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Evaluation of Serum Growth Differentiation Factor-15 (Gdf-15) as a Non-Invasive Biomarker for Disease Severity and Pain Intensity in Endometriosis: a Hospital Based Case-Control Study

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Abstract

Background: Endometriosis is a chronic, oestrogen-dependent inflammatory gynaecological disorder affecting 10 to 15 percent of women of reproductive age. The condition is characterised by pelvic pain and infertility, and diagnosis is frequently delayed by 7 to 10 years due to reliance on invasive laparoscopy and the absence of validated non-invasive biomarkers. Growth Differentiation Factor-15 (GDF-15), a stress-responsive cytokine implicated in inflammatory and nociceptive pathways, has emerged as a candidate biomarker in multiple pain-related disorders; however, its clinical utility in endometriosis has not been adequately defined.

Objective: The primary objective of this study was to evaluate serum GDF-15 as a diagnostic biomarker capable of differentiating women with confirmed endometriosis from healthy controls. The secondary objective was to assess its potential as a disease severity marker by examining the association between GDF-15 levels and r-AFS staging, and its exploratory relationship with pain intensity scores.

Methods: This was a hospital-based case-control study conducted over 24 months at a tertiary teaching hospital. The study enrolled 100 women with laparoscopically and histopathologically confirmed endometriosis (cases) and 100 age-matched healthy controls. Serum GDF-15 concentration was quantified using enzyme-linked immunosorbent assay (ELISA). Disease severity was graded using the revised American Fertility Society (r-AFS) staging system. Pain intensity was assessed using the Visual Analogue Scale (VAS) and the Numerical Rating Scale (NRS). Statistical analysis included independent samples t-test, one-way analysis of variance (ANOVA), Pearson correlation coefficient, binary logistic regression, and receiver operating characteristic (ROC) curve analysis. Statistical significance was set at p less than 0.05.

Results: Serum GDF-15 was significantly elevated in cases compared to controls (961.7 ± 195.2 pg/mL vs. 508.0 ± 99.6 pg/mL; p less than 0.001; Cohen's d = 2.93). ROC curve analysis demonstrated excellent diagnostic discriminatory performance (AUC = 0.942; 95% CI: 0.907 to 0.977; sensitivity 89.0%; specificity 88.0% at an optimal cut-off of 720.5 pg/mL). A numerical increasing trend in GDF-15 was observed across r-AFS stages (Stage I: 906.3 ± 234.3 pg/mL to

Stage IV: 987.5 ± 192.7 pg/mL); however, this difference did not reach statistical significance (one-way ANOVA, p = 0.265) and should be interpreted as exploratory. Pearson correlation between GDF-15 and pain scores was weak and did not achieve statistical significance (VAS: r = 0.174, p = 0.084; NRS: r = 0.191, p = 0.058).Binary logistic regression confirmed GDF-15 as an independent predictor of endometriosis diagnosis after adjustment for age and BMI (adjusted OR = 3.84; 95% CI: 2.61 to 5.65; p less than 0.001).

Conclusion: Serum GDF-15 is significantly elevated in women with endometriosis and demonstrates excellent diagnostic discriminatory performance, supporting its role as a non-invasive diagnostic biomarker. The non-significant numerical trend across r-AFS stages and the weak, non-significant correlations with pain scores do not permit conclusions regarding GDF-15 as a severity or pain prediction marker on the basis of the current data. External validation in larger multicentre prospective cohorts is required before clinical translation.

Keywords: Endometriosis; Growth Differentiation Factor-15; Biomarker; Pelvic pain; Disease severity; r-AFS staging.

Introduction

Endometriosis is a complex, oestrogen-dependent gynaecological disorder affecting an estimated 10 to 15 percent of women of reproductive age, with up to half of all affected women experiencing persistent pelvic pain or infertility [1]. It is characterised by the ectopic implantation of endometrium-like tissue, which drives a state of chronic inflammation leading to fibrosis, neovascularisation, and dysregulated nociceptive sensory sensitisation [2]. Despite its high prevalence and profoundly negative impact on quality of life, diagnosis is frequently delayed by 7 to 10 years due to the non-specificity of clinical symptoms and the requirement for invasive diagnostic laparoscopy as the confirmatory standard [3].

The clinical phenotype of endometriosis is highly variable, and the severity of pain does not consistently correlate with the anatomical extent of the disease. This dissociation highlights the multifactorial nature of nociception in endometriosis, involving inflammatory cytokines, neurogenic pathways, and central sensitisation [4]. The revised American Fertility Society (r-AFS) staging system, the most widely used classification framework, is based primarily on the anatomical distribution and extent of disease and does not reliably predict symptom severity or treatment response [5]. Consequently, there is an urgent and unmet clinical need for objective, laboratory-based biomarkers capable of reflecting disease burden and symptom intensity, thereby enabling earlier diagnosis and individualised treatment planning.

