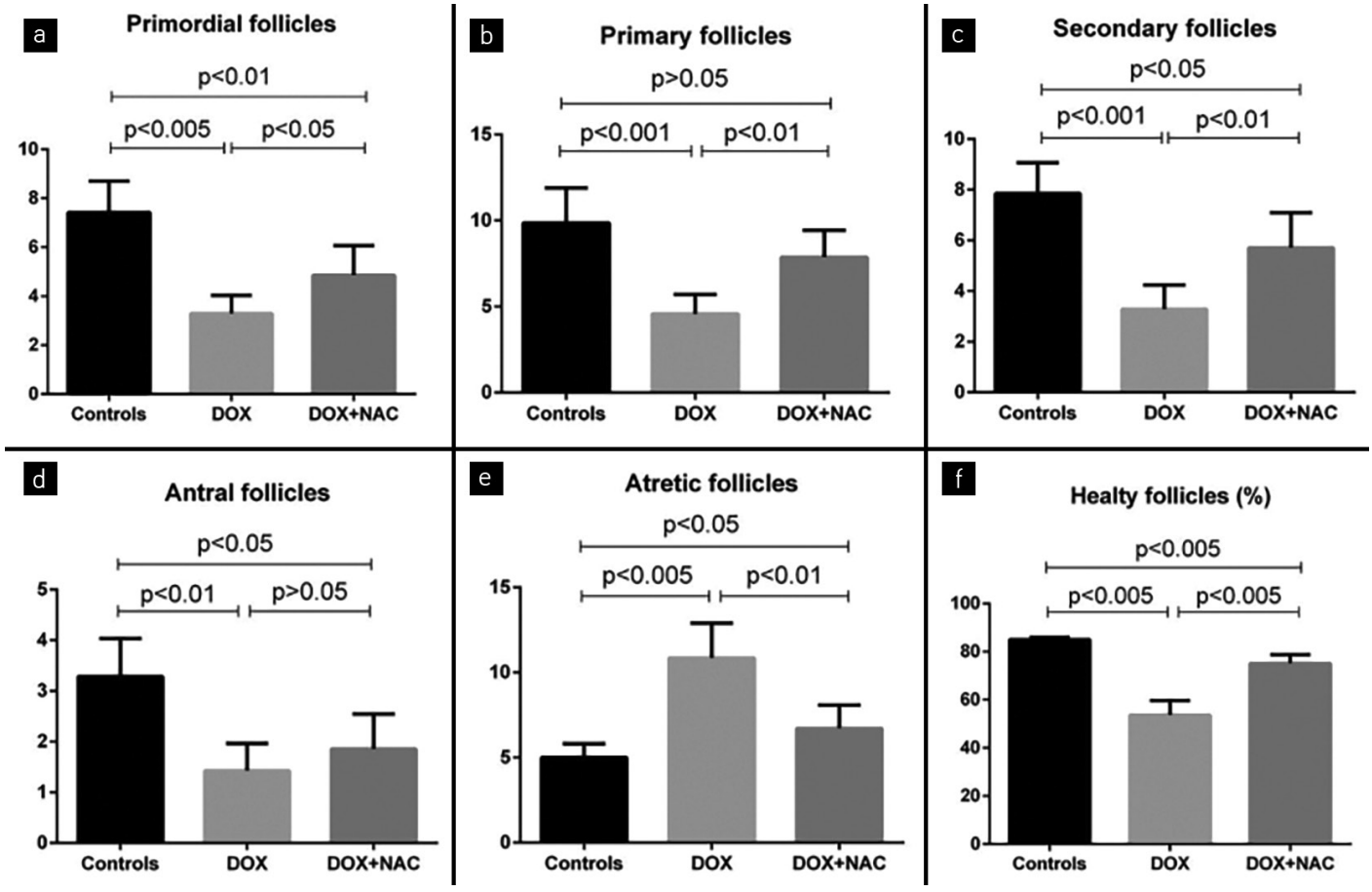


**Figure 2.** Effect of DOX and NAC-treatments on ovarian follicle numbers; **(a)** primordial, **(b)** primary, **(c)** secondary, **(d)** antral, **(e)** antretic follicles, and **(f)** healthy follicle percentage in control, DOX, DOX+NAC groups

*DOX: Doxorubicin, NAC: N-acetylcysteine*

in the medulla. Compared to the control group, the scores of edema, hemorrhage, and the infiltration increased score increased significantly in the DOX group. It was determined that these histopathological changes were significantly alleviated in the DOX + NAC group compared to the DOX group.

The percentage of healthy follicles and ovarian reserve of all groups is presented in Figure 2. The number of primordial, primary, secondary, and antral follicles was significantly lower in the DOX group than in the control group. The number of atretic follicles was significantly higher in the DOX group compared to the control group. A significant increase in the number of primordial, primary, secondary, and antral follicles was detected in the NAC treatment group compared to the DOX group. Additionally, the percentage of healthy follicles decreased significantly in the DOX group compared to the control group; it increased significantly in the DOX+NAC group compared to the DOX group.

### PCNA Immunoreactivity Findings

Findings of cell proliferation marker PCNA immunoreactivity in ovarian tissue are presented in Figure 3. PCNA immunoreactivity is observed in follicular granulosa cells in all groups. While PCNA immunoreactivity decreased in the DOX group compared to the control group, a significant increase in PCNA immunoreactivity was detected in the DOX+NAC group compared to the DOX group.

### Follicular Apoptosis Findings

Findings of follicular apoptosis are presented in Figure 4. The percentage of apoptotic granulosa cells was significantly increased in the DOX group compared to the control group. Granulosa cell apoptosis was significantly reduced in the NAC treatment group compared to the DOX group[15,16].

### Biochemical Analysis

As shown in Table 1, DOX treatment induced substantial oxidative stress, inflammation, and ovarian dysfunction, as evidenced by significant changes in AMH, GSH, TNF-α, TAS, and TOS levels. NAC co-treatment effectively mitigated these adverse effects, restoring oxidative balance and reducing inflammation, thereby demonstrating its protective potential in counteracting DOX-induced systemic and reproductive toxicity.

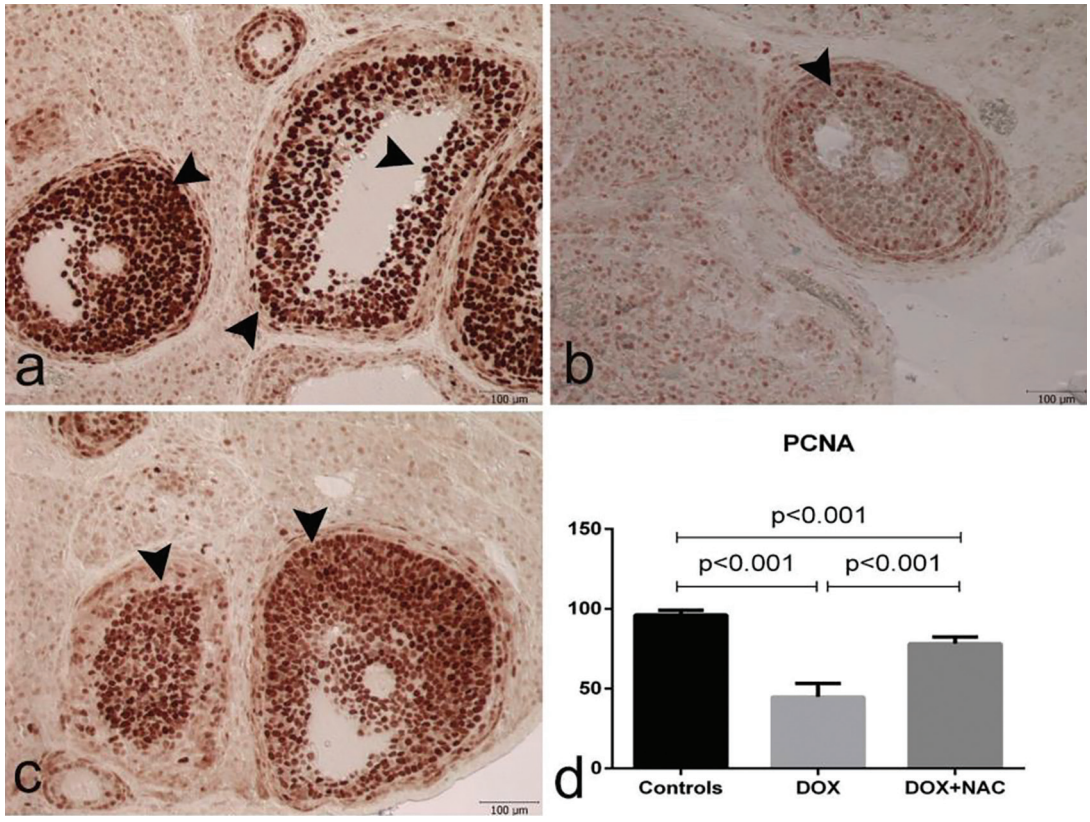


**Figure 3.** Effect of NAC and DOX treatments on PCNA immunoreactivity in granulosa cells of ovarian follicles. **(a)** control, **(b)** DOX, **(c)** DOX+NAC, **(d)** distribution of PCNA immunoreactivity of groups, arrowhead; PCNA-positive cells, magnification; 200X

*DOX: Doxorubicin, NAC: N-acetylcysteine, PCNA: Proliferating cell nuclear antigen*

**Table 1.** Serum biomarkers data

| Serum biomarkers | Control (mean ± SD) | DOX (mean ± SD) | DOX+NAC (mean ± SD) | Control vs. DOX p-value | DOX vs. DOX+NAC p-value |
|---|---|---|---|---|---|
| AMH (ng/mL) | 6.75±0.49 | 5.31±0.47 | 6.51±0.78 | <0.001 | 0.003931087 |
| GSH (mg/L) | 422.64±69.23 | 280.98±32.26 | 363.86±73.71 | <0.001 | 0.018416596 |
| TNF-α (ng/L) | 175.87±25.91 | 260.77±18.08 | 184.55±30.0 | <0.001 | 0.000109148 |
| TAS (U/mL) | 8.13±1.01 | 6.12±1.03 | 7.56±0.59 | 0.001 | 0.003392724 |
| TOS (U/mL) | 7.18±1.00 | 11.84±2.55 | 7.88±1.12 | 0.002 | 0.003191524 |

SD: Standard deviation, AMH: Anti-Müllerian hormone, GSH: Glutathione, TNF-α: Tumor necrosis factor alpha, TAS: Total antioxidant status, TOS: Total oxidant status, DOX: Doxorubicin, NAC: N-acetylcysteine

