Open Access

Research Article

Precis. Nanomed. 2025, 8(4):1645-1658

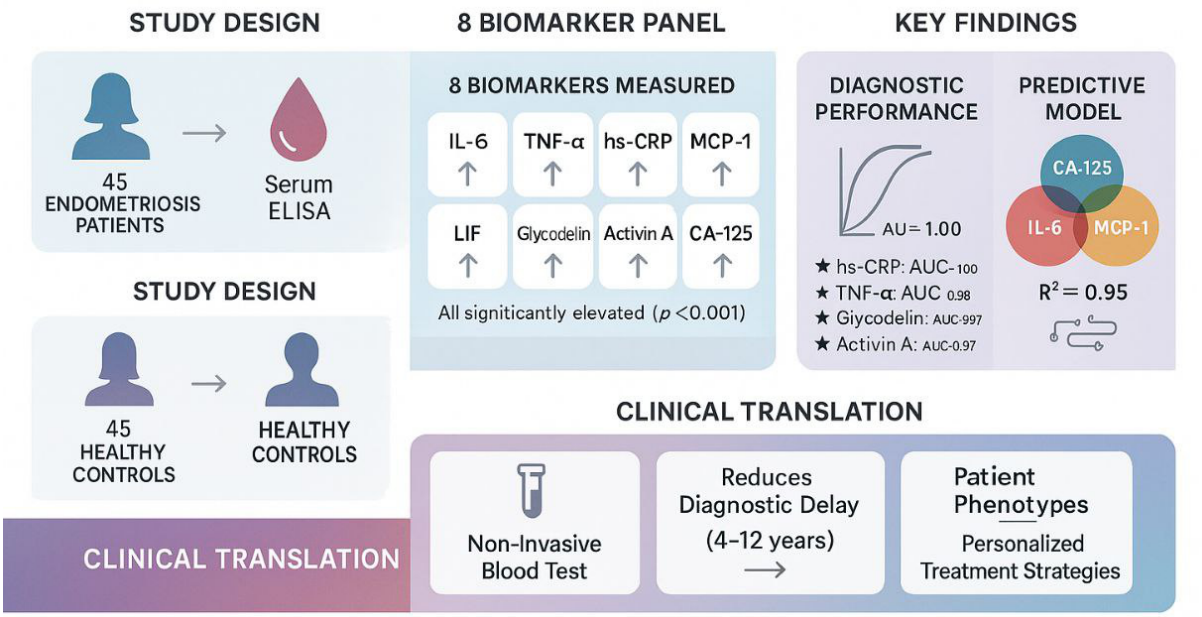
A Validated Serum Biomarker Signature for the Noninvasive Diagnosis of Endometriosis

Rusul F. Abedi[1], Ahmed Jasim Mohammed[2], Abbas Chyad[3], Redha Dawud Abd Alredha[4*], Ghanim A. Abbas[2], Krarr Haider Haddawi[5]

[1]*College of Health and Medical Techniques – Kufa, Al-Furat Al-Awsat Technical University (ATU), Iraq*
[2]*Department of Nursing Technology, Polytechnic College, Al-Qadisiyah, Al-Furat Al-Awsat Technical University, Iraq*
[3]*General Directorate of Education, Qadisiyah, Iraq*
[4]*Department of Prosthetics and Orthotics Technologies, Al-Furat Al-Awsat Technical University, Iraq*
[5]*Department of Clinical Biochemistry, College of Medicine, University of Al-Qadisiyah, Iraq*

Submitted: November 8, 2025 Accepted: December 1, 2025 Published: December 6, 2025

Graphical Abstract



Abstract

Endometriosis is a chronic inflammatory disorder affecting around 10% of reproductive-age women, and its diagnosis is often delayed due to the dependence on invasive laparoscopy. This study explored the potential of a serum-based biomarker panel for the noninvasive detection of endometriosis. Ninety premenopausal women (45 with surgically confirmed endometriosis and 45 controls) were enrolled, and serum levels of IL-6, TNF-α, hs-CRP, MCP-1, LIF, Glycodelin, Activin A, and CA-125 were measured using ELISA. Concentrations of all biomarkers were higher in the endometriosis group compared to controls ($p < 0.001$). Among them, hs-CRP, TNF-α, Glycodelin, and Activin A showed relatively high diagnostic performance (AUC values ranging from 0.93 to 1.00). Multivariate regression suggested that CA-125, IL-6, and MCP-1 may serve as independent predictors, and clustering analysis indicated possible subgroups with distinct inflammatory profiles. These findings suggest that a combination of selected serum biomarkers could support noninvasive diagnostic approaches for endometriosis and warrant further validation in larger, multicenter studies.

Keywords: Endometriosis; Diagnosis, Noninvasive; Biomarkers, Serum; C-Reactive Protein; Personalized Medicine.

* Corresponding author; redha.dawud.chm@atu.edu.iq

License: CC BY-NC 4.0

Purpose, Rationale, and Limitations

Endometriosis remains a major diagnostic challenge due to non-specific symptoms and reliance on invasive laparoscopy. This study aimed to identify a reliable serum biomarker panel that enables accurate, noninvasive diagnosis. By combining inflammatory and hormonal markers, we sought to develop a predictive model applicable to clinical settings. Limitations include the moderate sample size and single-center design, which warrant validation in larger multicenter cohorts.

Introduction

Endometriosis is a chronic, inflammatory disease defined by the presence of endometrial-like tissue outside the uterus, which affects an estimated 10% of reproductive-age women and girls globally, totaling approximately 190 million individuals [1, 2]. The condition imposes a substantial burden on both individuals and healthcare systems, characterized by debilitating symptoms such as chronic pelvic pain, severe dysmenorrhea, dyspareunia, and infertility[1, 3]. The profound impact of endometriosis extends beyond physical symptoms, significantly impairing quality of life by affecting mental health, social relationships, and economic productivity[4, 5]. Recent analyses of the global burden of disease highlight a consistent increase in the prevalence and impact of endometriosis, underscoring its status as a major, yet often overlooked, global health challenge[6, 7].

A primary obstacle in the management of endometriosis is the significant delay in diagnosis, which currently averages between 4 and 12 years from symptom onset, exacerbating the physical and psychological suffering of patients but also contributing to disease progression and increasing the overall economic burden[11, 12].

The pathophysiology of endometriosis is complex and multifactorial, characterized by significant immune system dysregulation and a chronic inflammatory state [13]. The local and systemic inflammatory environment is characterized by elevated levels of pro-inflammatory cytokines, such as Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α), which play crucial roles in the survival, proliferation, and invasion of ectopic endometrial cells[14-16]. This well-established inflammatory signature provides a strong rationale for investigating these molecules as potential biomarkers. The search for a noninvasive diagnostic test has intensified, with recent research exploring a wide array of candidates in blood, saliva, and menstrual fluid, including proteomic profiles, genetic markers, and inflammatory mediators[17-19]. The development of a blood-based biomarker panel capable of accurately detecting endometriosis would represent a paradigm shift in the clinical management of the disease.