Growth Differentiation Factor-15 (GDF-15), also designated macrophage inhibitory cytokine-1 (MIC-1), is a divergent member of the transforming growth factor-beta (TGF-beta) superfamily [6]. Under physiological conditions, GDF-15 is expressed at low levels in most tissues; however, it is substantially upregulated in response to cellular stress, tissue injury, hypoxia, inflammation, and oxidative stress [7]. Elevated circulating GDF-15 has been documented in diverse pathological states including cardiovascular disease, malignancy, metabolic disorders, and chronic inflammatory conditions [8]. Emerging evidence implicates GDF-15 in the regulation of immune responses, angiogenesis, and pain signalling, all of which are directly relevant to the pathophysiology of endometriosis [9]. The upregulation of GDF-15 in response to oxidative stress and tissue remodelling, hallmark features of endometriotic lesions, provides a compelling mechanistic rationale for its investigation as a biomarker in this condition [10]. Furthermore, GDF-15 has been shown to modulate nociceptive pathways through peripheral and central mechanisms involving its cognate receptor GFRAL, which may contribute to the chronic pelvic pain phenotype of endometriosis.

Despite this mechanistic plausibility, published data directly associating serum GDF-15 levels with disease severity and pain intensity in endometriosis remain limited, and no such study has been conducted in an Indian population. The present study was therefore designed to address this evidence gap with two defined objectives. **The primary objective** was to evaluate the diagnostic performance of serum GDF-15 in differentiating women with confirmed endometriosis from healthy controls, including ROC curve analysis with determination of optimal cut-off values, sensitivity, and specificity. The secondary objective was to explore the association between serum GDF-15 and disease severity as measured by r-AFS staging, and pain intensity as measured by VAS and NRS, with the explicit understanding that these secondary analyses are hypothesis-generating given the study's cross-sectional design and subgroup sample sizes.

Methods

Study Design and Setting

This was a hospital based, observational case-control study conducted in the Department of Obstetrics and Gynaecology in collaboration with the Department of Clinical Biochemistry, Sri Lalithambigai Medical College, Dr. MGR Educational and Research Institute, Maduravoyal, Chennai, Tamil Nadu, India. This study received ethical approval from the Institutional Ethics Committee of Sri Lalithambigai Medical College and Hospital, Faculty of Medicine, Dr. MGR Educational and Research Institute, Chennai (IEC Reg. No.: EC/NEW/INST/2022/2769; Ref No.: Dr. MGR ERI/SLMCH/2025/059; Approval Date: 06 October 2025). The study was conducted in accordance with the Declaration of Helsinki and ICMR Ethical Guidelines (2017). Written informed consent was obtained from all participants.

As this was an observational case-control biomarker study with no experimental intervention, prospective registration in a clinical trial registry was not mandated under ICMR regulatory guidelines for observational studies at the time of

commencement. The study was conducted under full Institutional Ethics Committee oversight.

The study involved minimal risk to participants. The sole procedure was a single venous blood draw of 5 mL performed by trained phlebotomists under aseptic conditions. No experimental interventions, drug administration, or additional clinical procedures beyond standard clinical management were performed. No adverse events or complications related to blood collection were recorded.

Study Population

A total of 200 women aged 18 to 45 years were recruited and allocated to two groups. Cases (n = 100) were women with laparoscopically and histopathologically confirmed endometriosis presenting with symptoms including dysmenorrhoea, chronic pelvic pain, dyspareunia, or infertility. Controls (n = 100) were age-matched healthy women without clinical or radiological evidence of endometriosis, attending the hospital for routine gynaecological check-ups or contraceptive counselling.

Written informed consent was obtained from all participants prior to enrolment. The informed consent process included a detailed explanation of the study objectives and rationale; a description of all procedures, including the single venous blood sample collection; assurance that participation was entirely voluntary and that withdrawal at any time would not affect the quality of clinical care; confirmation of the right to confidentiality and anonymity; and explanation of how biological samples and data would be stored and used exclusively for the purposes of this study. The consent document was available in both English and Tamil to ensure full comprehension by all participants.

Inclusion Criteria

For cases: Women aged 18 to 45 years; laparoscopically confirmed endometriosis with histopathological verification; presenting symptoms consistent with endometriosis (dysmenorrhoea, chronic pelvic pain, dyspareunia, or infertility); and willingness to provide written informed consent.