**Table 2.** Ovarian tissue biomarkers

| Ovarian biomarkers | Control (mean ± SD) | DOX (mean ± SD) | DOX+NAC (mean ± SD) | Control vs. DOX p-value | DOX vs. DOX+NAC p-value |
|---|---|---|---|---|---|
| GSH (mg/L) | 123.63±20.75 | 80.64±15.44 | 118.57±23.04 | 0.001 | 0.005495828 |
| TNF-α (ng/L) | 194.73±26.61 | 289.47±21.29 | 230.82±31.50 | <0.001 | 0.000955061 |
| TAS (U/mL) | 14.88±0.77 | 10.57±1.01 | 13.74±1.02 | <0.001 | 0.000177902 |
| TOS (U/mL) | 7.14±0.62 | 12.64±1.12 | 9.03±0.58 | <0.001 | 0.000000737 |
| Caspase-3 (ng/mL) | 2.06±0.29 | 3.14±0.33 | 2.17±0.28 | <0.001 | 0.000108463 |

SD: Standard deviation, GSH: Glutathione, TNF-α: Tumor necrosis factor alpha, TAS: Total antioxidant status, TOS: Total oxidant status, DOX: Doxorubicin, NAC: N-acetylcysteine

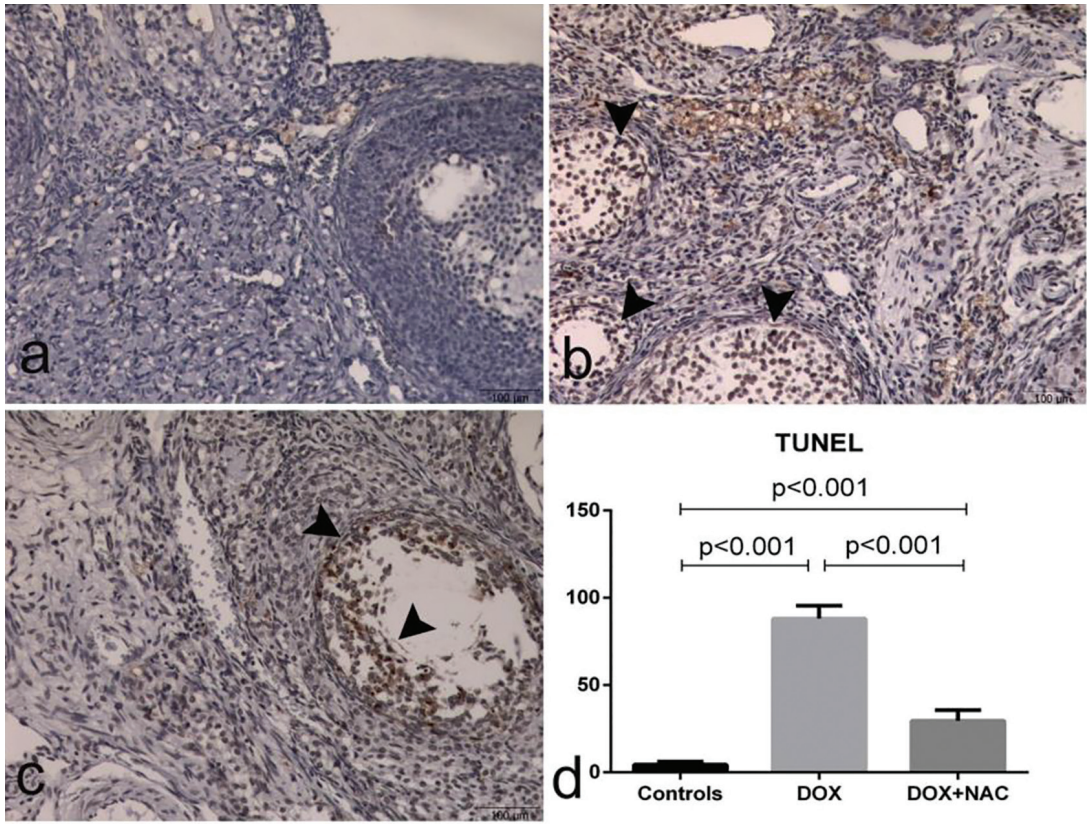


**Figure 4.** Effects of DOX and NAC treatments on granulosa cell apoptosis. **(a)** control, **(b)** DOX, **(c)** DOX+NAC, **(d)** distribution of TUNEL-positive cell percentages of groups, arrowhead; PCNA-positive cells, magnification; 200X

*DOX: Doxorubicin, NAC: N-acetylcysteine, PCNA: Proliferating cell nuclear antigen*

Table 2 summarizes the effect that DOX treatment significantly induces oxidative stress, inflammation, reduced antioxidant capacity, and apoptosis, as evidenced by changes in GSH, TNF-α, TAS, TOS, and Caspase-3 levels. NAC co-treatment effectively mitigates these adverse effects, restoring oxidative balance, reducing inflammation, and limiting apoptosis. These findings underscore the potential of NAC as a therapeutic agent to counteract DOX-induced ovarian toxicity.

## Discussion

The present study demonstrated that DOX exerts profound gonadotoxic effects in female rats, whereas NAC provides significant protection against ovarian injury through its antioxidant, anti-inflammatory, and anti-apoptotic properties. Consistent with earlier reports that chemotherapy can deplete ovarian reserve and impair follicular integrity[1,19,20], our findings confirmed that DOX markedly reduced serum AMH levels (6.75±0.49 → 5.31±0.47 ng/mL; p<0.001) and the percentage of healthy follicles; while increasing the number of atretic follicles. Importantly, NAC co-treatment preserved ovarian reserve, restoring AMH to 6.51±0.78 ng/mL (p=0.004 vs. DOX), which is clinically relevant given that AMH is a robust marker of ovarian function and fertility potential.

In line with the mechanistic role of oxidative stress in DOX-induced toxicity[19,21], our biochemical results showed that DOX significantly depleted systemic and tissue antioxidants, lowering serum GSH (422.64±69.23 → 280.98±32.26 mg/L; p<0.001) and TAS (8.13±1.01 → 6.12±1.03 U/mL; p=0.001), while increasing TOS (7.18±1.00 → 11.84±2.55 U/mL; p=0.002). NAC administration reversed these alterations, with GSH increasing to 363.86±73.71 mg/L (p=0.018 vs DOX) and TAS to 7.56±0.59 U/mL (p=0.003), accompanied by normalization of TOS (7.88±1.12 U/mL; p=0.003). These findings corroborate previous work where natural antioxidants such as gallic acid[5], resveratrol[6], and quercetin[16] attenuated DOX- or cisplatin-induced ovarian damage via restoration of redox balance. The present study extends this evidence to NAC, an aminothiol with potent GSH-replenishing capacity[7,8].

Inflammation and apoptosis are pivotal mediators of chemotherapy-induced ovarian injury[3,19,22]. Here, DOX elevated serum TNF-α by 48% (175.87±25.91 → 260.77±18.08 ng/L; p<0.001) and tissue caspase-3 levels (2.06±0.29 → 3.14±0.33 ng/mL; p<0.001), consistent with enhanced inflammatory signaling and apoptotic cell death. NAC significantly mitigated these effects, lowering TNF-α to 184.55±30.00 ng/L (p<0.001 vs. DOX) and caspase-3 to 2.17±0.28 ng/mL (p<0.001 vs. DOX). Parallel histological findings revealed a reduction in

edema, hemorrhage, and infiltration scores, together with decreased TUNEL-positive granulosa cells and enhanced PCNA immunoreactivity in the NAC-treated group. These results are consistent with prior reports showing that NAC alleviated oxidative and apoptotic injury in γ-radiation-induced ovarian failure[11] and in ovarian torsion models[9], further confirming its cytoprotective potential in diverse ovarian injury settings.

Moreover, a very recent study, using ultrasonographic and histopathological evaluation, confirmed that longterm NAC administration (100 mg/kg for 21 days) attenuated DOX-induced ovarian and uterine toxicity, preserving follicular counts and AMH levels in rats[23]. Additionally, emerging evidence indicates that NAC can counteract chemotherapy-induced ferroptosis by enhancing GPX4, Nrf2, and HO-1 expression and suppressing lipid ROS accumulation[22]. These findings reinforce the translational promise of NAC as an ovoprotective agent.

Taken together, the histological, immunohistochemical, and biochemical evidence indicates that NAC confers multifaceted ovarian protection against DOX. By scavenging ROS, replenishing GSH, suppressing inflammatory mediators, and limiting apoptosis, NAC preserved follicular integrity and granulosa cell proliferation. Compared with studies on other antioxidants such as gallic acid[5], resveratrol[6], and rutin[10], our results highlight NAC as a potent candidate with translational potential, especially given its long-established clinical safety profile in other indications[7,8]. The translational relevance of these findings is noteworthy. Fertility preservation is a critical concern for young women undergoing chemotherapy[20,21], and strategies that can be co-administered safely alongside anticancer drugs are urgently needed. By maintaining AMH levels, follicular health, and proliferative capacity, NAC may offer a feasible adjunct to protect ovarian reserve during chemotherapy.

In conclusion, this study provides the first comprehensive evidence that NAC significantly alleviates DOX-induced ovarian toxicity by improving redox balance, reducing inflammation, and inhibiting apoptosis, thereby preserving ovarian reserve. These findings, supported by both quantitative outcomes and comparison with recent, relevant preclinical studies, suggest that NAC merits further evaluation as a fertility-preserving agent in oncological settings.

### Study Limitations

Nevertheless, some limitations must be acknowledged. Estrous cycle staging was not performed, which may contribute to subtle variability in ovarian morphology and hormone levels. An NAC-only group was not included, preventing isolation of its independent effects. The study relied on a single DOX dose with short-term follow-up and a modest sample size (n=7/group). Furthermore, functional fertility outcomes, such as pregnancy rates and litter size, were not assessed. Future studies should evaluate dose–response and timing effects of NAC, incorporate long-term fertility outcomes, and explore additional mechanistic pathways including ferroptosis[22].

## Conclusion

This study highlights the protective role of NAC in a rat model of DOX-induced ovarian failure. By reducing oxidative stress, inflammation, and apoptosis, NAC preserves ovarian structure and function, emphasizing its potential as a therapeutic agent to safeguard reproductive health in patients undergoing chemotherapy. Future studies should expand upon these findings by exploring different NAC doses and administration schedules, (pre, co-, and post-treatment with DOX) to determine optimal timing and efficacy. Longer-term experiments incorporating functional fertility outcomes such as estrous cyclicity, pregnancy rates, and litter size are warranted to establish the true reproductive benefits of NAC. Mechanistic investigations focusing on ferroptosis, mitochondrial dysfunction, and antioxidant signaling pathways (e.g., Nrf2, HO-1, GPX4) may clarify the molecular basis of NAC's protective effects. Additionally, strategies combining NAC with other antioxidants or established fertility-preserving agents should be assessed. Finally, translational studies in women undergoing chemotherapy are needed to evaluate the clinical applicability and safety of NAC for fertility preservation.