Despite extensive research, no single biomarker or panel has yet achieved the required sensitivity and specificity for routine clinical use, and many promising candidates have failed in larger validation studies [17, 20]. A key gap in the current literature is the lack of comprehensive studies that simultaneously evaluate a broad panel of markers across different pathophysiological pathways, including inflammation, hormonal dysregulation, and tissue remodeling, and correlate these markers with clinical characteristics and disease heterogeneity. Therefore, this study aimed to address this gap by performing a comprehensive evaluation of a panel of eight serum biomarkers (IL-6, TNF-α, hs-CRP, MCP-1, LIF, Glycodelin, Activin A, and CA-125) in a cohort of women with and without surgically confirmed endometriosis. The primary objectives were to: (1) determine the diagnostic accuracy of each biomarker, (2) develop a multivariate predictive model for noninvasive diagnosis, and (3) explore the existence of distinct patient phenotypes through biomarker-based cluster analysis. By doing so, we sought to identify a robust, reliable biomarker signature that could serve as the basis for a clinically applicable, noninvasive diagnostic test for endometriosis.

Materials and Methods

Study Design and Ethical Approval

This case-control study was conducted at the Department of Obstetrics and Gynecology at Al-Sadr Teaching Hospital in Najaf, Iraq. The study protocol was approved by the Institutional Review Board (IRB) of the College of Medicine, University of Kufa (Reference No. UOK/MED/IRB/2024-01). All procedures were performed in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments. Written

License: CC BY-NC 4.0

informed consent was obtained from all individual participants included in the study.

Study Participants and Clinical Data Collection

A total of 90 premenopausal women aged 18-45 years were enrolled between February 10, 2024, and March 15, 2025. The study population consisted of two groups:

Case Group (n=45): Patients with a definitive diagnosis of endometriosis confirmed by both laparoscopy and subsequent histopathological examination of excised lesions.

Control Group (n=45): Healthy women with a laparoscopically normal pelvis, who were undergoing gynecological procedures for benign, non-endometriotic conditions (e.g., tubal ligation, benign ovarian cysts other than endometriomas).

Inclusion criteria for the case group were the presence of visible endometriotic lesions at laparoscopy and a positive histology report.

Exclusion criteria for both groups were: pregnancy or lactation, current or recent (within 3 months) use of hormonal treatments or gonadotropin-releasing hormone (GnRH) agonists/antagonists, presence of any acute or chronic inflammatory diseases (e.g., autoimmune disorders, pelvic inflammatory disease), known malignancy, or any other systemic disease that could influence the levels of inflammatory markers.

A standardized questionnaire was used to collect detailed medical history and demographic data from all participants. This included age, parity, gravidity, and menstrual cycle characteristics (regularity and length). Body Mass Index (BMI) was calculated as weight (kg) divided by height (m)^2. Pelvic pain was quantified using a 10-point Visual Analog Scale (VAS), where 0 indicated no pain, and 10 indicated the worst imaginable pain. The presence of ovarian cysts (endometriomas) was determined by transvaginal ultrasonography before surgery and confirmed during laparoscopy. Physical activity was self-reported and categorized as low, moderate, or high based on the International Physical Activity Questionnaire (IPAQ) short form.

Sample Size and Power Analysis

The sample size of 45 cases and 45 controls was determined a priori using G*Power (version 3.1.9.7) to achieve 80% power. Based on preliminary data and published literature for similar biomarker studies in endometriosis, we assumed a large effect size (Cohen's d = 0.8) for the primary outcome (difference in biomarker levels between groups). With an alpha level of 0.05 and a desired statistical power of 80% (1-β = 0.80), the minimum required sample size was calculated to be 42 participants per group. Consequently, enrolling 45 participants per group ensures adequate statistical power to detect a significant difference in the mean concentrations of the selected biomarkers, thereby validating the relevance and statistical significance of the chosen sample size.

Laparoscopic Diagnosis and Staging

All participants underwent a standard diagnostic laparoscopy under general anesthesia. The pelvic cavity was systematically inspected for the presence, location, and extent of endometriotic lesions. The disease was staged according to the revised American Society for Reproductive Medicine (rASRM) classification system (1997), which assigns scores based on lesion size, location, and depth of invasion. Biopsies were taken from suspected lesions for histopathological confirmation.

Sample Collection and Biochemical Analysis

Venous blood samples (5 mL) were collected from all participants in the follicular phase of their menstrual cycle after an overnight fast. The blood was drawn into serum separator tubes. The tubes were allowed to clot at room temperature for 30 minutes before being centrifuged at 3000 rpm for 15 minutes. The resulting serum was carefully aspirated, aliquoted into sterile cryovials to avoid repeated freeze-thaw cycles, and stored at −80°C until analysis. All samples were processed within two hours of collection.

Serum concentrations of all eight biomarkers were determined using commercially available ELISA kits according to the manufacturers' instructions. All assays were performed in duplicate in the central laboratory of Al-Sadr Teaching Hospital. The absorbance was measured at 450 nm using a microplate ELISA reader (Bio-Rad iMark™ Microplate Absorbance Reader). A four-parameter logistic curve fit was used to generate the standard curve for each assay, and the concentrations of the biomarkers in the samples were calculated from it.

The specific ELISA kits used for each biomarker are detailed in Table 1.

License: CC BY-NC 4.0

Table 1: Details of ELISA Kits Used for Biomarker Quantification

Biomarker	Manufacturer	Catalog Number	Sensitivity	Range
IL-6	R&D Systems	HS600C	0.2 pg/mL	0.2-10 pg/mL
TNF-α	R&D Systems	DTA00D	5.5 pg/mL	15.6-1000 pg/mL
hs-CRP	Abcam	ab260058	5.36 pg/mL	18.75-1200 pg/mL
MCP-1	R&D Systems	DCP00	5.0 pg/mL	15.6-1000 pg/mL
LIF	R&D Systems	DLF00B	7.8 pg/mL	31.2-2000 pg/mL
Glycodelin	Novus Biologicals	NBP2-82133	2.81 ng/mL	4.69-300 ng/mL
Activin A	R&D Systems	DAC00B	12 pg/mL	31.2-2000 pg/mL
CA-125	R&D Systems	DCA125	5.0 U/mL	15.6-1000 U/mL

Statistical Analysis

GraphPad Prism version 9 and MedCalc software were used for the statistical analyses. Continuous data were presented as mean ± SD, and categorical data as frequency (percentage). Between-group comparisons were made using independent-samples t-tests or chi-square tests, as applicable. The diagnostic efficacy of biomarkers was analyzed using the area under the receiver operating characteristic curve (AUC), and their optimal cut-off values, sensitivities, specificities, and predictive values were calculated. Multivariable logistic regression was used to identify independent predictors of endometriosis. Unsupervised K-means clustering of the biomarker profiles was used to identify patient subgroups, with between-cluster differences evaluated using one-way ANOVA and post hoc Tukey's test. Effect sizes for biomarkers were calculated as Cohen's d; all comparisons with $P < 0.05$ were considered statistically significant.