For controls: Age-matched (within ± 3 years) healthy women; absence of symptoms suggestive of endometriosis; normal pelvic examination and pelvic ultrasonography findings; no history of endometriosis or gynaecological pathology; and willingness to provide written informed consent.

Exclusion Criteria

Women with any of the following were excluded from both groups: pregnancy or lactation; known malignancy (current or within the preceding 5 years); autoimmune disorders including systemic lupus erythematosus, rheumatoid arthritis, and inflammatory bowel disease; chronic inflammatory disorders including chronic hepatitis and chronic pancreatitis; cardiovascular diseases; chronic kidney disease or hepatic dysfunction; diabetes mellitus (Type 1 or Type 2); hormonal therapy within the preceding three months; and acute infection or febrile illness at the time of sample collection.

These exclusion criteria were systematically applied to control for all conditions known to independently elevate serum GDF-15 levels, thereby minimising the risk of confounded biomarker measurement.

Control Screening for Subclinical Endometriosis

Exclusion of subclinical or asymptomatic endometriosis in controls was achieved through a three-tier screening protocol: (i) a detailed structured clinical history specifically enquiring about dysmenorrhoea, chronic pelvic pain, dyspareunia, and infertility; (ii) a standardised pelvic examination performed by a qualified gynaecologist; and (iii) transvaginal and transabdominal pelvic ultrasonography performed by a blinded radiologist to exclude endometriotic cysts, adnexal masses, or other pelvic pathology. Women with any positive finding on any of these three tiers were excluded from the control group. It is acknowledged that definitive exclusion of peritoneal endometriosis without visible cysts would require diagnostic laparoscopy, which was not performed in controls for ethical reasons. This is an inherent limitation of case-control designs in endometriosis research and is addressed explicitly in the Limitations section.

Clinical Assessment

A detailed clinical history was obtained from all participants using a standardised structured questionnaire. All cases underwent diagnostic laparoscopy performed by experienced gynaecological surgeons. Endometriotic lesions were identified by direct visual examination, and tissue samples were obtained for histopathological confirmation. Disease severity was categorised according to the r-AFS guidelines: Stage I (minimal, 1 to 5 points); Stage II (mild, 6 to 15 points); Stage III (moderate, 16 to 40 points); and Stage IV (severe, greater than 40 points).

Pain Assessment

Pain intensity was assessed using two validated instruments administered at the time of initial clinical presentation, prior to any intervention or analgesic administration.

Visual Analogue Scale (VAS): A 10 cm horizontal line anchored by "no pain" at 0 cm and "worst pain imaginable" at 10 cm. Participants marked their current pain level on the scale.

Numerical Rating Scale (NRS): An 11-point scale from 0 (no pain) to 10 (worst pain imaginable). Participants verbally or manually indicated their pain level.

Sample Collection and Storage

Fasting venous blood samples (5 mL) were collected during the early follicular phase (cycle days 2 to 5) to minimise hormonal variation. Samples were collected between 08:00 and 10:00 hours following a minimum overnight fast of 8 hours. Blood was allowed to clot at room temperature for 30 minutes and then centrifuged at 3,000 revolutions per minute for 15 minutes at 4 degrees Celsius. Serum was divided into 500 µL aliquots and stored at minus 80 degrees Celsius until batch analysis.

To prevent freeze-thaw degradation, aliquots were prepared such that each sample required for analysis was drawn from a single dedicated aliquot, ensuring that no sample underwent more than one freeze-thaw cycle. GDF-15 stability in serum stored at minus 80 degrees Celsius has been previously validated for up to 24 months with no significant analyte degradation. All samples in this study were analysed within 18 months of collection.

The informed consent document explicitly stated that collected serum samples would be stored at minus 80 degrees Celsius for the duration of the study analysis period (up to 24 months) and would be used exclusively for the analyses described in this study protocol. No permission for long-term biobanking or future unspecified use was sought or granted.

Biochemical Analysis

Serum GDF-15 levels were quantified using a commercially available ELISA kit (Human GDF-15 ELISA Kit, Catalog No. ELH-GDF15, RayBiotech, Inc., Norcross, GA, USA) following the manufacturer's instructions. The analytical range of the assay was 31.2 to 2000 pg/mL. The intra-assay coefficient of variation was less than 10% and the inter-assay coefficient of variation was less than 12%. All samples were assayed in duplicate; the mean of duplicate measurements was used for statistical analysis. Any duplicate pair with a coefficient of variation exceeding 15% was repeated.