### Ethics

**Ethics Committee Approval:** The current study followed the "Principles of laboratory animal care" (NIH publication no: 86-23, revised 1985) and, as well as the Aksaray University Experimental Animals Ethics Committee, and it was carried out in compliance with ethical standards (approval number: 14, date: 19.02.2024).

**Informed Consent:** Not necessary.

### Footnotes

### Authorship Contributions

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

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## References

1. Wang Y, Liu M, Johnson SB, Yuan G, Arriba AK, Zubizarreta ME, et al. Doxorubicin obliterates mouse ovarian reserve through both primordial follicle atresia and overactivation. Toxicol Appl Pharmacol. 2019;381:114714.
2. Al-Kawlani B, Murrieta-Coxca JM, Chaiwangyen W, Froehlich K, Fritzsche A, Winkler S, et al. Doxorubicin induces cytotoxicity and Mir-132 expression in granulosa cells. Reprod Toxicol. 2020;96:95-101.
3. Fabbri R, Macciocca M, Vicenti R, Caprara G, Piccinni M, Paradisi R, et al. Epigallocatechin-3-gallate inhibits doxorubicin-induced inflammation on human ovarian tissue. Biosci Rep. 2019;39:BSR20181424.

4. Zhang Z, Hou S-Q, He J, Gu T, Yin Y, Shen WH. PTEN regulates PLK1 and controls chromosomal stability during cell division. Cell Cycle. 2016;15:2476-85.
5. dos Santos Silva RL, Lins TLBG, do Monte APO, de Andrade KO, de Sousa Barberino R, da Silva GAL, et al. Protective effect of gallic acid on doxorubicin-induced ovarian toxicity in mouse. Reprod Toxicol. 2023;115:147-56.
6. Han J, Wang H, Zhang T, Chen Z, Zhao T, Lin L, et al. Resveratrol attenuates doxorubicin-induced meiotic failure through inhibiting oxidative stress and apoptosis in mouse oocytes. Aging (Albany N Y). 2020;12:7717.
7. Tardiolo G, Bramanti P, Mazzon E. Overview on the effects of N-acetylcysteine in neurodegenerative diseases. Molecules. 2018;23:3305.
8. Aldini G, Altomare A, Baron G, Vistoli G, Carini M, Borsani L, et al. N-acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. Free Radic Res. 2018;52:751-62.
9. Ersoy GS, Eken M, Tal R, Oztekin D, Devranoglu B, Kaygusuz EI, et al. N-acetylcysteine leads to greater ovarian protection than enoxaparin sodium in a rat ovarian torsion model. Reprod Biomed Online. 2016;33:93-101.
10. Lins TLBG, Gouveia BB, Barberino RS, Silva RLS, Monte APO, Pinto JGC, et al. Rutin prevents cisplatin-induced ovarian damage via antioxidant activity and regulation of PTEN and FOXO3a phosphorylation in mouse model. Reprod Toxicol. 2020;98:209-17.
11. Mantawy EM, Said RS, Kassem DH, Abdel-Aziz AK, Badr AM. Novel molecular mechanisms underlying the ameliorative effect of N-acetyl-l-cysteine against υ-radiation-induced premature ovarian failure in rats. Ecotoxicol Environ Saf. 2020;206:111190.
12. Hortu I, Ozceltik G, Ergenoglu AM, Yigitturk G, Atasoy O, Erbas O. Protective effect of oxytocin on a methotrexate-induced ovarian toxicity model. Arch Gynecol Obstet. 2020;301:1317-24.
13. Melekoglu R, Ciftci O, Eraslan S, Cetin A, Basak N. Beneficial effects of curcumin and capsaicin on cyclophosphamide-induced premature ovarian failure in a rat model. J Ovarian Res. 2018;11:1-8.
14. Ozdamar S, Taskin MI, Onder GO, Kaymak E, Baran M, Yay A. Progesterone decreases the extent of ovarian damage caused by cisplatin in an experimental rat model. Adv Clin Exp Med. 2019;28:25-33.
15. Kaygusuzoglu E, Caglayan C, Kandemir FM, Yildirim S, Kucukler S, Kilinc MA, et al. Zingerone ameliorates cisplatin-induced ovarian and uterine toxicity via suppression of sex hormone imbalances, oxidative stress, inflammation and apoptosis in female wistar rats. Biomed Pharmacother. 2018;102:517-30.
16. Algandaby MM. Quercetin attenuates cisplatin-induced ovarian toxicity in rats: emphasis on anti-oxidant, anti-inflammatory and anti-apoptotic activities. Arabian Journal of Chemistry. 2021;14:103191.
17. Ahmed KA, Shareef SH, Faraj TA, Abdulla MA, Najmaldin SK, Agha NFS, et al. Chemoprotective effect of arbutin on azoxymethane-induced aberrant crypt foci in rat colon via modulation of Pcna/Bax protein. Braz J Med Biol Res. 2024;57:e13306.
18. Kurt S, Seker U, Yazlik MO, Er E, Hürkul MM, Soker S. Identification of major phenolic compounds of aloe vera and its protective effect on ovaries under oxidative stress in diabetic rats. J Resn Pharm. 2023;27:652-64.
19. Nishi K, Gunasekaran VP, Arunachalam J, Ganeshan M. Doxorubicin-induced female reproductive toxicity: an assessment of ovarian follicular apoptosis, cyclicity and reproductive tissue histology in wistar rats. Drug Chem Toxicol. 2018;41:72-81.
20. Spears N, Lopes F, Stefansdottir A, Rossi V, De Felici M, Anderson R, et al. Ovarian damage from chemotherapy and current approaches to its protection. Hum Reprod Update. 2019;25:673-93.
21. Wang J, Zhang Y, Tang Q, Zhang Y, Yin Y, Chen L. Application of antioxidant compounds in bone defect repair. Antioxidants. 2024;13:789.
22. Zhang S, Liu Q, Chang M, Pan Y, Yahaya BH, Liu Y, et al. Chemotherapy impairs ovarian function through excessive ros-induced ferroptosis. Cell Death Dis. 2023;14:340.
23. Üstüner E, Yıldırım E, Macun HC, Ekici H, Şahin Y, Güncüm E, et al. Ultrasonographic and histopathological investigation of the effect of N-acetylcysteine on doxorubicin-induced ovarian and uterine toxicity in rats. J Ovarian Res. 2024;17:135.

*Review / Derleme*

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# The efficacy of single-incision laparoscopic surgery versus conventional laparoscopic surgery in the surgical management of ectopic pregnancy: A systematic review and meta-analysis

## *Ektopik gebeliğin cerrahi tedavisinde tek kesi laparoskopik cerrahinin etkinliği ile konvansiyonel laparoskopik cerrahinin etkinliğinin karşılaştırması: Sistematik bir inceleme ve meta-analiz*

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**Abstract**

To compare the efficacy and safety of single-incision laparoscopic surgery (SILS) versus conventional laparoscopic surgery (CLS) for the surgical management of ectopic pregnancy through a systematic review and meta-analysis. We searched Medline, PubMed, Scopus, Web of Science, ClinicalTrials.gov, and Cochrane databases from inception to May, 2023 for studies comparing SILS with CLS in ectopic pregnancy treatment. Included studies were controlled and observational, excluding single-arm studies, meta-analyses, and reviews. Quality was assessed using ROBINS-I for observational studies and the Cochrane tool for randomized trials. Data were analyzed with OpenMetaAnalyst and Review Manager 5.4.1, using odds ratios for dichotomous outcomes and mean differences (MD) for continuous outcomes. Twelve studies involving 880 women (372 SILS, 508 CLS) were included. SILS showed significantly less blood loss (MD=-51.01 mL, p=0.004), shorter postoperative hospital stay (MD=-0.24 days, p=0.003), and faster return of bowel function (MD=-1.03 hours, p<0.01), compared to CLS. No significant differences were found in total operative time, hemoglobin change, blood transfusion requirements, or number of patients needing transfusions. Patient satisfaction data were limited but suggested better cosmetic outcomes with SILS. SILS is a feasible and effective alternative to CLS for ectopic pregnancy, offering reduced blood loss, shorter hospital stays, and quicker bowel function recovery. These benefits, alongside potential cosmetic advantages, make SILS a promising option, particularly for young women. Further research is needed to confirm long-term outcomes and optimize patient selection.

**Keywords:** Ectopic pregnancy, laparoscopy, single-incision laparoscopic surgery, conventional laparoscopic surgery, hemoperitoneum, surgical outcomes

**Öz**

Ektopik gebeliğin cerrahi tedavisinde tek kesi laparoskopik cerrahinin (SILS) etkinliğini ve güvenliğini sistematik bir inceleme ve meta-analiz yoluyla konvansiyonel laparoskopik cerrahinin (CLS) etkinliği ve güvenliği ile karşılaştırmak amaçlanmıştır. Medline, PubMed, Scopus, Web of Science, ClinicalTrials.gov ve Cochrane veri tabanlarında, ektopik gebelik tedavisinde SILS'yi CLS ile karşılaştıran çalışmalar Mayıs 2023 tarihine kadar taranmıştır. Dahil edilen çalışmalar kontrollü ve gözlemsel olup, tek kollu çalışmalar, meta-analizler ve derlemeler hariç tutulmuştur. Kalite, gözlemsel çalışmalar için ROBINS-I ve randomize çalışmalar için Cochrane aracı kullanılarak değerlendirilmiştir. Veriler, ikili sonuçlar için olasılık oranları ve sürekli sonuçlar için ortalama farklar (OF) kullanılarak OpenMetaAnalyst ve Review Manager 5.4.1 ile analiz edildi. Sekiz yüz seksen kadını (372 SILS, 508 CLS) içeren on iki çalışma dahil edildi. SILS, CLS ile karşılaştırıldığında önemli ölçüde daha az kan kaybı (OF=-51,01 mL, p=0,004), daha kısa postoperatif hastanede kalış süresi (OF=-0,24 gün, p=0,003) ve bağırsak fonksiyonunun daha hızlı geri dönüşü (OF=-1,03 saat, p<0,01) ile ilişkili idi. Toplam ameliyat süresi, hemoglobin düzeyinde değişim, kan transfüzyonu gereksinimi veya transfüzyona ihtiyaç duyan hasta sayısı açısından önemli bir fark bulunamadı. Hasta memnuniyeti

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verileri sınırlıydı ancak SILS ile daha iyi kozmetik sonuçlar olduğunu düşündürmekteydi. SILS, ektopik gebelik tedavisinde CLS'nin uygulanabilir ve etkili bir alternatifidir; daha az kan kaybı, daha kısa hastanede kalış süresi ve daha hızlı bağırsak fonksiyonu iyileşmesi ile ilişkilidir. Bu faydalar, potansiyel kozmetik avantajlarının yanı sıra, SILS'yi özellikle genç kadınlar için umut verici bir seçenek haline getirir. Uzun vadeli sonuçları doğrulamak ve hasta seçimini optimize etmek için daha fazla araştırmaya ihtiyaç vardır.