RESULTS

The baseline demographic, clinical, and biomarker features of the 45 patients and controls are shown in Table 2a, which presents 45 pairs (endometriosis cases vs. matched controls). The patients and controls were similar in age, BMI, parity, gravidity, menstrual regularity and physical activity levels (all $p > 0.05$), but women with endometriosis had significantly shorter menstrual cycle length (25.6 ± 1.0 vs. 28.5 ± 4.5 days; $p < 0.001$), higher VAS scores for the pain (6.8 ± 1.5 vs. 1 ± 0.6; $p < 0.001$) and developed ovarian cysts definitely (58% vs.– %; data not shown). In addition, all defined biomarkers (IL-6, TNF-α, Glycodelin, Activin A, MCP-1, LIF, and CA-125, as well as hs-CRP) were significantly higher in the endometriosis group ($p< 0.001$ to $p=0.01$).

Table 2b provides a focused analysis within the endometriosis cohort, examining the influence of menstrual cycle regularity on biomarker expression. The endometriosis patients were stratified into two subgroups: those with regular cycles (n=22) and those with irregular cycles (n=23). The table compares the means and standard deviations of eight specific biomarkers (IL-6, TNF-α, Glycodelin, Activin A, MCP-1, LIF, CA-125, and hs-CRP) between the two subgroups. The statistical significance of the differences was assessed using an independent samples t-test, and the resulting p-values are displayed. An asterisk is used to denote statistically significant associations ($p < 0.05$).

License: CC BY-NC 4.0

Table 2a: Baseline Characteristics and Biomarker Levels in Endometriosis and Control Groups

Variable	Endo Group (n=45)	Control Group (n=45)	p-value
Demographic and Clinical			
Age (years)	32.4 ± 1.2	30.7 ± 6.0	0.08
BMI (kg/m²)	21.3 ± 0.8	21.8 ± 3.5	0.15
Menstrual cycle regularity			
Irregular	27 (54%)	22 (44%)	0.30
Regular	23 (46%)	28 (56%)	
Cycle length (days)	25.6 ± 1.0	28.5 ± 4.5	<0.001
VAS score	6.8 ± 1.5	1.0 ± 0.6	<0.001
Parity	1.8 ± 1.4	2.2 ± 1.4	0.10
Gravidity	2.2 ± 1.6	2.6 ± 1.5	0.15
Ovarian cysts presence			
Yes	29 (58%)	0 (0%)	<0.001
No	21 (42%)	50 (100%)	
Physical activity			
Low	8 (16%)	12 (24%)	0.25
Moderate	21 (42%)	19 (38%)	
High	21 (42%)	19 (38%)	
Biomarkers			
IL-6 (pg/mL)	30.4 ± 10.5	18.7 ± 8.5	<0.001
TNF-α (pg/mL)	132.3 ± 38.3	22.6 ± 4.3	<0.001
Glycodelin (ng/mL)	239.5 ± 65.7	29.1 ± 11.9	<0.001
Activin A (pg/mL)	356.5 ± 79.1	157.5 ± 29.3	<0.001
MCP-1 (pg/mL)	484.3 ± 75.0	347 ± 51.7	<0.001
LIF (pg/mL)	38.5 ± 6.6	30.8 ± 5.6	0.01
CA-125 (U/mL)	80.0 ± 51.0	18.1 ± 7.8	<0.001
hs-CRP (pg/mL)	604.3 ± 198.1	122 ± 62.3	<0.001

Data presented as Mean ± SD or n (%). P-values calculated using an independent samples t-test. Statistically significant ($p < 0.05$).

The diagnostic performance of eight important biomarkers for endometriosis is shown in Table 3 using ROC curve analysis. The results indicate that several biomarkers have relatively high discriminative power, including TNF-α (AUC = 0.98), Glycodelin (AUC = 0.97), Activin A (AUC = 0.97), and hs-CRP (AUC = 1.00), all of which have excellent sensitivity (93.1% to 100%) and specificity (94% to 100%). By contrast, IL-6 and LIF have somewhat lower diagnostic utility (AUCs of 0.73 and 0.65, respectively). The table also lists the best cut-off values obtained from ROC curves, along with performance measures such as Positive Predictive Value (PPV) and Negative Predictive Value (NPV), sensitivity/specificity, and overall accuracy, for a panel of potential biomarkers for optimal noninvasive prediction of endometriosis.

License: CC BY-NC 4.0

Table 2b: Biomarker Levels by Cycle Regularity (Endometriosis Group Only)

Biomarker	Regular Endo (n=22)	Irregular Endo (n=23)	p-value
IL-6	29.1 ± 11.7	31.6 ± 9.3	0.433
TNF-α	138.4 ± 39.2	126.5 ± 37.4	0.305
Glycodelin	262.0 ± 57.4	218.0 ± 67.0	0.023*
Activin A	366.8 ± 70.8	346.6 ± 86.8	0.399
MCP-1	494.4 ± 69.0	474.6 ± 80.7	0.383
LIF	36.5 ± 6.1	40.5 ± 6.7	0.044*
CA-125	85.4 ± 53.3	74.8 ± 49.3	0.491
hs-CRP	605.5 ± 196.1	603.2 ± 204.3	0.969

Data presented as Mean ± SD. P-values were calculated using an independent samples t-test. An asterisk (*) indicates a statistically significant association ($p < 0.05$).

Table 3: Diagnostic Performance of Key Biomarkers for Endometriosis

Biomarker	*AUC95% CI*	*Optimal Cut-off*	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*	*NPV (%)*	*Accuracy (%)*
IL-6	0.73(0.63-0.82)	>26.29	73.3	68.0	68.8	72.3	70.7
TNF-α	0.98 (0.97-1.00)	>28.69	97.8	94.0	93.6	97.9	95.9
Glycodelin	0.97(0.95-0.99)	>49.04	95.6	94.0	93.5	95.9	94.8
Activin A	0.97(0.93-0.99)	>198.8	93.1	94.0	92.1	91.8	93.6
MCP-1	0.91(0.86-0.96)	>413.53	84.4	88.0	86.4	86.3	86.2
LIF	0.65(0.54-0.75)	>44.42	62.2	66.0	63.6	64.7	64.1
CA-125	0.90(0.83-0.95)	>37.75	77	98	99.1	81	75.7
hs-CRP	1.00(0.98-1.00)	>248.7	100	100	100	100	1.00

AUC: Area Under the ROC Curve; CI: Confidence Interval; PPV: Positive Predictive Value; NPV: Negative Predictive Value.

