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Association of Vitamin D-Binding Protein (VDBP) and Endometriosis in Women with Dysmenorrhea: A Case-Control Study

Try Wahyudi Jeremi Loly^{1*}, Fatmawati Madya¹, Johnsen Mailoa¹, Rusdina Bte Ladju², Eddy R. Moeljono¹, Nuraeni Abidin¹

¹ Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

² Hasanuddin University Medical Research Center, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

*Corresponding author: Try Wahyudi Jeremi Loly, NTI Residence, Blok GE 17, Makassar, South Sulawesi, Indonesia, 90245. E-mail: tryloly@gmail.com. ORCID: <https://orcid.org/0009-0007-3856-6949>

ABSTRACT:

Background: Vitamin D-binding protein (VDBP) participates in vitamin D transport, actin clearance, and immune regulation, but its association with endometriosis and disease stage remains uncertain. **Objective:** This case-control study compared serum VDBP in women with dysmenorrhea with and without endometriosis and assessed paired serum-peritoneal concentrations, disease stage, and diagnostic value.

Methods: It included 24 women with histopathologically confirmed endometriosis and 24 controls without clinical or ultrasonographic evidence of endometriosis. VDBP was measured by enzyme-linked immunosorbent assay. Analyses used an independent-samples t-test without assuming equal variances, a paired-samples t-test, Spearman correlation, and receiver operating characteristic (ROC) analysis.

Results: Mean serum VDBP was higher in cases than controls (428.29 ± 138.60 versus 356.89 ± 71.07 $\mu\text{g/mL}$; mean difference 71.40 $\mu\text{g/mL}$; 95% confidence interval [CI] 6.81-135.99; $p = 0.031$). Peritoneal VDBP in cases was 185.14 ± 67.44 $\mu\text{g/mL}$ and lower than paired serum VDBP ($p < 0.001$). Serum VDBP correlated positively with stage II-IV disease ($\rho = 0.565$; $p = 0.004$). ROC analysis showed limited discrimination (area under the curve 0.615; 95% CI 0.449-0.781; $p = 0.176$); sensitivity and specificity were both 58.3%.

Conclusion: Serum VDBP concentrations were associated with endometriosis and disease stage, with higher mean concentrations in serum than in peritoneal fluid among cases. Serum VDBP showed limited diagnostic discrimination, and the findings do not support its use as a standalone diagnostic biomarker.

1. INTRODUCTION

Endometriosis is an estrogen-dependent inflammatory disorder characterized by endometrial-like tissue outside the uterus and is associated with dysmenorrhea, pelvic pain, and impaired fertility [1]. Persistent inflammation and altered immune responses contribute to the survival of ectopic tissue, tissue injury, and pain [2]. Dysmenorrhea is therefore both a clinically important presenting symptom and a reason to investigate proteins involved in the inflammatory environment of endometriosis.

Diagnostic delay remains common in women with endometriosis [3]. The European Society of Human Reproduction and Embryology (ESHRE) and National Institute for Health and Care Excellence (NICE) guidelines place symptoms, clinical examination, and imaging within the diagnostic pathway, while recognizing that normal imaging does not exclude all forms of disease [4, 5]. The American College of Obstetricians and Gynecologists (ACOG) also supports a presumptive clinical diagnosis to guide empirical treatment [6]. The limitations of imaging, particularly for superficial disease, sustain interest in complementary biomarkers [7].

A candidate biomarker should have a biological relationship with the disease and be evaluated in the population in which it would be used. For women presenting with dysmenorrhea, comparison with women who have similar symptoms is particularly relevant. Previous reviews have identified numerous candidate biomarkers, but consistent diagnostic performance remains difficult to establish [8, 9]. Vitamin D-binding protein (VDBP) is of interest because its functions connect vitamin D transport with processes involved in tissue damage and immune regulation.