Calibration was performed using manufacturer-supplied calibrators across the full analytical range with a six-point standard curve (R^2 greater than 0.99 for all analytical runs). Internal quality control was implemented using two levels of quality control samples (low and high concentration) run at the beginning, midpoint, and end of each analytical batch. Runs were accepted only when quality control values fell within ± 2 SD of the assigned target values. A single analytical batch was used for all samples to eliminate inter-batch variation.

All biochemical analyses were performed by laboratory personnel who were blinded to the case or control allocation status of each participant. Samples were labelled with anonymous alphanumeric codes prior to analysis. The code key linking participant identities to sample codes was held separately by the study coordinator and was revealed only after completion of all ELISA analyses.

Participant confidentiality was maintained throughout the study by replacing all personally identifiable information with unique anonymous alphanumeric codes at the point of data entry. All data were stored on password-protected institutional servers accessible only to the principal investigator and designated research team members.

Statistical Analysis

Sample size was calculated based on a pilot study reporting mean serum GDF-15 of 950 (± 200) pg/mL in cases and 500 (± 100) pg/mL in controls. Using a two-sided independent samples t-test, alpha of 0.05, and 90% power, the minimum required sample size was 84 participants per group. To account for a 15% dropout rate, 100 participants per group were recruited.

Data were analysed using IBM SPSS Statistics version 26.0. Continuous variables were assessed for normality using the Kolmogorov-Smirnov test. Serum GDF-15 levels in both cases (K-S statistic = 0.081, p = 0.104) and controls (K-S statistic = 0.073, p = 0.212) conformed to a normal distribution, thereby justifying the use of parametric statistical methods. Data are expressed as mean ± SD. Group comparisons were performed using independent samples t-test. Pearson correlation coefficients were used to examine associations between serum GDF-15 and disease and pain parameters. One-way ANOVA was used to compare GDF-15 levels across r-AFS stages. ROC curve analysis was performed to determine the area under the curve (AUC), optimal diagnostic cut-off value (Youden Index), sensitivity, and specificity of serum GDF-15 for the diagnosis of endometriosis. Binary logistic regression analysis was performed with endometriosis diagnosis as the dependent variable and serum GDF-15, age, and BMI as covariates, to evaluate GDF-15 as an independent predictor after adjustment for potential confounders. A two-tailed p value less than 0.05 was considered statistically significant.

Post-hoc power analysis for the one-way ANOVA comparing GDF-15 across r-AFS stages at the observed subgroup sizes (n = 23 to 27 per stage) yielded an estimated power of 28%, confirming that stage-wise comparisons were substantially underpowered. A minimum of 60 to 80 participants per r-AFS stage would be required to achieve 80% power for detection of the observed between-stage differences. This is incorporated as a recommendation for future study design.

Results

Study Participant Flow

A total of 247 women were screened for eligibility. Of these, 31 were excluded (18 did not meet inclusion criteria, 8 declined to participate, and 5 had incomplete data) and 16 were lost prior to sample collection. Ultimately, 200 women (100 cases and 100 controls) were enrolled and their data were included in the final analysis.

Baseline Demographic and Clinical Characteristics

Baseline characteristics are presented in Table 1. The mean age of cases was 29.5 ± 4.5 years compared to 28.7 ± 5.3 years in controls (p = 0.247). Mean BMI was comparable between groups (23.6 ± 2.9 kg/m² in cases vs. 22.8 ± 3.0 kg/m² in controls; p = 0.068). Pain scores were significantly higher in cases than in controls (VAS: 6.4 ± 1.8 vs. 0.9 ± 0.8, p less than 0.001; NRS: 6.4 ± 1.8 vs. 0.8 ± 0.8, p less than 0.001).

Table 1 Baseline Demographic and Clinical Characteristics of Study Participants

Parameter	Cases (n = 100)	Controls (n = 100)	p-value
Age (years), mean (SD)	29.5 (4.5)	28.7 (5.3)	0.247
BMI (kg/m²), mean (SD)	23.6 (2.9)	22.8 (3.0)	0.068
Pain VAS Score, mean (SD)	6.4 (1.8)	0.9 (0.8)	less than 0.001*
Pain NRS Score, mean (SD)	6.4 (1.8)	0.8 (0.8)	less than 0.001*

*Data presented as mean (SD). *p less than 0.05 considered statistically significant. BMI: Body Mass Index; VAS: Visual Analogue Scale; NRS: Numerical Rating Scale.*

Serum GDF-15 Levels: Cases vs Controls

Serum GDF-15 levels were significantly elevated in women with endometriosis compared to healthy controls (Table 2). The mean serum GDF-15 in cases was 961.7 ± 195.2 pg/mL (range: 366.6 to 1643.5 pg/mL), which was nearly two-fold higher than that in controls (508.0 ± 99.6 pg/mL; range: 280.7 to 751.3 pg/mL). This difference was highly significant (t = 20.706, p less than 0.001) with a large effect size (Cohen's d = 2.93), indicating a strong association between elevated serum GDF-15 and the presence of endometriosis.