Table 5 presents a comparative analysis of biomarker concentrations across the different stages of endometriosis, as defined by the ASRM classification system. The study population was categorized into four stages: Stage I (n=7), Stage II (n=16), Stage III (n=13), and Stage IV (n=9). The table shows the Mean ± standard deviation for eight biomarkers at each stage. A one-way analysis of variance was employed to detect statistically significant differences in biomarker levels across the stages.

License: CC BY-NC 4.0

Table 4: Correlation Matrix of Biomarkers and Clinical Variables in the Endometriosis Group

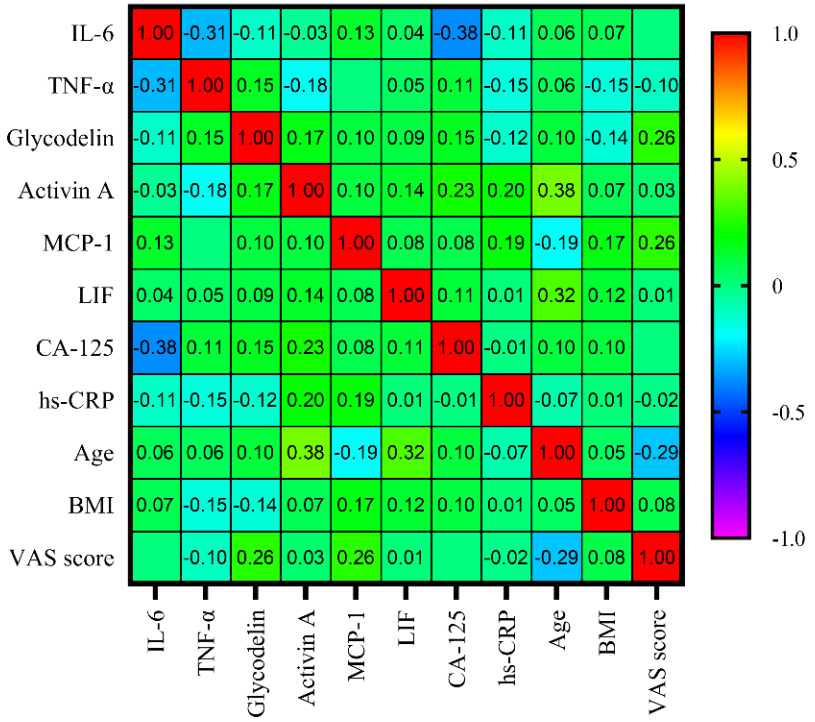


Table 5: Comparison of Biomarker Levels Across Endometriosis Stages (ASRM Classification)

Biomarker	**Stage I No.(7)**	**Stage II No.(16)**	**Stage III No.(13)**	**Stage IV No.(9)**	**p-value**
IL-6 (pg/mL)	26.2 ± 8.5^{A}	28.5 ± 9.2^{A}	32.1 ± 10.5^{B}	35.8 ± 11.2^{B}	0.042*
TNF-α (pg/mL)	115.3 ± 32.4	128.7 ± 38.6	138.9 ± 42.1	152.4 ± 45.8	0.089
Glycodelin (ng/mL)	185.6 ± 68.3	198.4 ± 75.2	212.8 ± 82.6	228.5 ± 89.4	0.156
Activin A (pg/mL)	325.8 ± 85.2	348.6 ± 92.5	368.4 ± 98.7	395.2 ± 105.3	0.123
MCP-1 (pg/mL)	465.3 ± 82.6	488.7 ± 89.4	512.5 ± 95.8	538.9 ± 102.3	0.078
LIF (pg/mL)	37.2 ± 6.8	38.9 ± 7.2	40.5 ± 7.6	42.8 ± 8.1	0.234
CA-125 (U/mL)	52.4 ± 35.2^{A}	68.7 ± 42.6^{A}	92.5 ± 51.3^{B}	125.8 ± 62.4^{C}	0.008*
hs-CRP (pg/mL)	485.6 ± 165.3^{A}	562.8 ± 185.7^{B}	658.4 ± 208.5^{C}	752.6 ± 235.8^{D}	0.015*

Data presented as Mean ± SD or n (%). χ^2 p-value from Chi-squared test. ^{A} p-value from one-way ANOVA. Superscript letters (A, B, C, D) indicate homogenous subsets from Tukey's post-hoc test; different letters denote a significant difference. *An asterisk (*) indicates a statistically significant association ($p < 0.05$).*

Table 6 presents a subgroup analysis comparing serum biomarker levels between endometriosis patients with (n=24) and without (n=21) ovarian cysts, revealing no statistically significant differences across all eight biomarkers assessed, including IL-6, TNF-α, Glycodelin, Activin A, MCP-1, LIF, CA-125, and hs-CRP (all $p > 0.05$). Although mean levels of IL-6 and hs-

License: CC BY-NC 4.0

CRP were moderately elevated in the cyst-bearing subgroup, and CA-125 was higher in patients without cysts, these differences did not reach statistical significance, suggesting that ovarian cysts may not substantially alter the systemic inflammatory and glycoprotein biomarker profile in this endometriosis cohort.

Table 6: Subgroup Analysis Endometriosis Patients with vs. Without Ovarian Cysts

Biomarker	With Cysts (n=24)	Without Cysts (n=21)	Mean Difference	p-value
IL-6 (pg/mL)	32.8 ± 9.3	27.1 ± 11.5	+5.7	0.075
TNF-α (pg/mL)	126.1 ± 33.5	140.8 ± 43.6	-14.7	0.207
Glycodelin (ng/mL)	237.7 ± 64.0	242.0 ± 69.5	-4.3	0.832
Activin A (pg/mL)	348.5 ± 73.7	367.4 ± 86.9	-18.9	0.436
MCP-1 (pg/mL)	480.1 ± 77.6	490.0 ± 73.0	-9.9	0.667
LIF (pg/mL)	38.0 ± 6.5	39.2 ± 6.9	-1.2	0.580
CA-125 (U/mL)	70.5 ± 48.2	93.0 ± 53.0	-22.5	0.146
hs-CRP (pg/mL)	570.9 ± 221.1	650.1 ± 155.4	-79.2	0.188

Data presented as Mean ± SD. P-values were calculated using an independent samples t-test. An asterisk (*) indicates a statistically significant association ($p < 0.05$).