VDBP, also known as group-specific component, is a predominantly liver-derived protein that transports vitamin D metabolites [10]. It also participates in extracellular actin scavenging and inflammatory responses [11], while hormonal and reproductive factors may influence its circulating concentration [12]. During tissue injury, actin released from damaged cells is bound by VDBP, facilitating its clearance; structural studies have demonstrated the VDBP-actin complex [13]. This function provides a biological link between VDBP and the repeated tissue injury associated with endometriosis.

A second proposed link involves macrophage regulation. VDBP has been described as a precursor of VDBP-derived macrophage-activating factor (DBP-MAF), generated through changes in glycosylation involving lymphocyte enzymes. This pathway has been proposed to influence macrophage phagocytosis and the clearance of ectopic endometrial cells and cellular debris [14, 15]. Because macrophages can contribute both to tissue clearance and to lesion maintenance [16], altered VDBP concentration or function could accompany the disturbed immune balance of endometriosis. The relevance of these mechanisms cannot be established from total VDBP concentration alone.

Previous studies provide a rationale for examining both systemic and local VDBP. A previous study reported higher concentrations in serum than in peritoneal fluid, but no significant case-control difference [17]. A later study likewise found no significant between-group difference in plasma or peritoneal VDBP, although peritoneal VDBP correlated with lactoferrin, linking the protein to the local inflammatory environment [18]. A previous study reported increased VDBP expression in ectopic endometrial tissue [15]. These findings suggest that circulating concentration, peritoneal distribution, and tissue expression may capture different aspects of VDBP biology.

This study compared serum VDBP between women with dysmenorrhea and histopathologically confirmed endometriosis and women with dysmenorrhea without clinical evidence of endometriosis. It also compared paired serum and peritoneal fluid concentrations, examined the relationship between serum VDBP and surgical stage, and explored diagnostic discrimination. These analyses address whether VDBP concentration is associated with endometriosis and its clinical extent, while considering its proposed role in inflammation and immune regulation.

2. MATERIALS AND METHODS

2.1. Study design and setting

This observational, comparative case-control study was conducted from September 2025 to April 2026 at Dr. Wahidin Sudirohusodo Hospital and affiliated clinical centers in Makassar, Indonesia, within the Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University. Consecutive sampling was used to enroll eligible women until the intended group sizes were reached. The two clinical groups were independent and were not individually matched. Reporting followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [19].

2.2. Participants and disease classification

Eligible participants were women aged 15-49 years who presented with dysmenorrhea and agreed to participate. The case group comprised women with dysmenorrhea who underwent surgery and had endometriosis confirmed by histopathological examination of an endometriotic cyst. The control group comprised women with dysmenorrhea who had no clinical evidence of endometriosis based on history taking, physical examination, and appropriate diagnostic investigations, consistent with ESHRE guideline for endometriosis [4]. Accordingly, endometriosis was histopathologically confirmed in cases, whereas

controls were clinically classified as not having endometriosis. Exclusion criteria were chronic inflammatory disease, hepatic or renal disease, pregnancy, and estrogen therapy during the preceding three months. Damaged or uninterpretable specimens were considered unsuitable for analysis.

Endometriosis stage was recorded from operative findings using the revised American Society for Reproductive Medicine (rASRM) classification [20]. The analyzed cases included stages II, III, and IV. Demographic and clinical variables included age, body mass index (BMI), marital status, parity, education, smoking, and pain score. BMI categories were normal (18.5 to <25 kg/m²), overweight (25 to <30 kg/m²), and obese (≥30 kg/m²). Pain intensity was recorded on a 0-10 scale described in the study protocol as a visual analog scale (VAS), with mild pain defined as 1-3, moderate pain as 4-7, and severe pain as 8-10.

2.3. Specimen collection and VDBP measurement

A 3-mL venous blood sample was collected from each participant for serum VDBP measurement. Peritoneal fluid was collected directly during surgery without lavage in women with endometriosis. Specimens were transported to the laboratory in a cooler box. VDBP was measured using the Human DBP (Vitamin D-binding protein) ELISA Kit (Elabsience Biotechnology Inc., Houston, TX, USA; catalog no. E-EL-H6167), according to the manufacturer's instructions. Final VDBP concentrations were corrected for the sample dilution factor and reported in µg/mL. Serum and peritoneal fluid measurements from the same woman were linked for the paired analysis.