**Anahtar Kelimeler:** Ektopik gebelik, laparoskopi, tek kesili laparoskopik cerrahi, konvansiyonel laparoskopik cerrahi, hemoperiton, cerrahi sonuçlar

## Introduction

Ectopic pregnancy, where a pregnancy implants outside the uterine cavity, occurs in approximately 1-2% of pregnancies[1]. The presentation of ectopic pregnancy may vary among patients ranging from an asymptomatic condition to lower abdominal pain, to rupture of internal organs resulting in massive hemoperitoneum and severe hemorrhagic shock[2]. Despite its rarity, ectopic pregnancy is the number one cause of maternal mortality in pregnancy[3]. Thus, early diagnosis and management of ectopic pregnancy are essential. It is usually diagnosed by ultrasonography with or without the use of human chorionic gonadotropin titers, and serum progesterone levels can also aid in the diagnosis[4]. Although medical treatment with methotrexate is effective in many cases with a 75% tubal patency rate, surgical management using laparoscopy remains first line treatment for many patients with ectopic pregnancies when medical management is contraindicated or fails[5]. The comparison between single-incision laparoscopic surgery (SILS) and conventional laparoscopic surgery (CLS) is particularly relevant for ectopic pregnancies due to the urgent nature of surgical intervention and the unique patient demographic, often young women of reproductive age. SILS' potential to minimize abdominal wall trauma and improve cosmetic outcomes aligns with patient priorities, such as reduced scarring and faster recovery, which can enhance both physical and psychological outcomes in this population. CLS has become the most commonly used procedure in the management of ectopic pregnancy compared to laparotomy[5]. CLS is associated with less tissue injury, fewer adhesions, less bleeding, shorter total operative time, and shorter hospital stay and a rapid return to daily activities, according to many studies[6-8]. Unlike CLS, SILS uses a multi-channel single port system with articulating instruments through a single skin incision. The single incision is usually at the umbilicus, which may leave no new scar after the operation. This decrease in the number of ports has the potential to reduce the perioperative morbidity and improve the cosmetic results of the procedure[9-11]. However, SILS has some disadvantages including impaired visualization, instrument interference, and loss of laparoscopic triangulation[12]. Our search of previous meta-analyses on this topic reported no considerable differences between CLS and SILS in the treatment of ectopic pregnancy[13,14]. In this study, we aim to compare the surgical outcomes and effectiveness of SILS with CLS in the surgical treatment of women with ectopic pregnancies.

## Methods

We searched Medline, Pubmed, Scopus, Web of Science, ClinicalTrials.Gov, and the Cochrane database from each database's inception until June 15th, 2024. We included only English language studies. We included both controlled studies and observational studies. We excluded single-arm studies, meta-analyses, review articles, and studies that did not report any of our selected outcomes. We utilized the PRISMA guidelines in performing our study[15]. We searched in the online databases using this strategy: ("single port laparoscop*" OR "laparoendoscopic single-site surgery" OR "single-incision laparoscopic surgery" OR "single incision laparoscopic" OR "single site laparoscopy") AND ("ectopic pregnancy" OR "tubal pregnancy" OR "tubal ectopic pregnancy") till May 2023 to retrieve the relevant studies.

### Studies Selection and Eligibility Criteria

The selection of the included items involved two steps. Step one was the screening of titles and abstracts. Then, the selected relevant articles underwent a full-text screening according to the inclusion criteria of our study. We included studies that investigated the surgical outcomes of SILS compared with CLS in the surgical management of women with different types of ectopic pregnancies. We did not exclude studies based on the types of ectopic pregnancies they included or excluded, as long as the inclusion and exclusion groups were treated equally. Our main outcomes were the duration of postoperative hospitalization, total operative time, the surgeon estimated blood loss, hemoglobin change, the number of women and amount of blood transfusions needed, the patient's satisfaction, and return of bowel function.

### Data Extraction

We collected data from the included articles. We extracted the baseline information and the included studies' characteristics. Moreover, we extracted data on our selected outcomes including total operative time, length of hospital stay, the surgeon's estimated blood loss, hemoglobin change, the number of blood transfusions needed, the number of women in need of blood transfusions, and return of bowel function. For outcomes such as surgeon-estimated blood loss and return of bowel function, some studies provided indirect measures, such as ranges or averages, rather than exact values. In these cases, we used standardized methods to estimate mean values and standard deviations, ensuring consistency across studies for inclusion in the meta-analysis.

### Quality Assessment

Our study included both observational studies and randomized controlled trials. Thus, the risk of bias in observational articles was measured utilizing the ROBINS-I tool[16]. The Cochrane risk of bias tool was utilized to assess the randomized controlled trials[17].

### Statistical Methods

We extracted data for both dichotomous and continuous outcomes. OpenMetaAnalyst and Review Manager 5.4.1 software was used to analyze all the data retrieved. Regarding the dichotomous data, we used an odds ratio (OR) using the Mantel-Haenszel analysis method. For continuous outcomes, we used a mean difference (MD) under the inverse variance analysis method. A fixed effects analysis model was utilized if outcomes were homogeneous, while a random effects model was used if we observed heterogeneity. The heterogeneity was measured by the I2 and the p-value. Heterogeneity is identified if $p<0.1$, or $I^2>50\%$.

## Results

### Summary of Our Included Studies

Our search results in the online databases are presented in the PRISMA diagram, as seen in Figure 1. We included 12 studies in our analysis[18-29]. In total, these included 880 women experiencing ectopic pregnancies. Of these, 372 women underwent SILS, while 508 women underwent CLS for the surgical management of ectopic pregnancy. The characteristics of the included studies and included participants are illustrated in Table 1 and Table 2.

### Risk of Bias Assessment Results

According to the ROBINS-I risk of bias tool, the overall risk of bias in the observational studies was moderate. Table 3 shows all domains. Regarding the randomized studies, the risk of bias assessment was illustrated in Table 4[18].

### Analysis of Outcomes

### Total Operative Time (in Minutes)

When comparing SILS with CLS utilizing data retrieved from all included studies[18-29], our analysis of the prospective studies showed comparable total operative time in both procedures [MD=1.81 (-10.71, 14.33), (p=0.78), $I^2$=93%]. The retrospective subgroup analysis also showed similar total operative time in both procedures [MD=0.07 (-1.62, 1.76), p=0.94, $I^2$=93%]. The overall analysis of both subgroups showed a comparable total operative time in both procedures [MD=0.87 (-6.10, 7.84), p=0.81, $I^2$=93%], as seen in Figure 2.

### Surgeon Estimated Blood Loss (in mL)

We analyzed this outcome using data from four included studies[19,21,22,26]. Regarding the prospective subgroup, there was a significantly lower blood loss in the SILS group [MD=-62.29 (-99.56, -25.02), p=0.001, $I^2$=98%]. Nasu et al.[26] in the retrospective subgroup showed similar results in both procedures [MD=-51.01 (-86.18, -15.85), (p=0.004), $I^2$=98%]. However, the overall analysis showed a significantly decreased blood loss among patients in the SILS group [MD=-51.01 (-86.18, -15.85) (p=0.004), $I^2$=98%], as seen in Figure 3.

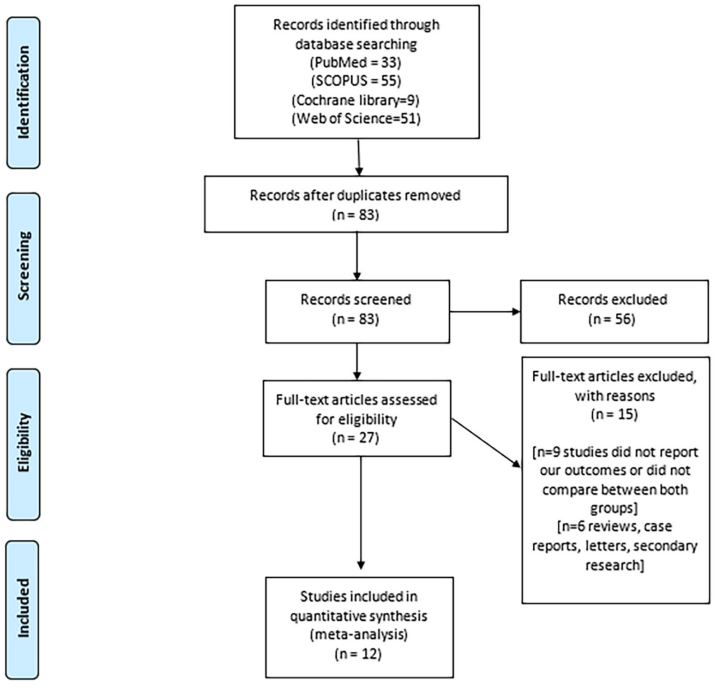


**Figure 1.** PRISMA workflow diagram illustrating our literature search

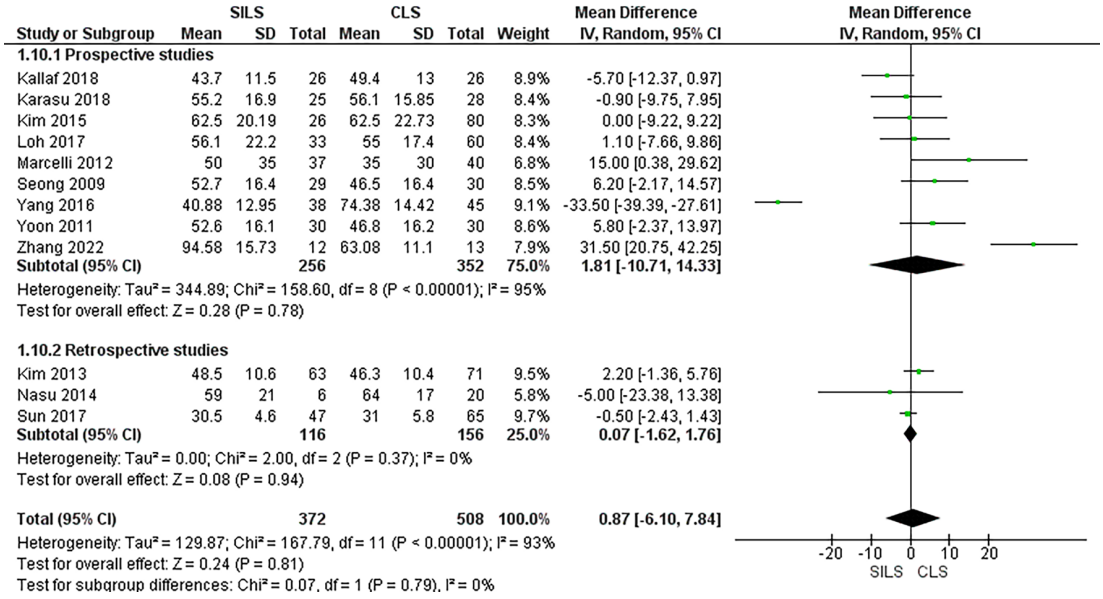


**Figure 2.** Meta-analysis of total operative time (in minutes)

*CI: Confidence interval, SD: Standard deviation, SILS: Single incision laparoscopic surgery, CLS: Conventional laparoscopic surgery*