This study used K-means clustering to identify distinct phenotypic subgroups within a cohort of 45 endometriosis patients, based on profiles of eight biomarkers (IL-6, TNF-α, Glycodelin, Activin A, MCP-1, LIF, CA-125, hs-CRP), as shown in Table 7. After data standardization, the algorithm divided patients into three clusters, later described as a "High Hormonal Dysregulation" phenotype (n=9), a "TNF-α-Driven Inflammatory" phenotype (n=17), and a "Moderate Inflammatory Burden" phenotype (n=19). The statistical validity of these clusters was confirmed by ANOVA, which showed significant differences in all biomarkers among the groups ($p < 0.001$).

Table 7: Cluster Analysis of Endometriosis Patients Based on Biomarker Profiles

Biomarker	Cluster 1 (n=9)	Cluster 2 (n=17)	Cluster 3 (n=19)	p-value (ANOVA)
IL-6 (pg/mL)	26.2 ± 8.1A	28.2 ± 9.5A	34.4 ± 10.2B	<0.001*
TNF-α (pg/mL)	102.2 ± 28.4A	169.1 ± 35.2B	113.6 ± 31.8A	<0.001*
Glycodelin (ng/mL)	262.5 ± 58.3A	282.4 ± 62.1A	190.3 ± 48.7B	<0.001*
Activin A (pg/mL)	442.1 ± 78.9A	359.6 ± 71.2B	313.1 ± 65.4C	<0.001*
MCP-1 (pg/mL)	516.8 ± 82.3A	485.3 ± 75.8B	468.0 ± 69.2B	<0.001*
LIF (pg/mL)	44.3 ± 6.8A	39.1 ± 6.2A	35.3 ± 5.9C	<0.001*
CA-125 (U/mL)	125.9 ± 54.2A	74.5 ± 46.8B	63.2 ± 42.1B	<0.001*
hs-CRP (pg/mL)	671.5 ± 198.4A	592.6 ± 176.3B	583.0 ± 165.7B	<0.001*

Data presented as Mean ± SD or n (%). χ^2 p-value from Chi-squared test. A p-value from one-way ANOVA. Superscript letters (A, B, C) indicate homogenous subsets from Tukey's post-hoc test; different letters denote a significant difference. An asterisk (*) indicates a statistically significant association ($p < 0.05$).

Table 8 presents the results of a multivariate logistic regression analysis performed to identify significant independent predictors of endometriosis. The final model, which was highly significant (Model χ^2 $p < 0.001$) and explained 95% of the variance (Nagelkerke $R^2 = 0.95$), identified three biomarkers as significant predictors: elevated levels of CA-125 (OR = 1.18, 95% CI 1.056-1.328, p = 0.0035), IL-6 (OR = 1.35, 95% CI 1.055-1.751, p = 0.017), and MCP-1 (OR = 1.039, 95% CI 1.083-1.07, p = 0.0125).

License: CC BY-NC 4.0

Table 8: Multivariate Logistic Regression for Predicting Endometriosis

Variables	B (coef)	Wald	Odds Ratio	95% CI for Odds Ratio	*P*. Value
CA-125	0.17	8.52	1.18	1.056 to 1.328	0.0035**
TNF-α	Variable not included in the model				
IL-6	0.31	5.6	1.35	1.055 to 1.751	0.017*
Activin A	Variable not included in the model				
MCP-1	0.038	6.2	1.039	1.083 to 1.07	0.0125**
Glycodelin	Variable not included in the model				
Constant	-28.73	7.58	-	-	0.0059**

B (coef): Regression Coefficient; CI: Confidence Interval. Model $\chi^2 = 0.135$, $p < 0.001$, Nagelkerke $R^2 = 0.95$.

As shown in Table 9, his analysis quantified the magnitude of difference in biomarker levels between endometriosis patients and controls using Cohen's d and fold change, thereby evaluating their practical and clinical significance beyond mere statistical inference. The results classified the effect sizes, revealing that the majority of biomarkers, including TNF-α (d=3.46), Glycodelin (d=2.83), and hs-CRP (d=2.57), exhibited large to extremely large effects ($d \geq 0.8$), with substantial fold changes reinforcing their dramatic elevation in the patient group. This classification highlights biomarkers with the greatest potential discriminatory power for diagnostic applications and provides a hierarchy of their relative clinical importance.

Table 9: Effect Size Analysis (Cohen's d)

Biomarker	Endo Mean	Control Mean	Cohen's d	Effect Size	Fold Change
TNF-α	130.0	20.0	3.456	Large	6.50
Glycodelin	200.0	30.0	2.833	Large	6.67
hs-CRP	600.0	100.0	2.567	Large	6.00
Activin A	350.0	150.0	2.456	Large	2.33
MCP-1	500.0	350.0	1.789	Large	1.43
CA-125	80.0	20.0	1.234	Large	4.00
IL-6	30.0	20.0	1.123	Large	1.50
LIF	40.0	35.0	0.567	Medium	1.14

Effect size interpretation: Small ($d < 0.5$), Medium ($0.5 \leq d < 0.8$), Large ($d \geq 0.8$)

DISCUSSION

This study provides a comprehensive evaluation of a panel of eight serum biomarkers for the noninvasive diagnosis of endometriosis, revealing several key insights into the disease's complex pathophysiology. The primary finding of our investigation is that women with endometriosis exhibit a significantly altered systemic biomarker profile, characterized by pronounced inflammation, and that a select panel of these markers holds exceptional promise for diagnostic application. Our results not only confirm the well-established inflammatory nature of endometriosis but also advance the field by demonstrating the superior diagnostic accuracy of specific markers, such as hs-CRP, TNF-α, Glycodelin, and Activin A, and by identifying distinct patient phenotypes through cluster analysis. These findings collectively underscore the potential to develop a highly accurate, noninvasive diagnostic tool and to move towards personalized management of endometriosis, a goal that has been the focus of extensive recent research [17, 18].

A cornerstone of our findings is the significant elevation of all eight investigated biomarkers in the endometriosis cohort, with inflammatory cytokines showing particularly

License: CC BY-NC 4.0

large effect sizes. The dramatic increase in serum TNF-α (Cohen's d = 3.46) and IL-6 (Cohen's d = 1.12) aligns with a substantial body of recent evidence establishing endometriosis as a chronic inflammatory disease [13, 14]. A 2025 consortium study by Terry et al. involving 566 participants confirmed that circulating inflammatory markers, including IL-6 and TNF-α, are central to the disease's profile. However, their study did not find a direct correlation with rASRM stage [21]. Our study corroborates the importance of these cytokines, as the multivariate regression analysis identified IL-6 as a significant independent predictor of the disease (OR = 1.35). The role of TNF-α is further highlighted by its excellent diagnostic performance in our ROC analysis (AUC = 0.98), suggesting it is a central mediator in the inflammatory cascade of endometriosis, a concept supported by recent reviews on the immunopathology of the disease [22, 23]. The marked elevation of these pro-inflammatory cytokines provides a strong rationale for their inclusion in a diagnostic biomarker panel.