2.4. Statistical analysis

Statistical processing was undertaken in SPSS after the dataset had been checked for missing entries, duplication, implausible values, and data-entry errors. Categorical variables were summarized as frequencies and percentages, and continuous variables as mean ± standard deviation (SD) with 95% confidence intervals (CIs), where appropriate. Age, parity, education, and pain categories were compared using Pearson's chi-square test without continuity correction; Fisher's exact test was used for marital status and for normal versus elevated BMI, combining overweight and obesity for the latter comparison. Normality was assessed using the Shapiro-Wilk test for serum concentrations in each group and for within-person serum-peritoneal differences.

Serum VDBP concentrations were compared between the endometriosis and control groups using an independent-samples t-test without assuming equal variances. Serum and peritoneal fluid VDBP concentrations within women with endometriosis were compared using a paired-samples t-test. Between-group and paired mean differences are reported with 95% CIs. Spearman's rank correlation coefficient (ρ) assessed the association between rASRM stage and serum VDBP.

Receiver-operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic value, including sensitivity and specificity [21, 22]. Values of $p < 0.05$ were considered to be statistically significant.

All tests were two-sided, with $p < 0.05$ considered statistically significant. Analyses included 48 participants with serum measurements and 24 complete serum-peritoneal pairs. No VDBP measurements were imputed or excluded as outliers. Results were unadjusted, and no correction for multiple comparisons was applied. The stage and diagnostic analyses were interpreted as exploratory.

2.5. Ethical considerations

The Research Ethics Committee of Hasanuddin University approved the study protocol and related documents on 11 August 2025 (approval no. 574/UN4.6.4.5.31/PP36/2025; protocol no. UH25070473). The approval followed expedited review and was valid through 11 August 2026, encompassing the reported data-collection period. All participants provided written informed consent before enrollment.

3. RESULTS AND DISCUSSION

3.1. Participant characteristics

The study included 24 women with histopathologically confirmed endometriosis and 24 controls with dysmenorrhea but no clinical or ultrasonographic evidence of endometriosis. Most participants were aged ≤35 years and had normal BMI (Table 1). Age, BMI, marital status, parity, and education did not differ significantly between groups (all $p > 0.05$). Pain severity differed significantly ($p < 0.001$): 21 controls (87.5%) reported mild pain, whereas all cases reported moderate or severe pain. All participants were nonsmokers. Among cases, eight had stage II, nine stage III, and seven stage IV disease.

Table 1. Study characteristics

Characteristic	Endometriosis (n = 24)	Controls (n = 24)	p-value
Age (years)			0.763
≤35	15 (62.5)	16 (66.7)	
>35	9 (37.5)	8 (33.3)	
Body mass index (kg/m ²)			0.188
18.5 to <25	19 (79.2)	23 (95.8)	
25 to <30	4 (16.7)	1 (4.2)	
≥30	1 (4.2)	0 (0.0)	
Marital status			0.609
Unmarried	1 (4.2)	3 (12.5)	
Married	23 (95.8)	21 (87.5)	
Parity			0.432
Nulliparous	7 (29.2)	4 (16.7)	
Primiparous	9 (37.5)	8 (33.3)	
Multiparous	8 (33.3)	12 (50.0)	
Education			0.768
<9 years	10 (41.7)	9 (37.5)	
≥9 years	14 (58.3)	15 (62.5)	
Pain score category			<0.001
Mild (1-3)	0 (0.0)	21 (87.5)	
Moderate (4-7)	13 (54.2)	3 (12.5)	
Severe (8-10)	11 (45.8)	0 (0.0)	
Nonsmoker	24 (100.0)	24 (100.0)	—