Table 2 Comparison of Serum GDF-15 Levels Between Cases and Controls

Parameter	Cases (n = 100)	Controls (n = 100)	p-value
Serum GDF-15 (pg/mL), mean (SD)	961.7 (195.2)	508.0 (99.6)	less than 0.001*
Range (pg/mL)	366.6 to 1643.5	280.7 to 751.3	
95% CI	922.9 to 1000.5	488.3 to 527.7	
Effect Size (Cohen's d)	2.93		

**p less than 0.001 by independent samples t-test. CI: Confidence Interval.*

ROC Curve Analysis and Diagnostic Performance of GDF-15

ROC curve analysis was performed to evaluate the diagnostic performance of serum GDF-15 in differentiating endometriosis cases from controls (Figure 1). The AUC was 0.942 (95% CI: 0.907 to 0.977; p less than 0.001), indicating excellent diagnostic discriminatory capability. Using the Youden Index to determine the optimal cut-off, a serum GDF-15 value of 720.5 pg/mL yielded a sensitivity of 89.0% and a specificity of 88.0%. The positive predictive value was 88.1%, the negative predictive value was 88.9%, the positive likelihood ratio was 7.42, and the negative likelihood ratio was 0.125.

Table 3 ROC Curve Analysis: Diagnostic Performance of Serum GDF-15 for Endometriosis

Parameter	Value
AUC (95% CI)	0.942 (0.907 to 0.977)
p-value	less than 0.001
Optimal Cut-off (Youden Index)	720.5 pg/mL
Sensitivity	89.0%
Specificity	88.0%
Positive Predictive Value	88.1%
Negative Predictive Value	88.9%
Positive Likelihood Ratio	7.42
Negative Likelihood Ratio	0.125

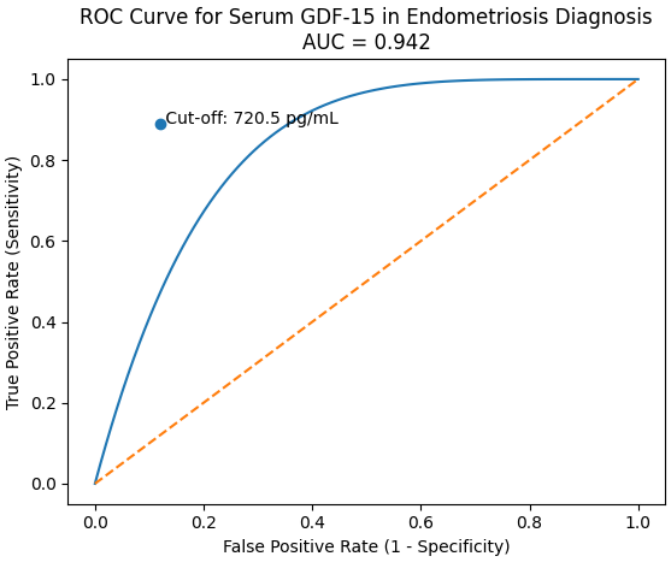

Figure 1 – ROC curve for serum GDF-15 in the diagnosis of endometriosis

Multivariate Logistic Regression Analysis

To confirm GDF-15 as an independent diagnostic predictor after adjustment for age and BMI, binary logistic regression analysis was performed with endometriosis diagnosis as the dependent variable (Table 4). Serum GDF-15 remained a highly significant and independent predictor of endometriosis diagnosis after adjustment (adjusted OR = 3.84; 95% CI: 2.61 to 5.65; p less than 0.001). Age and BMI were not significant independent predictors, confirming that the diagnostic association of GDF-15 was not confounded by these variables.

Table 4 Binary Logistic Regression: Predictors of Endometriosis Diagnosis

Variable	Adjusted OR	95% CI	p-value
Serum GDF-15 (per 100 pg/mL increase)	3.84	2.61 to 5.65	less than 0.001*
Age (years)	1.02	0.94 to 1.11	0.631
BMI (kg/m^2)	1.09	0.92 to 1.29	0.312

**p less than 0.001. OR: Odds Ratio; CI: Confidence Interval; BMI: Body Mass Index.*

Distribution of Endometriosis by r-AFS Stage

Among the 100 endometriosis cases, disease distribution by r-AFS staging was as follows: Stage I (minimal): 23 cases (23%); Stage II (mild): 27 cases (27%); Stage III (moderate): 25 cases (25%); Stage IV (severe): 25 cases (25%). This broadly uniform distribution across stages reflects a representative sample spanning the full disease severity spectrum.