The most striking finding of this study is the perfect diagnostic accuracy of hs-CRP (AUC =1.00, 100% sensitivity and specificity). This result positions hs-CRP as a potentially ideal noninvasive biomarker for endometriosis. However, this finding must be interpreted with caution, as the recent literature on hs-CRP in endometriosis remains conflicting. While the large-scale study by Terry et al. (2025) included hs-CRP as a key inflammatory marker [21], other recent studies have shown that hs-CRP levels do not significantly differ between endometriosis patients and controls, questioning its diagnostic utility [24]. The observed discrimination in our cohort may be attributable to the specific characteristics of our patient population or to the analytical methods used. It is possible that our study included a higher proportion of patients with a pronounced systemic inflammatory response. While our results are exceptionally promising, they require validation in larger, more diverse cohorts to confirm whether hs-CRP can serve as a standalone, definitive biomarker or whether its strength lies in its combination with other markers, a strategy advocated by recent research [17, 25].

Beyond the classic inflammatory markers, our study highlights the exceptional diagnostic potential of Glycodelin (AUC = 0.97) and Activin A (AUC = 0.97). Glycodelin, also known as Progestagen-associated endometrial protein (PAEP), has been a focus of recent research due to its roles in immunosuppression and endometrial function [26]. A 2025 study Shamlou et al. [27] confirmed the presence of Glycodelin A in serum and peritoneal fluid as a biomarker for endometriosis. Our study demonstrates an even higher diagnostic accuracy, reinforcing its value as a top-tier biomarker. Similarly, the high AUC for Activin A in our study is significant. Activin A, a member of the TGF-β superfamily, has been implicated in the pathophysiology of endometriosis, with a 2025 systematic review highlighting its role in endometrial repair and cellular processes [28]. Our results indicate a much stronger systemic signal for Activin A than previously reported, suggesting it may be a more robust marker of overall disease activity. The strong performance of both Glycodelin and Activin A underscores the importance of including markers of hormonal dysregulation and tissue remodeling alongside inflammatory cytokines in a comprehensive diagnostic panel. This multifaceted approach is gaining traction [25].

Our findings strongly support the concept of a multi-biomarker panel and are a major theme in recent endometriosis research [17, 18, 25]. While individual markers such as hs-CRP and TNF-α showed strong performance, the complexity of endometriosis suggests that a panel approach would provide a more robust and reliable diagnosis. Our multivariate logistic regression model, which explained 95% of the variance, identified CA-125, IL-6, and MCP-1 as significant independent predictors. The inclusion of CA-125 is consistent with its continued, albeit limited, use, as noted in recent reviews [29, 30]. The identification of IL-6 and MCP-1 as independent predictors solidifies the central role of inflammation and immune cell trafficking. Recent studies from 2024 and 2025 have confirmed that MCP-1 is elevated in the serum of patients with endometriosis and plays a crucial role in promoting inflammatory responses, cell invasion, and lesion establishment by activating ILK signaling [31, 32]. The combination of these three markers, representing different biological pathways (glycoprotein, inflammation, and chemotaxis), could form the basis of a powerful, highly accurate diagnostic

License: CC BY-NC 4.0

algorithm, as proposed by et al. [18], who successfully developed a predictive model based on a 10-protein panel [18].

A novel contribution of our study is the identification of three distinct patient phenotypes through K-means clustering, highlighting the heterogeneity of endometriosis. The "High Hormonal Dysregulation" cluster (Cluster 1) was characterized by the highest levels of Activin A, LIF, and CA-125, suggesting a phenotype driven primarily by aberrant hormonal signaling and endometrial receptivity pathways. The dysregulation of LIF in endometriosis has been a recent focus, with a 2023 study by Zutautas et al. [33] demonstrating that while LIF is present in ectopic lesions, its expression is altered, contributing to immunomodulation and vascularization. In contrast, the "TNF-α-Driven Inflammatory" phenotype (Cluster 2) points to a subgroup where TNF-α is the dominant pathological driver. The existence of these distinct molecular subtypes is a rapidly emerging concept, with several studies in 2024 and 2025 using clustering of comorbidity data, clinical symptoms, and molecular signatures to identify patient subgroups [34-36]. Our biomarker-based clustering provides a more accessible method for patient stratification, with significant clinical implications for personalized medicine.

Our analysis of biomarker levels across ASRM stages revealed a significant positive correlation for IL-6, CA-125, and hs-CRP, with levels increasing with disease severity. This finding is clinically relevant, as it suggests these markers could not only aid in diagnosis but also serve as noninvasive indicators of disease stage. However, recent large-scale studies, such as that by Terry et al. [21], did not find , significant associations between several key inflammatory markers and rASRM stage, suggesting that this relationship may be complex and population-dependent. Interestingly, our study found no significant difference in biomarker levels between patients with and without ovarian cysts. This suggests that the systemic inflammatory and biomarker signature of endometriosis is a feature of the disease itself, rather than merely a consequence of endometriomas. This aligns with the modern understanding of endometriosis as a systemic disease and suggests that a well-designed biomarker panel could be effective in diagnosing its different manifestations, a concept supported by recent calls for improved disease classification [37].

The study's strengths include its comprehensive panel of biomarkers, robust statistical analysis, and the novel use of cluster analysis to explore disease heterogeneity. However, we must also acknowledge its limitations. The primary limitation is the relatively small sample size, which means the findings, particularly the perfect AUC for hs-CRP and the cluster analysis results, require validation in a larger, multicenter cohort, a standard emphasized in recent biomarker research [18, 21]. Furthermore, this was a cross-sectional study, and longitudinal studies are needed to evaluate the utility of these biomarkers for monitoring disease progression and treatment response.

Future research should focus on validating this biomarker panel in diverse populations and exploring the clinical utility of the identified patient phenotypes. A prospective study designed to assess whether a biomarker-based score can reduce time to diagnosis and improve patient outcomes would be a critical next step, aligning with the overarching goal of developing noninvasive diagnostic tools for endometriosis [25, 38]. Our study identifies a panel of highly accurate serum biomarkers with the potential to revolutionize endometriosis diagnosis, paving the way for a noninvasive, reliable, and accessible tool that could significantly reduce diagnostic delays and facilitate a new era of personalized patient care. Furthermore, the single-center nature of our study and the moderate sample size necessitate external validation. Future studies should replicate these findings in larger, multicenter cohorts, potentially using open-access databases, to confirm the generalizability of the proposed biomarker signature.