Values are n (%), with percentages calculated within each group. Pearson's chi-square test was used for age, parity, education, and pain; Fisher's exact test was used for marital status and BMI. For the BMI comparison, overweight and obesity were

combined as elevated BMI. No participant had BMI <18.5 kg/m². Pain categories followed the study protocol: mild, 1-3; moderate, 4-7; severe, 8-10. No test was performed for smoking because all participants were nonsmokers. The predominance of reproductive-age women is consistent with the estrogen-dependent nature of endometriosis [1, 2, 23]. Most women in both groups had normal BMI, and the absence of a significant difference in demographic characteristics should be interpreted in the context of the small sample. The greater pain severity among cases is consistent with the inflammatory and neural mechanisms of endometriosis-associated pain [2]. Using controls who also had dysmenorrhea provides a clinically relevant comparison, although the difference in symptom severity remains an important feature of the study population. Other menstrual pain studies illustrate why symptomatic women require careful characterization. One study associated dysmenorrhea with stress and urinary prostaglandin levels in adolescents [24], and another study reported associations with underweight and urinary PGF2 α [25]. A separate study examined stress and urinary 8-hydroxy-2-deoxyguanosine in relation to premenstrual syndrome [26]. Although these populations and outcomes differ from endometriosis, the findings provide complementary context for hormonal, inflammatory, and stress-related influences on menstrual symptoms. They do not directly establish the role of VDBP in endometriosis.

3.2. Serum VDBP in endometriosis cases and controls

Mean serum VDBP was higher in women with endometriosis than in controls (428.29 $\pm$ 138.60 versus 356.89 $\pm$ 71.07 μ g/mL; Table 2). The between-group mean difference was 71.40 μ g/mL (95% CI 6.81-135.99; p = 0.031).

Table 2. Serum VDBP in endometriosis cases and symptomatic controls

Group	n	Mean $\pm$ SD (μ g/mL)	95% CI for mean (μ g/mL)
Endometriosis	24	428.29 $\pm$ 138.60	369.77-486.82
Controls	24	356.89 $\pm$ 71.07	326.88-386.90

VDBP, vitamin D-binding protein; SD, standard deviation; CI, confidence interval. Independent-samples t -test without assuming equal variances: t = 2.246, df = 34.31, p = 0.031. Mean difference (endometriosis minus controls): 71.40 μ g/mL (95% CI 6.81-135.99).

The higher serum VDBP concentration may reflect a systemic response to the chronic inflammatory environment of endometriosis. Repeated tissue injury and immune-cell activity create a need for the clearance of extracellular proteins and cellular debris. In the actin-scavenger system, VDBP binds actin released by damaged cells and facilitates its removal [13]. The increased circulating concentration observed here is therefore compatible with a compensatory response: VDBP may participate in the response to tissue injury and inflammation. This interpretation remains a biological hypothesis because actin release, VDBP-actin complexes, and clearance activity were not measured. Macrophage regulation provides a further possible explanation. Conversion of VDBP to DBP-MAF has been proposed to enhance phagocytic activity, potentially affecting the removal of ectopic endometrial cells and debris [14, 15]. However, macrophages in endometriosis have diverse functions, including tissue remodeling and support of lesion growth [16]. Higher total VDBP could thus coexist with altered or ineffective immune clearance. It does not by itself demonstrate DBP-MAF formation, a particular macrophage phenotype, or successful resolution of inflammation. The direction of the serum difference is consistent with the nonsignificantly higher serum concentrations reported in a previous study [17]. A later study also found no statistically significant case-control difference in circulating VDBP [18]. Variation in hormonal exposure, genetic background, sampling conditions, disease characteristics, and inflammatory regulation may contribute to these differences [10, 11, 12, 17, 18]. The present finding supports further investigation of VDBP within the inflammatory biology of endometriosis, while the inconsistency across studies indicates that concentration alone is unlikely to explain its role. The biological effect of VDBP may also depend on its molecular form. A previous study reported greater abundance of the GC*2 allele product in women with endometriosis and proposed that differences in conversion to DBP-MAF could influence macrophage function [14]. GC*1 and GC*2 are allele products of the GC gene. A Korean study, however, found no significant case-control differences in GC genotype frequencies or circulating VDBP [27]. In addition, VDBP measurements may vary with assay method and isoform recognition [28]. These findings support the interpretation that total VDBP abundance and its immunological activity should be considered separately.