**Table 1.** The characteristics of the included studies and demographic data of participants

| Study | Study design | Sample size | | Age, years | | BMI (kg/m²) | | Parity | |
|---|---|---|---|---|---|---|---|---|---|
| | | SILS | CLS | SILS | CLS | SILS | CLS | SILS | CLS |
| Kallaf[18] 2018 | Prospective randomized study | 26 | 26 | 24.3±3.6 | 24.7±3.5 | 28.6±3 | 28.2±3.5 | NR | NR |
| Karasu and Akselim[19] 2018 | Prospective case control study | 25 | 28 | 31.8±5.9 | 33.4±5.9 | 26.6±4.1 | 24.4±3.0 | 1.75±0.76 | 2.75±1.98 |
| Kim et al.[23] 2013 | Retrospective study | 63 | 71 | 31.2±5.2 | 30.4 ±5.0 | 21.0±2.1 | 21.3 ± 2.3 | | |
| Kim et al.[22] 2015 | Prospective observational case–control study | 26 | 80 | 30.7±4.8 | 30.25±5.16 | 20.61±1.86 | 22.5±2.9 | 0.75±0.75 | 1±0.82 |
| Loh et al.[24] 2017 | Prospective observational study | 33 | 60 | 30.0±5.95 | 31.0±5.65 | 23.8±2.84 | 25.2±2.08 | 1.06±1.46 | 0.77±0.87 |
| Marcelli et al.[25] 2012 | Prospective observational case-control | 37 | 40 | 29.3±3 | 28.7±2.8 | 23±4 | 24±4.5 | 1±1.1 | 1.2±1.5 |
| Nasu et al.[26] 2014 | Retrospective observational study | 6 | 20 | 29.3±6.2 | 31.2±5.4 | NR | NR | NR | NR |
| Seong et al.[27] 2009 | Prospective observational study | 29 | 30 | 31.1±5.3 | 32.6±4.9 | NR | NR | NR | NR |
| Sun et al.[28] 2017 | Retrospective cohort study | 47 | 65 | 35.3±5.9 | 36.9±6.0 | NR | NR | NR | NR |
| Yang et al.[29] 2016 | Prospective observational case-control study | 38 | 45 | 30±2.01 | 29.14±1.49 | 22.69±0.87 | 22.55±0.69 | 0.62±0.24 | 0.52±0.20 |
| Yoon et al.[20] 2011 | Prospective observational case-control study | 30 | 30 | 30.9±5.4 | 32.1±5.0 | 20.6±2.6 | 20.1±2.2 | 0.3±0.5 | 0.2±0.6 |
| Zhang and Zhu[21] 2022 | Retrospective observational study | 12 | 13 | 34.5±6.5 | 36.7±7.2 | 22.6±3.6 | 21.7±4.1 | NR | NR |

Data are presented as mean ± standard deviation.
SILS: Single incision laparoscopic surgery, CLS: Conventional laparoscopic surgery, BMI: Body mass index, NR: Not reported

**Table 2.** The characteristics of the included studies and demographic data of participants (continued)

| Study | Prior abdominal surgery | | Presence of hemoperitoneum | | Duration of amenorrhea (days) | | Gestational age (w) | | hCG level (mIU/mL) | | Size of ectopic mass (cm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SILS | CLS | SILS | CLS | SILS | CLS | SILS | CLS | SILS | CLS | SILS | CLS |
| Kallaf[18] 2018 | NR | NR | 12 (46.2) | 15 (57.7) | NR | NR | 7.1±1.1 | 6.8±1 | NR | NR | NR | NR |
| Karasu and Akselim[19] 2018 | 7 (28) | 5 (17.85) | 25 (100) | 28 (100) | NR | NR | 8.5±1.5 | 7±1 | 5685.6±5929.6 | 4894.9±3074.6 | 4.7±2.3 | 4.6±1.4 |
| Kim et al.[23] 2013 | 8 (12.7) | 11 (15.5) | NR | NR | 53.3±11.5 | 50.4 ± 10.3 | NR | NR | NR | NR | 4.0±0.9 | 3.8±0.8 |
| Kim et al.[22] 2015 | NR | NR | 18 (69.2) | 53 (74.6) | 40.25±10.34 | 52.75±15.9 | NR | NR | NR | NR | NR | NR |
| Loh et al.[24] 2017 | 11 (33.3) | 21 (35) | NR | NR | NR | NR | NR | NR | 9657.3±11734 | 11053.1±22350 | 3.48±1.53 | 3.86±1.16 |
| Marcelli et al.[25] 2012 | NR | NR | 28 (75.7) | 30 (75) | NR | NR | 7.4±3 | 7.3±3 | 3500±1100 | 3650±1200 | NR | NR |
| Nasu et al.[26] 2014 | NR | NR | 2 (33) | 1 (5) | NR | NR | 6.3±1.2 | 7.2 ± 1.3 | NR | NR | NR | NR |
| Seong et al.[27] 2009 | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR |
| Sun et al.[28] 2017 | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR |
| Yang et al.[29] 2016 | 6 (15) | 7 (14.5) | 38 (100) | 45 (100) | NR | NR | 7±0.46 | 7.75±0.68 | NR | NR | NR | NR |
| Yoon et al.[20] 2011 | 3 (10.0) | 7 (23.3) | 9 (30) | 11 (36.7) | 52.0±14.0 | 48.4±9.9 | NR | NR | 5442±7802 | 6921±10366 | 3.4±1.6 | 3.2±1.3 |
| Zhang and Zhu[21] 2022 | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR |

Data are presented as mean ± standard deviation or number (%).
SILS: Single incision laparoscopic surgery, CLS: Conventional laparoscopic surgery, BMI: Body mass index, NR: Not reported

**Table 3.** The risk of bias assessment of the included studies by ROBINS-I tool

| Study | Bias due to confounding | Selection bias | Bias in classification of interventions | Bias due to deviations from intended intervention | Bias due to missing data | Bias in measurement of outcomes | Bias in selection of reported result |
|---|---|---|---|---|---|---|---|
| Kallaf[18] 2018 | Moderate | Moderate | Low | Low | Low | Moderate | Moderate |
| Karasu and Akselim[19] 2018 | Low | Moderate | Low | Low | Low | Moderate | Low |
| Kim et al.[22] 2015 | Moderate | Moderate | Low | Low | Moderate | Moderate | Low |
| Loh et al.[24] 2017 | Low | Moderate | Low | Low | Low | Moderate | Low |
| Marcelli et al.[25] 2012 | Moderate | Low | Low | Moderate | Low | Moderate | Low |
| Nasu et al.[26] 2014 | Moderate | Low | Low | Low | Low | Moderate | Low |
| Seong et al.[27] 2009 | Low | Low | Low | Low | Low | Moderate | Low |
| Sun et al.[28] 2017 | Low | Low | Low | Moderate | Low | Moderate | Moderate |
| Yang et al.[29] 2016 | Moderate | Low | Low | Low | Low | Moderate | Low |
| Yoon et al.[20] 2011 | Moderate | Low | Low | Low | Low | Moderate | Low |
| Zhang and Zhu[21] 2022 | Low | Low | Low | Low | Low | Moderate | Low |

**Table 4.** The risk of bias assessment of the randomized controlled study by the Cochrane tool

| Study | Randomization | Allocation concealment | Blinding of participants and personnel | Blinding of outcome assessment | Attrition bias | Selective reporting |
|---|---|---|---|---|---|---|
| Kallaf[18] 2018 | Low | Low | High | High | Low | Low |

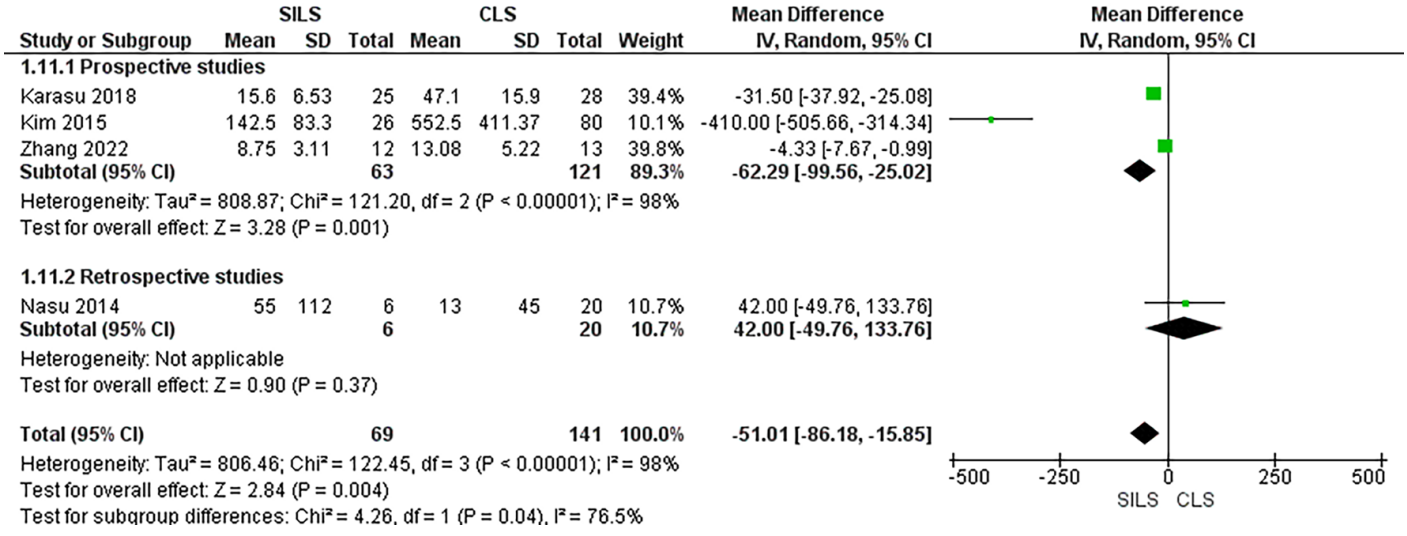


**Figure 3.** Meta-analysis of the surgeon declared estimated blood loss (in mL)

*CI: Confidence interval, SD: Standard deviation, SILS: Single incision laparoscopic surgery, CLS: Conventional laparoscopic surgery*

### Length of Postoperative Hospital Stay (in Days)

Most studies assessed this outcome[18-29]. The prospective subgroup analysis yielded a significantly decreased duration of hospital stay in the SILS group [MD=-0.30 (-0.51, -0.09) (p=0.005), $I^2$=76%]. The retrospective subgroup analysis demonstrated no difference between the two procedures [MD=-0.11 (-0.40, 0.17) p=0.43, $I^2$=76%]. The pooled analysis showed a significantly decreased duration of postoperative hospitalization among patients who underwent SILS compared to those who underwent CLS [MD=-0.24 (-0.39, -0.08) (p=0.003), $I^2$=76%], as shown in Figure 4.