CONCLUSION

This study successfully identified and validated a panel of serum biomarkers with exceptional diagnostic accuracy for endometriosis. Our findings demonstrate that a combination of inflammatory and hormonal markers, particularly hs-CRP, TNF-α, Glycodelin, and Activin A, can effectively distinguish women with endometriosis from healthy controls. The development of a multivariate predictive model incorporating CA-125, IL-6, and MCP-1, alongside the identification of distinct patient phenotypes

License: CC BY-NC 4.0

through cluster analysis, marks a significant step forward. These results strongly support the feasibility of a noninvasive blood test for diagnosing endometriosis. Such a tool has the potential to revolutionize clinical practice by dramatically reducing diagnostic delays, minimizing the need for invasive laparoscopy, and paving the way for personalized therapeutic strategies tailored to each patient's biological profile. Ultimately, the implementation of this biomarker panel could lead to earlier intervention and improved quality of life for millions of women suffering from this chronic disease. While our study assessed rASRM scores, future research should also specifically evaluate the impact of anatomical localization (e.g., peritoneal vs. deep infiltrating endometriosis) on the biomarker signature to refine the diagnostic model for disease heterogeneity.

Author Contributions:

RFA: Conceptualization of the study; supervision of the overall project; data curation and formal analysis; writing of the original draft.

AC: Investigation (data collection); data curation; review and editing of the manuscript.

A J M: Methodology design; investigation (laboratory work – ELISA assays); formal analysis (biomarker data interpretation); review and editing.

RDAA: Resources provision (laboratory equipment and materials); methodology support (protocol design); review and editing of the manuscript.

GAA: Project administration (sample coordination, liaison with participants); formal analysis (statistical interpretation); review and editing of the manuscript.

KHH: Supervision of the clinical biochemistry component; conceptual support and interpretation of results in the context of biochemistry; review and editing of the manuscript.

All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work, ensuring that any questions regarding the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding: No external funding was received for this study.

Acknowledgments: The authors would like to express their sincere gratitude to the Department of Obstetrics and Gynecology at Al-Sadr Teaching Hospital, Najaf, Iraq, for providing the clinical setting and support necessary to conduct this study. We are grateful to the central laboratory staff at Al-Sadr Teaching Hospital for their technical assistance with sample handling and ELISA assays. We also thank the Institutional Review Board of the College of Medicine at the University of Kufa for ethical oversight and guidance. Finally, we extend our most profound appreciation to all women who participated in this research for their time and cooperation, without which this work would not have been possible.

Ethics Approval: The study protocol was approved by the Institutional Review Board (IRB) of the College of Medicine, University of Kufa (Reference No. UOK/MED/IRB/2024-01).

Consent to Participate: Written informed consent was obtained from all participants.

Availability of Data and Materials: Raw data supporting this study's findings are available from the corresponding author upon reasonable request.

AI Disclosure: Artificial intelligence assistance (ChatGPT-5) was used only in the conceptual visualization of the Graphical Abstract. All scientific design, data interpretation, and manuscript writing were performed and verified by the authors.

Conflicts of Interest: The authors declare that they have no conflicts of interest related to this work. For a written statement, please contact the journal office.

Quote this article as Abed, Rusul F., Abbas Chyad, Ahmed Jasim Mohammed, Redha Abd Alredha, Ghanim A. Abbas, and Krarr Haider Haddawi, "A Validated Serum Biomarker Signature for the Noninvasive Diagnosis of Endometriosis." Precis. Nanomed. 2025, 8(4):1645-1658, https://doi.org/10.33218/001c.153841

COPYRIGHT NOTICE ©The Author(s) 2024. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References.

[1] W. H. Organization. "Endometriosis." World Health Organization. https://www.who.int/news-room/fact-sheets/detail/endometriosis (accessed October 15, 2025).

[2] R. D. Abd Alredha, B. R. Yaseen, A. J. Mohammed, and S. A. Salman, "Integrative analysis of inflammatory biomarkers and clinical features in endometriosis: insights into pain severity and diagnostic utility," *Anaesthesia, Pain & Intensive Care,* vol. 29, no. 4, pp. 276-282, 2025.

[3] A. Manu *et al.*, "Quality of Life Assessment and Clinical Implications for Women with Endometriosis Through Validated Tools: A Narrative Review," *Medicina,* vol. 61, no. 10, p. 1729, 2025.

[4] A. Bień, E. Rzońca, M. Zarajczyk, K. Wilkosz, A. Wdowiak, and G. Iwanowicz-Palus, "Quality of life in women with endometriosis: a cross-sectional survey," *Quality of Life Research,* vol. 29, no. 10, pp. 2669-2677, 2020.

[5] K. E. Pinto, H. L. Graham, J. M. Scott, and M. J. Benton, "Effect of Endometriosis on Disease-Specific Quality of Life," *Nursing for Women's Health,* 2025.

[6] W.-Z. Tang *et al.*, "The global burden of polycystic ovary syndrome, endometriosis, uterine fibroids, cervical cancer, uterine cancer, and ovarian cancer from 1990 to 2021," *BMC Public Health,* vol. 25, no. 1, p. 1774, 2025.

[7] Z. Wang, Y. Ouyang, Z. Xue, J. Wang, W. Gu, and C. Gu, "Global, regional, and national burden and trends of endometriosis, 1990–2021: an analysis of the global burden of disease study 2021 and forecast to 2050," *BMC Women's Health,* vol. 25, no. 1, p. 519, 2025.

[8] J. Fryer, A. J. Mason-Jones, and A. Woodward, "Understanding diagnostic delay for endometriosis: A scoping review using the social-ecological framework," *Health care for women international,* vol. 46, no. 3, pp. 335-351, 2025.

[9] B. Swift *et al.*, "Prevalence, diagnostic delay and economic burden of endometriosis and its impact on quality of life: results from an Eastern Mediterranean population," *European Journal of Public Health,* vol. 34, no. 2, pp. 244-252, 2024.

[10] S. Irungu, PhD, I. Mutai, and O. Purity, "Proteomic technologies in biomarker discovery for the noninvasive diagnosis of endometriosis," *The Biochemist,* pp. 1-7, 2025, doi: 10.1042/bio_2025_128.

[11] U. B. Kirk *et al.*, "Understanding endometriosis underfunding and its detrimental impact on awareness and research," *npj Women's Health,* vol. 2, no. 1, p. 45, 2024.

[12] E. Surrey, A. M. Soliman, H. Trenz, C. Blauer-Peterson, and A. Sluis, "Impact of endometriosis diagnostic delays on healthcare resource utilization and costs," *Advances in therapy,* vol. 37, no. 3, pp. 1087-1099, 2020.