3.3. Paired serum and peritoneal fluid VDBP

Within the endometriosis group, mean serum VDBP exceeded mean peritoneal fluid VDBP (428.29 ± 138.60 versus 185.14 ± 67.44 $\mu\text{g/mL}$; Table 3). The paired mean difference was 243.16 $\mu\text{g/mL}$ (95% CI 171.27-315.05; paired-samples t-test, $p < 0.001$).

Table 3. Paired serum and peritoneal fluid VDBP in women with endometriosis

Compartment	n	Mean $\pm$ SD ($\mu\text{g/mL}$)	95% CI for mean ($\mu\text{g/mL}$)
Serum	24	428.29 ± 138.60	369.77-486.82
Peritoneal fluid	24	185.14 ± 67.44	156.66-213.61

SD, standard deviation; CI, confidence interval. Measurements were paired within the same 24 women. Mean serum-peritoneal difference: 243.16 $\mu\text{g/mL}$ (95% CI 171.27-315.05); paired-samples t-test: $t = 6.997$, $df = 23$, $p < 0.001$.

The difference between serum and peritoneal fluid indicates distinct systemic and local distributions of VDBP. Predominant hepatic synthesis and the exchange of proteins between the circulation and extravascular fluid provide physiological explanations for a higher serum concentration [10, 11]. A previous study similarly reported higher VDBP in serum than in peritoneal fluid [17]. Another study found reduced expression of a specific VDBP isoform in the peritoneal fluid of untreated endometriosis patients [29], supporting interest in local VDBP biology, although isoform expression is not equivalent to the total concentration measured here. Local binding, utilization, or modification of VDBP within the inflamed peritoneal environment could contribute to its lower concentration. Actin scavenging and the proposed DBP-MAF pathway offer possible links to debris clearance and macrophage-mediated tissue remodeling [13, 14, 16]. These processes may operate alongside differences in protein transfer and clearance. Because this study did not measure VDBP turnover, actin complexes, or DBP-MAF, local consumption remains a hypothesis rather than a demonstrated explanation for the concentration gradient. The peritoneal relationship between VDBP and lactoferrin provides another link to local inflammation. A previous study found a positive correlation between these proteins in peritoneal fluid from women with endometriosis ($r = 0.399$; $p = 0.002$) [18]. Lactoferrin is released from neutrophil granules and participates in iron handling and inflammatory responses. The association supports examining VDBP within a network of local immune proteins, although it does not establish a direct molecular interaction or prove that either protein reduces inflammation in endometriosis.

3.4. Association between serum VDBP and endometriosis stage

Mean serum VDBP increased across stages II, III, and IV: 320.68 ± 68.63 , 471.59 ± 141.51 , and 495.61 ± 133.78 $\mu\text{g/mL}$, respectively (Table 4). Spearman's analysis showed a positive correlation between endometriosis stage and serum VDBP concentration ($\rho = 0.565$; $p = 0.004$; $n = 24$). The permutation sensitivity analysis supported this association ($p = 0.005$).

Table 4. Serum VDBP by rASRM endometriosis stage

Stage	n	Mean $\pm$ SD ($\mu\text{g/mL}$)	Spearman ρ (p-value)
II	8	320.68 ± 68.63	0.565 (0.004)
III	9	471.59 ± 141.51	
IV	7	495.61 ± 133.78	

rASRM, revised American Society for Reproductive Medicine; SD, standard deviation. Spearman's correlation between ordinal stage and serum VDBP: $\rho = 0.565$, $p = 0.004$ ($n = 24$). No stage I cases were included.