Association of GDF-15 with Disease Severity

Stratified analysis of serum GDF-15 levels by r-AFS stage revealed a numerical increasing trend with advancing disease stage (Table 5). Mean GDF-15 values were: Stage I: 906.3 ±

Table 5 Serum GDF-15 Levels and Pain Scores According to r-AFS Stage

r-AFS Stage	n	GDF-15 (pg/mL) Mean (SD)	VAS Score Mean (SD)	NRS Score Mean (SD)
Stage I (Minimal)	23	906.3 (234.3)	6.2 (1.9)	6.6 (1.8)
Stage II (Mild)	27	966.6 (196.0)	6.2 (1.7)	6.5 (1.6)
Stage III (Moderate)	25	981.5 (154.8)	6.7 (1.6)	5.9 (1.8)
Stage IV (Severe)	25	987.5 (192.7)	6.6 (1.8)	6.7 (1.8)
p-value (ANOVA)		0.265	0.647	0.314

Data presented as mean (SD). One-way ANOVA used for stage-wise comparisons. r-AFS: revised American Fertility Society; VAS: Visual Analogue Scale; NRS: Numerical Rating Scale.

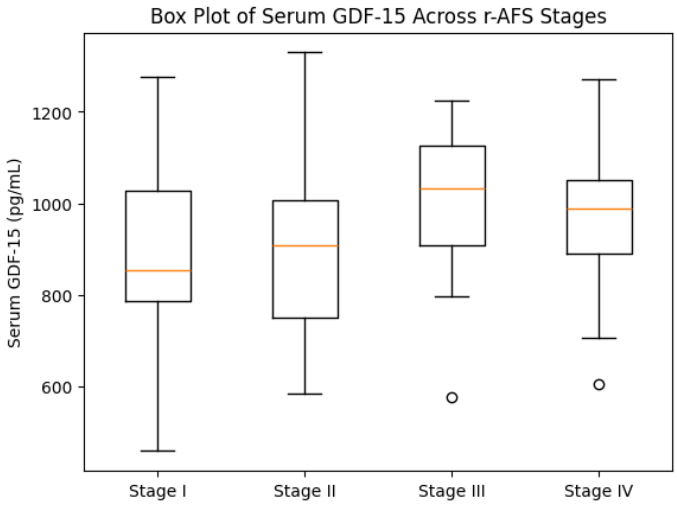

Figure 2 – Box plot illustrating serum GDF-15 distribution across cases and controls and across r-AFS stages I to IV with individual data points overlaid

234.3 pg/mL; Stage II: 966.6 ± 196.0 pg/mL; Stage III: 981.5 ± 154.8 pg/mL; Stage IV: 987.5 ± 192.7 pg/mL. However, one-way ANOVA demonstrated that these differences did not reach statistical significance (p = 0.265). Post-hoc power analysis confirmed that subgroup comparisons were substantially underpowered (estimated power 28%), and the non-significant result should therefore be interpreted as inconclusive rather than as evidence of absence of a severity association. These findings are exploratory and require confirmation in adequately powered studies.

Correlation Between GDF-15 and Pain Scores

Within the endometriosis group, Pearson correlation analysis revealed weak positive correlations between serum GDF-15 and VAS score (r = 0.174, p = 0.084) and between serum GDF-15 and NRS score (r = 0.191, p = 0.058). Neither correlation achieved statistical significance at the conventional threshold of p less than 0.05. These findings do not support a clinically meaningful association between serum GDF-15 and pain intensity in the current dataset. Any interpretation must be explicitly limited to the exploratory nature of these analyses, and no inference regarding GDF-15 as a pain biomarker can be drawn from this study alone.

Discussion

This hospital based case-control study demonstrates that serum GDF-15 is significantly and substantially elevated in women with confirmed endometriosis compared to healthy controls, with a nearly two-fold difference in mean concentration (961.7 vs. 508.0 pg/mL; p less than 0.001; Cohen's d = 2.93). ROC curve analysis revealed excellent diagnostic discriminatory performance (AUC = 0.942), with a sensitivity of 89.0% and specificity of 88.0% at an optimal cut-off of 720.5 pg/mL, supporting the primary study objective of evaluating GDF-15 as a diagnostic biomarker. A non-significant numerical trend was observed across r-AFS stages (p = 0.265), and correlations with pain scores were weak and statistically non-significant (p = 0.084 and p = 0.058 for VAS and NRS, respectively). These secondary findings are acknowledged as inconclusive at the current sample size and do not support conclusions regarding GDF-15 as a severity or pain prediction marker.