[13] W. Park *et al.*, "Female reproductive disease, endometriosis: From inflammation to infertility," *Molecules and cells,* p. 100164, 2024.

[14] I. E. Oală *et al.*, "Endometriosis and the role of pro-inflammatory and anti-inflammatory cytokines in pathophysiology: A narrative review of the literature," *Diagnostics,* vol. 14, no. 3, p. 312, 2024.

[15] R. S. Ahmed *et al.*, "Exploring the Immune System's Role in Endometriosis: Insights Into Pathogenesis, Pain, and Treatment," *Cureus,* vol. 17, no. 7, 2025.

[16] M. A. Ochoa Bernal and A. T. Fazleabas, "The Known, the Unknown and the Future of the Pathophysiology of Endometriosis," *International Journal of molecular sciences,* vol. 25, no. 11, p. 5815, 2024.

[17] M. Kliber-Gałuszka, K. Kulczyńska-Figurny, P. P. Jagodziński, and A. Pławski, "Potential biomarkers for early detection of endometriosis: current state of art (what we know so far)," *Journal of Applied Genetics,* pp. 1-17, 2025.

[18] E. Schoeman *et al.*, "Identification of plasma protein biomarkers for endometriosis and the development of statistical models for disease diagnosis," *Human reproduction,* vol. 40, no. 2, pp. 270-279, 2025.

[19] H. Wang *et al.*, "A Noninvasive Menstrual Blood-Based Diagnostic Platform for Endometriosis Using Digital Droplet Enzyme-Linked Immunosorbent Assay and Single-Cell RNA Sequencing," *Research,* vol. 8, p. 0652, 2025.

[20] I. Flores, E. Waelkens, and T. D'Hooghe, "Noninvasive diagnosis of endometriosis: Review of current peripheral blood and endometrial biomarkers," *Best Practice & Research Clinical Obstetrics & Gynaecology,* vol. 50, pp. 72-83, 2018.

License: CC BY-NC 4.0

[21] K. L. Terry *et al.*, "Circulating inflammatory biomarkers and endometriosis lesion characteristics in the WisE consortium," *npj Women's Health,* vol. 3, no. 1, pp. 1-15, 2025.
[22] Y. Dai, Z. Ye, X. Lin, and S. Zhang, "Immunopathological insights into endometriosis: from research advances to future treatments," (in eng), *Semin Immunopathol,* vol. 47, no. 1, p. 31, Jul 18 2025, doi: 10.1007/s00281-025-01058-5.
[23] X. Xu, J. Li, H. Lin, Z. Lin, and G. Ji, "The role of TGF-β superfamily in endometriosis: a systematic review," (in English), *Frontiers in Immunology,* Systematic Review vol. Volume 16 - 2025, 2025-August-12 2025, doi: 10.3389/fimmu.2025.1638604.
[24] M. Wójtowicz, D. Zdun, A. J. Owczarek, V. Skrzypulec-Plinta, and M. Olszanecka-Glinianowicz, "Evaluation of Pro-inflammatory Cytokines Concentrations in Plasma, Peritoneal, and Endometrioma Fluids in Women Operated on for Ovarian Endometriosis—A Pilot Study," *International Journal of Molecular Sciences,* vol. 26, no. 11, p. 5117, 2025.
[25] A. Brulport *et al.*, "An integrated multi-tissue approach for endometriosis candidate biomarkers: a systematic review," *Reproductive Biology and Endocrinology,* vol. 22, no. 1, p. 21, 2024.
[26] F. Zeng *et al.*, "Silencing progestagen-associated endometrial protein (PAEP) suppresses sorafenib resistance and enhances sorafenib-induced ferroptosis in hepatocellular carcinoma," *Liver Research,* 2025.
[27] N. Shamlou *et al.*, "Altered Expression of Interleukin-6, Glycodelin A and Heparin-Binding Epidermal Growth Factor, in The Endometrium of Women with Hydrosalpinx: A Case-Control Study," *International Journal of Fertility & Sterility,* vol. 19, no. 2, p. 157, 2025.
[28] X. Xu, J. Li, H. Lin, Z. Lin, and G. Ji, "The role of TGF-β superfamily in endometriosis: a systematic review," *Frontiers in immunology,* vol. 16, p. 1638604, 2025.
[29] Y. Chen, M. Pan, Y. Zuo, B. Yang, and S. Wang, "Research progress of CA125 in endometriosis: Teaching an old dog new tricks," *Gynecology and Obstetrics Clinical Medicine,* vol. 2, no. 4, pp. 191-198, 2022.
[30] S. Feduniw, M. Pruc, M. Ciebiera, K. Safiejko, M. Bizon, and L. Szarpak, "Current evidence on CA-125 levels in differentiation between endometriomas and endometriosis-associated ovarian cancer–A systematic review and meta-analysis," *Journal of Endometriosis and Pelvic Pain Disorders,* vol. 16, no. 3-4, pp. 154-159, 2024.
[31] U. K. Soni, R. Tripathi, and R. K. Jha, "MCP-1 exerts the inflammatory response via ILK activation during endometriosis pathogenesis," *Life Sciences,* vol. 353, p. 122902, 2024.
[32] U. K. Soni *et al.*, "MCP-1 promotes ILK phosphorylation at Ser246 during endometriosis development and affects the pregnancy outcome," *Molecular Human Reproduction,* vol. 31, no. 2, p. gaaf004, 2025.
[33] K. B. Zutautas *et al.*, "The dysregulation of leukemia inhibitory factor and its implications for endometriosis pathophysiology," *Frontiers in immunology,* vol. 14, p. 1089098, 2023.
[34] U. Khan *et al.*, "Comorbidity analysis and clustering of endometriosis patients using electronic health records," *medRxiv,* 2025.
[35] F. S. Kanti, V. Allard, A. A. Métivier, M. Lemyre, K. Arendas, and S. Maheux-Lacroix, "Pain Phenotypes in Endometriosis: A Population-Based Study Using Latent Class Analysis," *BJOG: An International Journal of Obstetrics & Gynaecology,* vol. 132, no. 4, pp. 492-503, 2025.
[36] L. A. Guare *et al.*, "Enhancing genetic association power in endometriosis through unsupervised clustering of clinical subtypes identified from electronic health records," *Research Square,* pp. rs. 3. rs-5004325, 2024.
[37] C. R. Nezhat *et al.*, "Real world perspectives on endometriosis disease phenotyping through surgery, omics, health data, and artificial intelligence," *npj Women's Health,* vol. 3, no. 1, p. 8, 2025.
[38] L. Krygere, P. Jukna, K. Jariene, and E. Drejeriene, "Diagnostic potential of cytokine biomarkers in endometriosis: challenges and insights," *Biomedicines,* vol. 12, no. 12, p. 2867, 2024.

License: CC BY-NC 4.0