The positive stage association is compatible with the interpretation that VDBP may accompany a greater burden of inflammation and tissue injury in more extensive disease. Increased cellular turnover and tissue remodeling could increase demand for actin clearance and alter macrophage-related pathways [13, 16]. Such a response could raise circulating VDBP without implying that VDBP itself drives lesion progression. Stage was assessed at one operation, so the observed association describes differences between women rather than changes over time.

A previous study observed a nonsignificant tendency toward higher serum VDBP in advanced disease [17], whereas another study found no association between VDBP isoform expression and stage [29]. A tissue study reported increased VDBP expression in ectopic endometrial tissue and stage-related changes in the proteomic analysis [15], suggesting a potential local relationship with disease extent. A previous study did not find circulating VDBP to be associated with severity [30]. Differences between tissue expression, peritoneal isoforms, and total circulating protein may help explain these inconsistent results. The stage association in this small sample consequently requires confirmation.

3.5. ROC Curve Analysis

Serum VDBP had an AUC of 0.615 (95% CI 0.449-0.781; $p = 0.176$), indicating limited diagnostic discrimination (Table 5 and Figure 1). At the study cutoff of 364.86 $\mu\text{g/mL}$, sensitivity and specificity were both 58.3%. Fourteen of 24 cases were classified as positive and 14 of 24 controls as negative; there were 10 false-negative and 10 false-positive results.

Table 5. Exploratory ROC analysis of serum VDBP

Metric	Estimate	95% CI
AUC	0.615	0.449-0.781
p-value for AUC versus 0.50	0.176	Not applicable
Study cutoff ($\mu\text{g/mL}$)	≥ 364.86	Not applicable
Sensitivity	58.3%	38.8%-75.5%
Specificity	58.3%	38.8%-75.5%
True positive/false negative	14 / 10	Not applicable
True negative/false positive	14 / 10	Not applicable

AUC, area under the receiver operating characteristic curve; CI, confidence interval. The AUC CI and p-value use DeLong's nonparametric variance estimate with a normal approximation [31]. Sensitivity and specificity CIs were calculated using the Wilson method. The cutoff was selected by the intersection method and evaluated in the same dataset; it was not independently validated.

These results support the conclusion that VDBP has limited accuracy as a standalone diagnostic biomarker. A significant difference in group means can coexist with considerable overlap between individual measurements. The AUC did not differ significantly from 0.50, and sensitivity and specificity were below 60%. The biological involvement of VDBP in inflammation therefore does not necessarily confer sufficient disease specificity for diagnosis. This is consistent with the ESHRE recommendation against using blood biomarkers to diagnose endometriosis [4]; clinical assessment and imaging remain central to the diagnostic pathway.

VDBP may be more informative as part of a biomarker panel reflecting complementary inflammatory and immune pathways. Findings of elevated urinary VDBP in another study [32] also indicate that its potential value may depend on the biological compartment examined, although evidence for urinary biomarkers remains insufficient for routine diagnosis [33]. The present results justify investigating VDBP alongside other markers, but do not establish that a combined panel is accurate or clinically useful. Any proposed panel requires direct evaluation and independent validation.

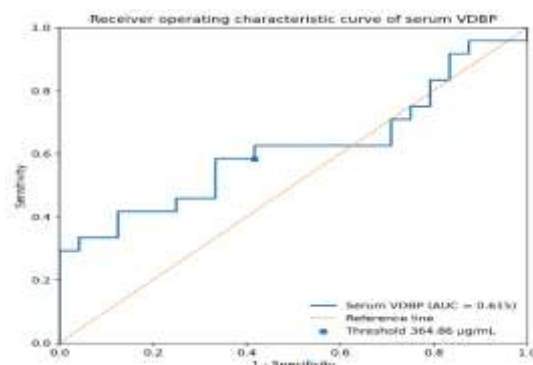


Figure 1. Receiver operating characteristic curve for serum VDBP in 24 endometriosis cases and 24 controls. AUC = 0.615 (95% CI 0.449-0.781; $p = 0.176$). The open circle marks the study cutoff of 364.86 $\mu\text{g/mL}$. The dashed diagonal represents chance discrimination.