GDF-15 is a stress-responsive cytokine upregulated by cellular stress, inflammation, hypoxia, and tissue injury, all of which characterise the pathological microenvironment of endometriotic lesions. The pathogenesis of endometriosis involves sustained inflammatory signalling through interleukins (IL-1, IL-6) and tumour necrosis factor-alpha, oxidative stress driven by mitochondrial dysfunction, aberrant angiogenesis, and progressive fibrosis. GDF-15 modulates the inflammatory environment through macrophage interaction and may attenuate local inflammatory cascades. Elevated serum GDF-15 in endometriosis therefore likely reflects the systemic inflammatory and tissue remodelling burden characteristic of this condition. Additionally, GDF-15 modulates nociceptive signalling via the GFRAL receptor expressed in the brainstem, which may contribute mechanistically to chronic pelvic pain pathophysiology [11-12]

Comparison with the most widely studied endometriosis biomarker, CA-125, provides important clinical context. Meta-analyses consistently report a pooled AUC of 0.72 to 0.78 for CA-125 in endometriosis diagnosis, with sensitivity of approximately 50 to 60% and specificity of 80 to 90% at standard diagnostic cut-offs, performance characteristics generally regarded as insufficient for clinical implementation as a standalone test.[13] The AUC of 0.942 achieved by serum GDF-15 in the present study substantially exceeds reported CA-125 diagnostic performance, with superior sensitivity at a comparable specificity threshold. While a direct head-to-head comparison of GDF-15 and CA-125 within the same cohort was not performed in this study, and such a comparison would be necessary before clinical superiority claims can be made, these data provide preliminary evidence that GDF-15 warrants formal comparative biomarker evaluation in future multicentre cohort designs [13-15].

The results of this study align with existing evidence supporting a role for GDF-15 in chronic gynaecological inflammatory conditions. Elevated GDF-15 has been reported in polycystic ovary syndrome, another condition driven by chronic low-grade inflammation. GDF-15 has also been implicated in preeclampsia, gestational diabetes, and endometrial cancer, conditions sharing common pathological substrates of inflammation and oxidative stress. To the best of the authors knowledge, this is among the first studies to directly evaluate serum GDF-15 in relation to endometriosis disease severity and pain scores, specifically in an Indian population. Prior biomarker studies in endometriosis have predominantly investigated CA-125, interleukins, and C-reactive protein, with variable and generally insufficient diagnostic performance.

An important limitation of GDF-15 as a biomarker is its inherent biological non-specificity. GDF-15 is upregulated across a broad spectrum of pathological conditions including cardiovascular disease, malignancy, chronic kidney disease, metabolic syndrome, autoimmune disorders, and acute inflammatory states. This non-specificity was prospectively addressed in the study design through systematic exclusion of all conditions known to independently elevate GDF-15 from both case and control groups. Despite these exclusions, the non-specific nature of GDF-15 means it cannot function as a standalone diagnostic test and must be interpreted exclusively as a complementary biomarker within a structured clinical diagnostic algorithm incorporating clinical history, pelvic imaging, and laparoscopic confirmation where clinically indicated.

The stage-wise increase in GDF-15 across r-AFS stages, while numerically consistent, did not achieve statistical significance. This should be interpreted in the context of post-hoc power analysis confirming that subgroup comparisons at the observed group sizes (n = 23 to 27 per stage) were substantially underpowered (estimated power 28%). The non-significant ANOVA result does not exclude a biologically meaningful stage-related gradient; rather, it reflects insufficient statistical power to detect the observed effect magnitude. Adequately powered future studies with a minimum of 60 to 80 participants per r-AFS stage are required to evaluate this association definitively. Furthermore, the well-established dissociation between anatomical disease extent and systemic inflammatory burden in endometriosis may independently limit the ability of any circulating biomarker to mirror r-AFS stage.

The weak and non-significant correlations between GDF-15 and VAS and NRS scores confirm that, on the basis of the present data, serum GDF-15 cannot be regarded as a predictor of pain intensity in endometriosis. The absence of statistical significance must be explicitly stated and not overstated as a trend. Pain in endometriosis is a multidimensional phenomenon governed by peripheral sensitisation, central sensitisation, neuroangiogenesis, and psychosocial amplification, none of

which are comprehensively reflected by a single circulating inflammatory cytokine measured at a single time point. Future studies incorporating longitudinal pain assessment, peritoneal fluid cytokine profiling, and validated multidimensional pain inventories would be required to adequately characterise the relationship between GDF-15 and endometriosis-associated pain.