### Hemoglobin Change (in g/L)

When comparing SILS with CLS, the prospective studies analysis demonstrated no significant difference in the hemoglobin change between the two techniques [MD=-0.08 (-0.22, 0.05)

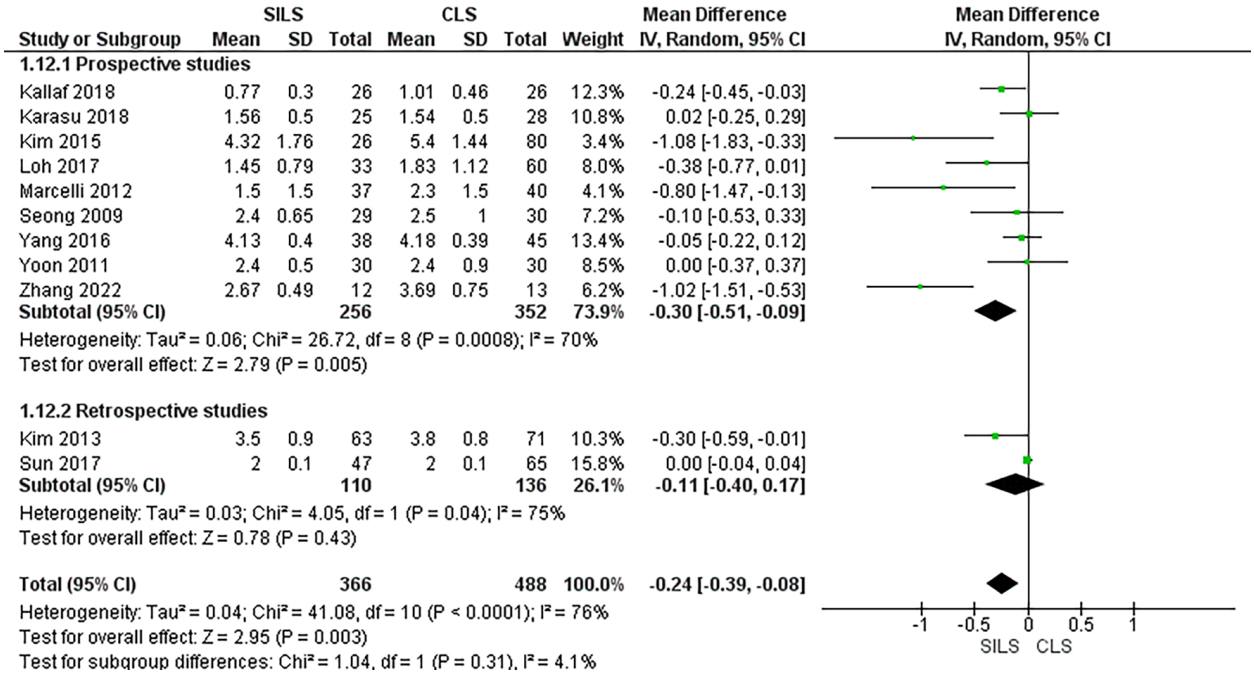


**Figure 4.** Meta-analysis of the length of the postoperative hospital stay (in days)

*CI: Confidence interval, SD: Standard deviation, SILS: Single incision laparoscopic surgery, CLS: Conventional laparoscopic surgery*

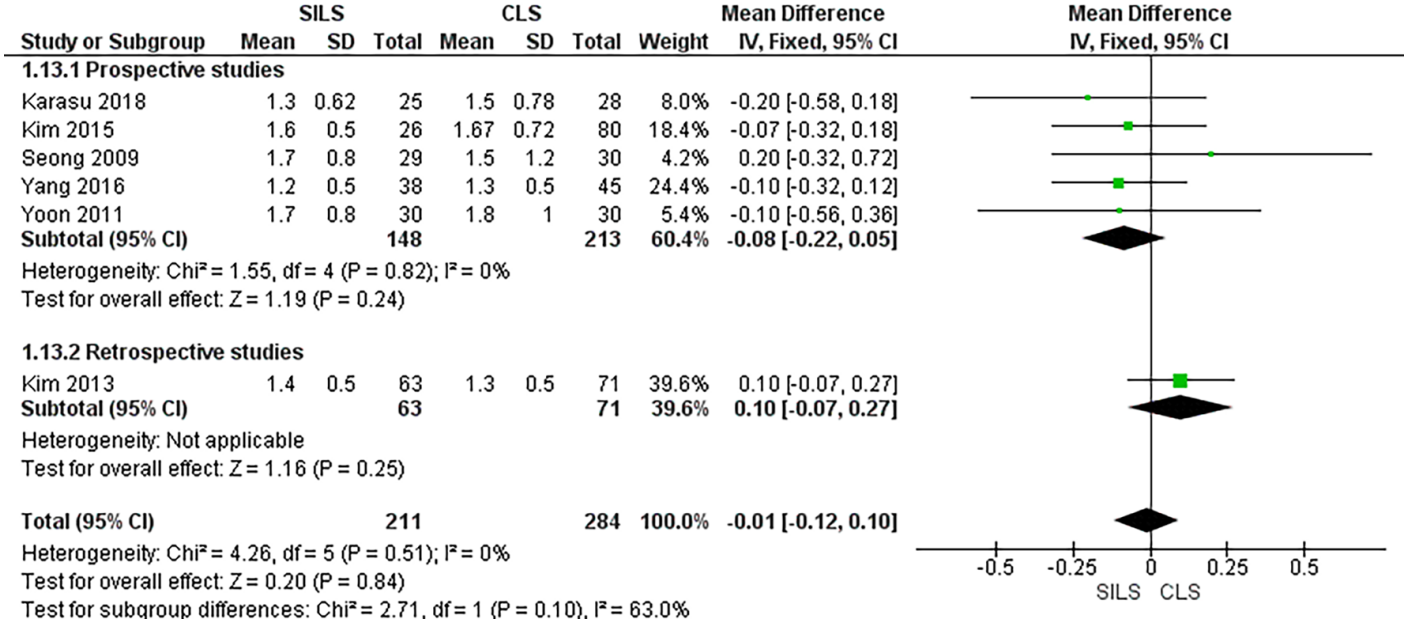


**Figure 5.** Meta-analysis of the change in hemoglobin levels (in g/L)

*CI: Confidence interval, SD: Standard deviation, SILS: Single incision laparoscopic surgery, CLS: Conventional laparoscopic surgery*

(p=0.24), $I^2$=0%]. The overall analysis from the six included studies also showed no differences between the two procedures [MD=-0.01 (-0.12, 0.10), p=0.84, $I^2$=0%], as seen in Figure 5.

### Amount of Blood Transfused (in Units)

We compared SILS and CLS, analyzing data from three studies that reported this outcome[18,23,29]. Both prospective and retrospective subgroups showed similar amounts of blood transfused in both groups [MD=-0.02 (-0.12, 0.07) (p=0.60), $I^2$=53%]. Pooled analysis showed no difference between the two procedures regarding the amount of blood needed [MD=-0.02 (-0.12, 0.07); p=0.60, $I^2$=53%], as seen in Figure 6.

### Number of Patients Requiring a Blood Transfusion

Eight studies reported this outcome[18-20,22-25,29]. The prospective studies subgroup showed a comparable number of patients who needed blood transfusions in both groups [OR=0.885 (0.524, 1.496) (p=0.649), $I^2$=0%]. Our overall analysis of prospective and retrospective subgroups showed a similar incidence of blood transfusion in both groups as well [OR=0.986 (0.635, 1.531), p=0.951, $I^2$=0%], as seen in Figure 7.

### Return of Bowel Function (in Hours)

The time needed for the bowel to return to normal function was reported in three studies[18,22,28]. The prospective subgroup analysis demonstrated similar results in both cohorts [MD=-1.29 (-2.62, 0.05) p=0.06, $I^2$=0%). However, the overall analysis

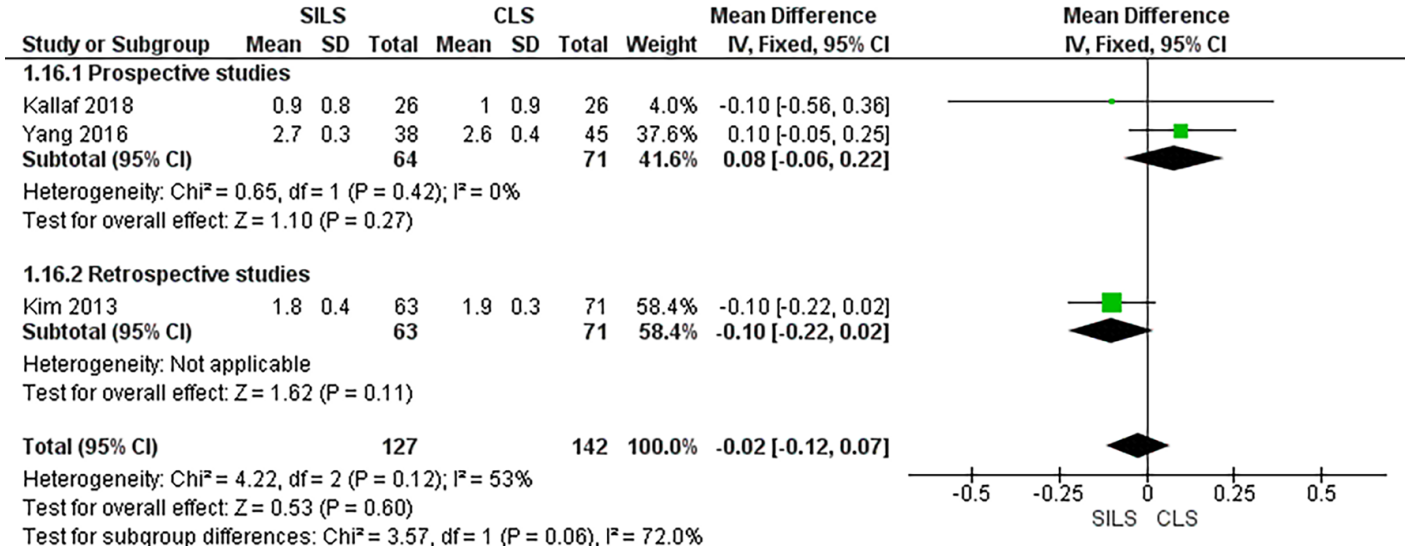


**Figure 6.** Meta-analysis of the number of units of blood transfused (in units)

*CI: Confidence interval, SD: Standard deviation, SILS: Single incision laparoscopic surgery, CLS: Conventional laparoscopic surgery*