3.6. Interpretation, study contribution, and limitations

This study combines a clinically relevant comparison of women with dysmenorrhea with paired measurements from systemic and peritoneal compartments and an assessment of surgical stage. Its contribution is the observation that higher serum VDBP, a serum-peritoneal concentration difference, and a positive stage association can occur together. These findings can be interpreted in relation to VDBP-mediated actin clearance, proposed macrophage regulation, and responses to chronic inflammation. They provide a basis for mechanistic research while also showing the limited discrimination of total serum VDBP as a single diagnostic marker.

The small sample limits statistical precision and generalizability. Only 24 cases were available for the paired and stage analyses, and stage I disease was absent. The analyses were unadjusted, and the groups differed in pain severity, leaving potential confounding. VDBP was assessed as a single protein; actin complexes, DBP-MAF, GC genotype, macrophage function, lactoferrin, and inflammatory mediators such as IL-6, TNF- α , and TGF- β were not evaluated. Thus, the study cannot determine whether the proposed compensatory or immunological pathways account for the concentration differences.

Cases had histopathological confirmation, whereas controls were evaluated by history taking, physical examination, and ultrasonography, without identified masses or anatomical abnormalities. Normal examination and imaging cannot exclude all endometriosis [4, 5], so occult disease in controls remains possible. Peritoneal fluid was not collected from controls, preventing assessment of whether the serum-peritoneal gradient was specific to endometriosis. Menstrual timing, vitamin D supplementation, and other relevant laboratory covariates were not available in the analyzed dataset. These limitations warrant cautious interpretation of both the biological associations and the exploratory ROC results.

Further studies should include larger and more diverse populations and measure VDBP together with inflammatory mediators and markers of its biological activity. Paired compartment measurements, isoform characterization, and assessment of macrophage-related pathways could test the mechanistic explanations discussed here. Prospective evaluation of a biomarker panel would then be needed to determine whether these biological relationships improve clinical discrimination beyond symptoms and imaging.

4. CONCLUSION

Women with dysmenorrhea and histopathologically confirmed endometriosis had significantly higher mean serum VDBP concentrations than symptomatic controls without clinical or ultrasonographic evidence of endometriosis. Within the endometriosis group, mean serum VDBP was significantly higher than paired peritoneal fluid VDBP, and serum VDBP correlated positively with stage II-IV disease.

Together, these findings document an association between serum VDBP and endometriosis, a positive relationship with disease stage, and a difference between systemic and peritoneal concentrations. They provide a basis for investigating VDBP in the inflammatory and immune biology of endometriosis. Its limited diagnostic discrimination does not support standalone diagnostic use. Larger studies with well-characterized controls are needed to confirm these associations and evaluate VDBP function.

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AUTHOR CONTRIBUTIONS

Try Wahyudi Jeremi Loly: Conceptualization, Methodology, Investigation, Writing - Original Draft.

Fatmawati Madya: Conceptualization, Methodology, Investigation, Resources, Validation, Writing - Original Draft, Writing - Review & Editing, Supervision.

Johnsen Mailoa: Conceptualization, Methodology, Investigation, Resources, Validation, Writing - Original Draft, Writing - Review & Editing, Supervision.

Rusdina Bte Ladju: Methodology, Formal Analysis, Writing - Review & Editing, Supervision.

Eddy R. Moeljono: Validation, Writing - Review & Editing, Supervision.

Nuraeni Abidin: Validation, Writing - Review & Editing, Supervision.

All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

The Research Ethics Committee of Hasanuddin University approved the protocol and related documents on 11 August 2025 (approval no. 574/UN4.6.4.5.31/PP36/2025; protocol no. UH25070473). All participants provided written informed consent before enrollment.

DATA AVAILABILITY STATEMENT

The de-identified data supporting the findings of this study may be made available from the corresponding author upon reasonable request, subject to applicable ethical and institutional requirements.

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