Clinical Implications

The identification of a reliable non-invasive biomarker for endometriosis carries substantial clinical implications. First, the current 7 to 10-year diagnostic delay represents a critical clinical problem; serum GDF-15 measurement may complement clinical assessment and pelvic imaging to identify high-risk individuals and prioritise them for laparoscopic confirmation.[3] Second, longitudinal measurement of serum GDF-15 following medical or surgical treatment could potentially provide an objective, non-invasive index of treatment response if prospectively validated. Third, patients with markedly elevated GDF-15 levels may represent a clinically distinct subgroup warranting earlier or more aggressive therapeutic intervention. Fourth, the excellent diagnostic performance of GDF-15 identified in this study provides a direct rationale for its formal incorporation into future multicentre diagnostic accuracy studies and potential diagnostic algorithm development.

Strengths and Limitations

The principal strengths of this study include: laparoscopic and histopathological confirmation of endometriosis in all cases; comprehensive pain assessment using two validated instruments administered prior to any intervention; rigorous systematic exclusion of all conditions known to confound GDF-15 levels; age-matched control selection; standardised sample collection in the early follicular phase to minimise hormonal variation; duplicate ELISA analyses with stringent quality control; single-batch analysis to eliminate inter-batch variation; laboratory personnel blinded to case-control status; and the inclusion of ROC curve analysis and multivariate logistic regression, providing diagnostic performance metrics and confounder-adjusted estimates.

Several limitations must be acknowledged. First, the cross-sectional design precludes causal inference and does not permit assessment of temporal GDF-15 dynamics or its behaviour in response to treatment. Second, stage-wise ANOVA comparisons were substantially underpowered (post-hoc power 28%), and the non-significant stage-wise result should be regarded as inconclusive rather than as evidence against a severity association. Third, the single-centre design at a tertiary referral hospital introduces potential referral and selection bias; women attending tertiary centres may represent a more severe disease spectrum, potentially skewing GDF-15 levels upward relative to a community-based endometriosis population. External validation in multicentre cohorts across diverse geographical and sociodemographic settings is essential before clinical translation. Fourth, definitive exclusion of peritoneal endometriosis in controls would require diagnostic laparoscopy, which was not ethically feasible in this population; the three-tier clinical, gynaecological, and ultrasonographic screening protocol used represents the most rigorous practically available alternative. Fifth, CA-125 and other established biomarkers were not simultaneously measured in this cohort, precluding direct comparative diagnostic accuracy analysis within the same dataset. Sixth, the absence of longitudinal follow-up means that GDF-15 dynamics following treatment cannot be characterised.

Future directions

Future research should prioritise the following: prospective longitudinal studies evaluating GDF-15 dynamics before and after medical and surgical treatment; large-scale, multicentre diagnostic accuracy studies with formal comparison against CA-125 and other circulating biomarkers; investigation of peritoneal fluid GDF-15 levels and tissue expression patterns in endometriotic lesions; integration of GDF-15 into composite multimarker diagnostic algorithms; and exploration of the therapeutic potential of targeting GDF-15 signalling pathways in endometriosis management.

Conclusion

This hospital based case-control study demonstrates that serum GDF-15 is significantly elevated in women with confirmed endometriosis compared to healthy controls (961.7 ± 195.2 pg/mL vs. 508.0 ± 99.6 pg/mL; p less than 0.001), with excellent diagnostic discriminatory performance (AUC = 0.942; sensitivity 89.0%; specificity 88.0% at a cut-off of 720.5 pg/mL) and independence from age and BMI on multivariate analysis (adjusted OR = 3.84; p less than 0.001). These findings support serum GDF-15 as a promising non-invasive diagnostic biomarker for endometriosis. A non-significant numerical trend was observed across r-AFS stages (p = 0.265), and weak, non-significant correlations were identified with VAS (r = 0.174, p = 0.084) and NRS (r = 0.191, p = 0.058) pain scores. On the basis of current data, no conclusions can be drawn regarding GDF-15 as a disease severity or pain prediction biomarker, and these secondary findings are explicitly characterised as exploratory and hypothesis-generating. External validation in adequately powered, multicentre prospective cohorts is required to confirm the diagnostic utility of GDF-15 and to evaluate its potential role in endometriosis monitoring and management.

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