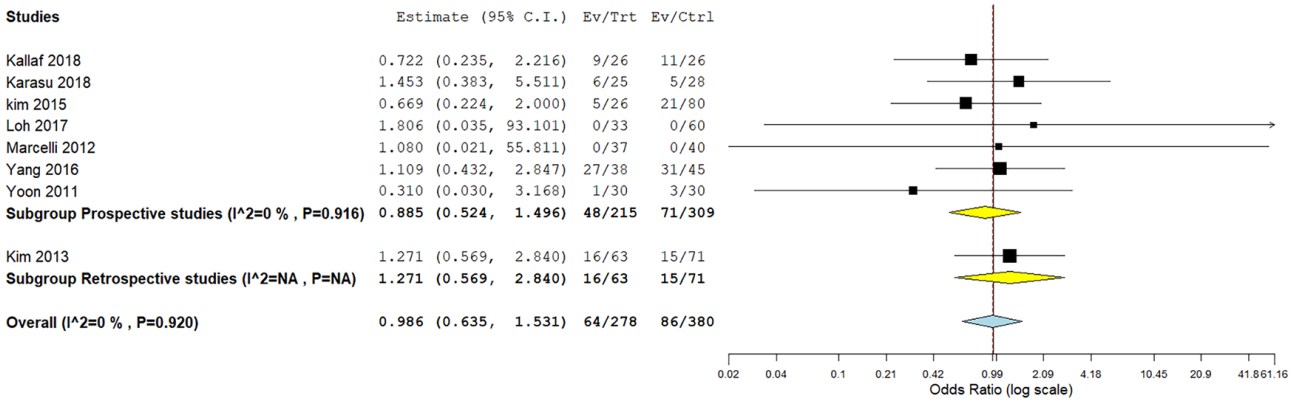


**Figure 7.** Meta-analysis of the number of patients requiring any blood transfusion

of both prospective and retrospective subgroups showed that patients who underwent the SILS operation required less time to return to normal bowel function [MD=-1.03 (-1.45, -0.61), p<0.01 $I^2$=0%]. Pooled analysis was homogeneous (p=0.78), $I^2$=0%, as seen in Figure 8.

### Patient Satisfaction Scores

As only two studies reported this outcome, a quantitative synthesis was not possible[18,24]. Kallaf[18] reported higher satisfaction rates in the SILS group versus the CLS group. A total of 46.2% of patients in the SILS group were very satisfied with the wound cosmesis, compared to 19.3% of patients in the CLS group. Loh et al.[24] assessed the satisfaction score after both procedures as well. The satisfaction score was slightly higher in the SILS group (8.5) compared to the CLS group (7.9), with no statistically significant difference.

## Discussion

In the last decade, great efforts have been made to enhance surgical techniques and improve patient care through advancing minimally invasive gynecologic surgery (MIGS). One important aspect of MIGS is minimizing the abdominal wall injury, caused by using multiple and larger trocars in conventional laparoscopy[30,31]. SILS is a type of surgical procedure in which surgeons use a single port through a small skin incision, usually at the umbilicus[32,33]. Since it was first described by Peters et al.[34] in the management of cholecystectomy, SILS has been utilized for various other indications including appendectomy[35], adrenalectomy[36], and ectopic pregnancy[37]. SILS has been recognized as a reliable alternative to CLS with the possible advantages of reduced abdominal wall trauma, less postoperative pain, less bleeding, quicker recovery, and improved cosmetic results, especially in young women in the reproductive age[38,39]. Unfortunately, there are limitations to this method as having only a single port for the instruments and camera may reduce the field of vision and impair depth perception[40]. In our meta-analysis, we compared the surgical results of SILS with CLS in the management of women with ectopic pregnancies. Our study demonstrated overall comparable surgical outcomes in both procedures. We found no significant differences between the two procedures regarding the total operative time, hemoglobin change, the number of blood transfusions needed or the percent of women who needed blood transfusions. However, SILS was associated with significantly lower surgeon-estimated blood loss, a shorter duration of postoperative hospitalization, and quicker return to normal bowel function. A notable gap in the literature is the lack of cost-effectiveness data comparing SILS and CLS for ectopic pregnancy management. While our analysis found that SILS is associated with a shorter postoperative hospital stay, which could potentially reduce healthcare costs, the initial investment in specialized single-port equipment may pose a financial barrier. Future studies should include formal cost-effectiveness analyses to better inform clinical decision-making and healthcare policy regarding the adoption of SILS. Optimizing patient selection for SILS is critical, particularly in stratifying outcomes by ectopic location and the presence of hemoperitoneum. Such stratification could identify subgroups of patients, such as those with tubal ectopic pregnancies or minimal hemoperitoneum, who may derive greater benefits from SILS, thereby guiding clinicians in tailoring surgical approaches to individual patient needs.

### Comparison with Existing Literature

In 2018, Gasparri et al.[13] conducted a meta-analysis of 460 women with ectopic pregnancy. This study reported no advantages of SILS over CLS in terms of surgeon-estimated

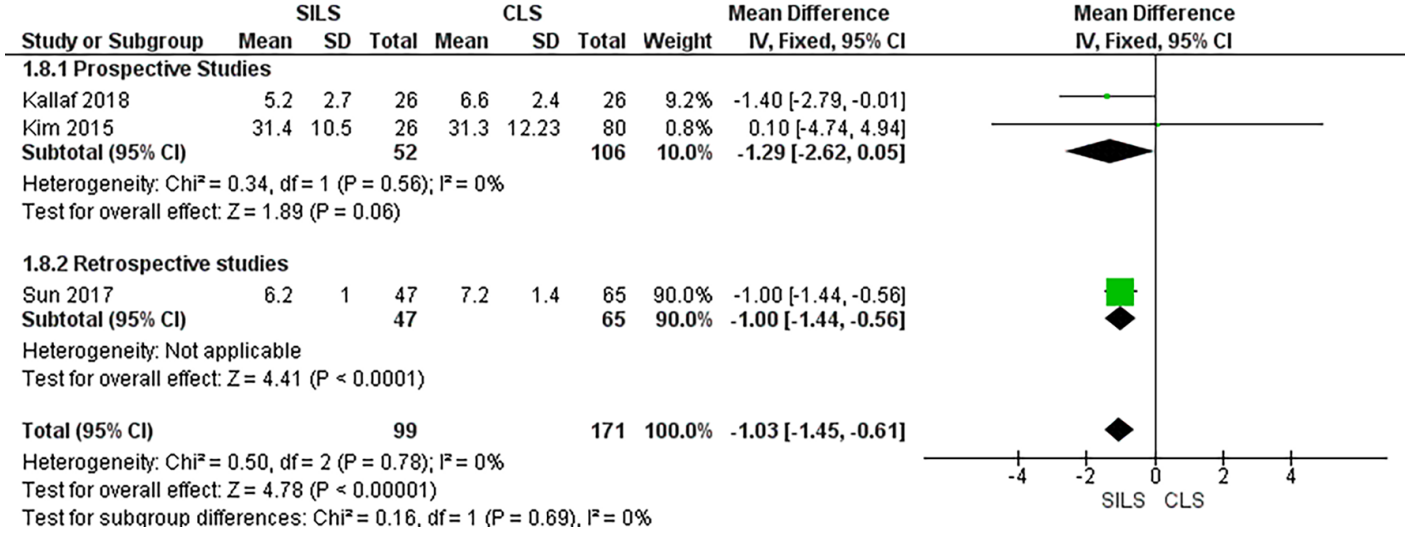


**Figure 8.** Meta-analysis of the amount of time until the return of bowel activity (in hours)

*CI: Confidence interval, SD: Standard deviation, SILS: Single incision laparoscopic surgery, CLS: Conventional laparoscopic surgery*

blood loss in both procedures. Another meta-analysis of 2,085 women evaluating the surgical outcomes of SILS compared to CLS in various gynecologic surgeries showed no considerable difference between the two procedures in the total operative time or the duration of postoperative hospitalization, which is in line with our findings[14]. Kallaf[18] conducted a randomized comparative study evaluating the intraoperative and immediate postoperative results of SILS compared to CLS for the management of ectopic pregnancy. They concluded that there is a significant advantage of the SILS procedure over CLS in short-term outcomes. Additionally, they found significantly better satisfaction with cosmetic in the SILS group. These results go hand in hand with Yang et al.[29], Karasu and Akselim[19], and Kim et al.[22], all of which superiority of SILS over CLS in managing women with tubal ectopic pregnancy even if complicated with massive hemoperitoneum. Contrary to these results, Loh et al.[24] reported no differences between the conventional laparoscopy and the SILS regarding the total operative time, length of hospital stay, and the satisfaction score. However, this study included only tubal ectopic pregnancies and excluded other types of ectopic pregnancies. In 2022, Zhang and Zhu[21] reported better satisfactory cosmetic results, reduced pain, decreased bleeding, and quicker recovery in patients who underwent SILS for ectopic pregnancy or leiomyoma. However, they also reported that SILS required a longer total operative time compared to CLS. They attributed this prolonged total operative time to the difficulty in the fixation of the multi-channel laparoscopic devices[21]. A previous meta-analysis of six randomized controlled trials comparing SILS with CLS in adnexal surgery showed no significant difference between both techniques in all surgical outcomes except the total operative time, which was longer in women who underwent SILS. This may be explained by the limited triangulation and frequent instrument collisions[41]. In another study, Sun et al.[28] retrospectively evaluated 112 patients with tubal pregnancies operated on by a single surgeon. They reported almost identical surgical results in both procedures. However, they found that patients who underwent SILS experienced quicker resumption of normal bowel function, which is consistent with our findings.

### Strengths

Our study is the largest meta-analysis to date to compare SILS and CLS head-to-head in the laparoscopic treatment of ectopic pregnancy. In addition, the quality of the included studies was found to be relatively high according to the grading scales.

### Limitations

Our main limitations include the relatively small sample size, the paucity of included randomized controlled trials, and the inclusion of studies with different study designs. As a result, we faced heterogeneity in some forest plots, which could not be solved by subgroup analysis. Another limitation is the lack of data on surgeon experience, which may influence outcomes such as operative time and complication rates due to the technical challenges of SILS, including instrument triangulation and visualization. Future studies should evaluate the impact of surgeon expertise and training on SILS outcomes to better understand its learning curve and broader applicability. The reliance on surgeon-estimated blood loss introduces potential bias due to its subjective nature. Future studies should adopt more objective and standardized methods, such as gravimetric or volumetric techniques, to measure blood loss, thereby improving the reliability and comparability of results. Additionally, we did not consider the experience of the surgeons in our analysis since this information was not reported in the studies. Another possible source of bias is the subjective definition of blood loss. Although we specifically defined our blood loss as "surgeon estimated", we cannot be certain in some studies that this did not include at least some preexisting hemoperitoneum, which could skew our results. Lastly, because of the limited number of ectopic pregnancies not in the ampullary portion of the fallopian tubes or with a hemoperitoneum, we were unable to perform a subgroup analysis for these events. As a result, these special circumstances could have influenced our findings.

## Conclusion

SILS is a reliable and effective alternative to CLS for the surgical management of ectopic pregnancy. Our meta-analysis demonstrates that SILS offers significant clinical benefits, including reduced blood loss and a shorter postoperative hospital stay, being approximately 0.24 days, which can enhance patient recovery and reduce healthcare costs. These advantages, combined with potential cosmetic benefits from a single umbilical incision, are particularly valuable for young women of reproductive age seeking minimal scarring. Additionally, the quicker return of bowel function with SILS may improve postoperative comfort. We recommend that clinicians consider SILS as a preferred approach when surgical expertise and resources are available, especially for uncomplicated ectopic pregnancies. Future research should focus on long-term outcomes, such as tubal patency and fertility, and stratify results by ectopic location and hemoperitoneum to optimize surgical techniques and patient selection.

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### Footnotes

### Authorship Contributions

Surgical and Medical Practices: G.J.M., A.A., Concept: G.J.M., Data Collection or Processing: D.H.G., B.H., M.R., E.K., S.M., N.P., G.J., Literature Search: A.A., Writing: G.J.M.

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

1. Gaskins AJ, Missmer SA, Rich-Edwards JW, Williams PL, Souter I, Chavarro JE. Demographic, lifestyle, and reproductive risk factors for ectopic pregnancy. Fertil Steril. 2018;110:1328-37.
2. Hendriks E, Rosenberg R, Prine L. Ectopic pregnancy: diagnosis and management. Am Fam Physician. 2020;101:599-606.
3. Mullany K, Minneci M, Monjazeb R, C Coiado O. Overview of ectopic pregnancy diagnosis, management, and innovation. Womens Health (Lond). 2023;19:17455057231160349.
4. Di Gennaro D, Damiani GR, Muzzupapa G, Stomati M, Cicinelli R, Gaetani M, et al. Ectopic pregnancy: an overview. Clin Exp Obstet Gynecol. 2022;49:262.
5. Ranchal S, Dunne C. Diagnosis and treatment of ectopic pregnancy. Br Columbia Med J. 2021;63:112-6.
6. Mann Mann LM, Kreisel K, Llata E, Hong J, Torrone EA. Trends in ectopic pregnancy diagnoses in United States emergency departments, 2006-2013. Matern Child Health J. 2020;24:213-21.
7. Hirschler LE, Soti V. The utility of monitoring beta-human chorionic gonadotropin levels in an ectopic pregnancy. Cureus. 2023;15:e34063.
8. Marchand GJ, Masoud AT, Coriell C, Ulibarri H, Parise J, Arroyo A, et al. Treatment of cesarean scar ectopic pregnancy in China with uterine artery embolization-a systematic review and meta-analysis. J Clin Med. 2022;11:7393.
9. Detrie C, Milord S, Marchand G, Sainz K, Azadi A, Thweat J. Design and implementation of an 11mm improvised laparoscopic system for performing a single-port laparoscopic hysterectomy through the smallest incision ever reported. Surg Technol Int. 2020;37:149-53.
10. Huang Y, Duan K, Koythong T, Patil NM, Fan D, Liu J, et al. Application of robotic single-site surgery with optional additional port for endometriosis: a single institution's experience. J Robot Surg. 2022;16:127-35.
11. Marchand G, Masoud AT, Galitsky A, Azadi A, Ware K, Vallejo J, et al. Management of interstitial pregnancy in the era of laparoscopy: a meta-analysis of 855 case studies compared with traditional techniques. Obstet Gynecol Sci. 2021;64:156-73.
12. Marchand GJ, Massoud A, Ulibarri H, Arroyo A, Blanco M, Herrera DG, et al. 11933 comparison of the surgical treatment of pediatric adnexal masses by gynecologic surgeons versus pediatric surgeons, a systematic review. J Minim Invasive Gynecol. 2024;31:S120.
13. Gasparri ML, Mueller MD, Taghavi K, Papadia A. Conventional versus single port laparoscopy for the surgical treatment of ectopic pregnancy: a meta-analysis. Gynecol Obstet Invest. 2018;83:329-37.
14. Marchand GJ. Single-port technology in gynecologic surgery. Single port gynecologic laparoscopic and robotic-assisted surgery [Internet]. London: IntechOpen; 2021. Chapter 1. Available from: https://www.intechopen.com/chapters/82734
15. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71.
16. Walpita YN, Nurmatov U. ROBINS-I: a novel and promising tool for risk of bias assessment of nonrandomized studies of interventions. J. Coll. Community Physicians Sri Lanka. 2020;26.
17. Bossuyt PM, Deeks JJ, Leeflang MM, Takwoingi Y, Flemyng E. Evaluating medical tests: introducing the Cochrane Handbook for Systematic Reviews of Diagnostic Test Accuracy. Cochrane Database Syst Rev. 2023;7:ED000163.
18. Kallaf H. Laparoscopic management of tubal ectopic pregnancy: single-port versus conventional multi-port laparoscopy. Int J Adv Res. 2018;6:856-63.
19. Karasu Y, Akselim B. Feasibility of single-incision laparoscopy for ruptured ectopic pregnancies with hemoperitoneum. Minim Invasive Ther Allied Technol [Internet]. 2018;28:46-50.
20. Yoon BS, Park H, Seong SJ, Park CT, Jun HS, Kim IH. Single-port versus conventional laparoscopic salpingectomy in tubal pregnancy: a comparison of surgical outcomes. Eur J Obstet Gynecol Reprod Biol. 2011;159:190-3.
21. Zhang Y, Zhu Y. Comparison of conventional versus single port laparoscopy for surgical treatment of gynecological diseases: a pilot study. Wideochir Inne Tech Maloinwazyjne. 2022;17:252-60.
22. Kim MK, Kim JJ, Choi JS, Eom JM, Lee JH. Prospective comparison of single port versus conventional laparoscopic surgery for ectopic pregnancy. J Obstet Gynaecol Res. 2015;41:590-5.
23. Kim YW, Park BJ, Kim TE, Ro DY. Single-port laparoscopic salpingectomy for surgical treatment of tubal pregnancy: comparison with multi-port laparoscopic salpingectomy. Int J Med Sci. 2013;10:1073-8.
24. Loh AZH, Torrizo MP, Ng YW. Single incision laparoscopic surgery for surgical treatment of tubal ectopic pregnancy: a feasible alternative to conventional laparoscopy. J Gynecol Surg. 2017;33:61-7.
25. Marcelli M, Lamourdedieu C, Lazard A, Cravello L, Gamerre M, Agostini A. Salpingectomy for ectopic pregnancy by transumbilical single-site laparoscopy with the SILS® system. Eur J Obstet Gynecol Reprod Biol [Internet]. 2012;162:67-70.
26. Nasu K, Yuge A, Nishida M, Kawano Y, Hirakawa T, Narahara H. Evaluation of postsurgical pain in single- port versus three-port laparoscopic surgery for ectopic pregnancy: a preliminary study. Gynecol Obstet [Internet]. 2014;4:1-3.
27. Seong SJ, Park H, Park CT, Yoon BS, Kim IH. Single port versus conventional laparoscopic salpingectomy for surgical treatment of tubal pregnancy. J Minim Invasive Gynecol [Internet]. 2009;16:S129.
28. Sun HD, Horng HC, Liu CH, Hsiao SM, Chen YJ, Chang WH, et al. Comparison of single-port and three-port laparoscopic salpingectomy in the management for tubal pregnancy. J Chinese Med Assoc [Internet]. 2017;81:469-74.
29. Yang J, Na YJ, Song YJ, Choi OH, Lee SK, Kim HG. The effectiveness of laparoendoscopic single-site surgery (LESS) compared with conventional laparoscopic surgery for ectopic pregnancy with hemoperitoneum. Taiwan J Obstet Gynecol [Internet]. 2016;55:35-9.
30. Stone R, Carey E, Fader AN, Fitzgerald J, Hammons L, Nensi A, et al. Enhanced recovery and surgical optimization protocol for minimally invasive gynecologic surgery: an AAGL white paper. J Minim Invasive Gynecol. 2021;28:179-203.
31. Chao L, Lin E, Kho K. Enhanced recovery after surgery in minimally invasive gynecologic surgery. Obstet Gynecol Clin North Am. 2022;49:381-95.

32. Wesevich V, Webster EM, Baxley SE. Overcoming challenges in minimally invasive gynecologic surgery. Gynecol Pelvic Med. 2020;3.
33. Antonilli M, Sevas V, Gasparri ML, Farooqi AA, Papadia A. Minimally invasive surgery in gynecology. Advances in Minimally Invasive Surgery. 2021:1-6.
34. Peters A, Siripong N, Wang L, Donnellan NM. Enhanced recovery after surgery outcomes in minimally invasive nonhysterectomy gynecologic procedures. Am J Obstet Gynecol. 2020;223:234-e1.
35. Gitas G, Hanker L, Rody A, Ackermann J, Alkatout I. Robotic surgery in gynecology: is the future already here? Minim Invasive Ther Allied Technol. 2022;31:815-24.
36. Arcieri M, Romeo P, Vizzielli G, Restaino S, Driul L, Stabile G, et al. Robotic single-port da vinci surgical system (SP1098) in gynecologic surgery: a systematic review of literature. Clin Exp Obstet Gynecol. 2023;50:158.
37. Li J, Wu T, Lin K. An innovative practice of single-port endoscopic surgery: abdominal wall endometrioma resection through the scar. J Minim Invasive Gynecol. 2022;29:700-1.
38. Bonollo M, Bellaminutti S, Gasparri ML, Papadia A. Is it time to leave multi-for single-port laparoscopy in benign gynecologic surgery? A systematic review of randomized clinical trials. Minerva Obstet Gynecol. 2021;74:434-43.
39. Misal M, Delara RM, Yi J, Magtibay PM. Tips and tricks for adapting to single port robotic surgery. J Minim Invasive Gynecol. 2020;27:S54.
40. Yu J, Peng Y, Yu L, Shi S. Laparoscopic surgery for ectopic pregnancy: a comparative study on the clinical benefits and impact on tubal patency and reproductive outcomes. Technol Health Care. 2024;29:2183-92.
41. Schmitt A, Crochet P, Knight S, Tourette C, Loundou A, Agostini A. Single-port laparoscopy vs conventional laparoscopy in benign adnexal diseases: a systematic review and meta-analysis. J Minim Invasive Gynecol [Internet]. 2017;24:1083-95.