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Diagnostic and Staging Value of Inter-Alpha-Trypsin Inhibitor Heavy Chain Family Member 4, Neutrophil Gelatinase-Associated Lipocalin, and Fibronectin Type III Domain-Containing Protein 5 as Non-Invasive Biomarkers for Endometriosis

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ABSTRACT

Background: *Endometriosis remains a diagnostic challenge owing to the reliance on invasive surgical confirmation. The identification of reliable serum biomarkers could facilitate earlier, non-invasive diagnosis and staging. This study aimed to evaluate the diagnostic and staging value of serum inter-alpha-trypsin inhibitor heavy chain family member 4 (ITIH4), neutrophil gelatinase-associated lipocalin (NGAL), and fibronectin type III domain-containing protein 5 (FNDC5) in women with endometriosis.* ***Methods:*** *This case–control study included 100 women: 50 with surgically confirmed endometriosis (27 stage I–II and 23 stage III–IV according to the revised American Society for Reproductive Medicine classification) and 50 age-matched healthy controls. Serum levels of ITIH4, NGAL, and FNDC5 were measured using ELISA.* ***Results:*** *Serum ITIH4 and NGAL levels were significantly elevated in the endometriosis group compared with controls (43.60 ± 11.96 vs. 14.30 ± 2.74 ng/mL; and 135.69 ± 41.89 vs. 41.02 ± 5.82 ng/mL, respectively; both $p < 0.001$), whilst FNDC5 was significantly reduced (4.17 ± 1.45 vs. 8.44 ± 1.23 ng/mL, $p < 0.001$). For diagnosis, the AUC values were 0.998 for ITIH4, 0.954 for FNDC5, and 0.984 for CA-125. For staging, FNDC5 achieved an AUC of 0.965, ITIH4 achieved 0.916, and NGAL achieved 0.794. The combined biomarker panel yielded an AUC of 0.995 for staging.* ***Conclusion:*** *Serum ITIH4, NGAL, and FNDC5 demonstrate excellent diagnostic and staging performance for endometriosis and may serve as promising non-invasive biomarkers to complement current diagnostic approaches.*

Keywords: Endometriosis; ITIH4; NGAL; FNDC5; Non-invasive diagnosis; Staging; rASRM.

Article Information

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INTRODUCTION

Endometriosis is a chronic inflammatory disease that is estrogen-dependent and characterizes, a chronic inflammatory illness that is estrogen-dependent. Roughly 10% of reproductive-age women throughout the globe are affected. [1].this ectopic tissue can cause tissue spread invasion organ damage or neoangiogenesis; it's found more common with clear cell carcinoma and endomatrioid neoplasm in comparison with healthy women as there is some cytokine are found increased level in both cancer and endometriosis; pro-inflammatory and anti-inflammatory cytokine influencing proliferation and differentiation of endometriosis tissue[2]

The ailment presents with incapacitating symptoms such as persistent pelvic discomfort, painful menstruation, painful intercourse, and reduced fertility, significantly impacting the quality of life and reproductive capabilities of the women afflicted. Notwithstanding its widespread occurrence, endometriosis continues to pose challenges for prompt identification, with a delay of 6.7 years from the

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beginning of symptoms to a conclusive diagnosis. This extended duration is mostly due to the dependence on laparoscopic visualization coupled with histological validation as the definitive diagnostic criterion [5].

The revised ASRM classification categorises endometriosis into four stages based upon the location, extent, and depth of implants and adhesions [6]. Whilst this staging system provides a standardised framework, it necessitates invasive intervention, precluding its utility as a screening tool. Therefore, there is a critical need for trustworthy, non-invasive biomarkers that can identify endometriosis and differentiate between phases of the illness without the need for surgery. A meta-analysis demonstrated that CA-125 at a threshold of 30 U/mL yields a sensitivity of only 52% for all stages of endometriosis [7,8]. These limitations underscore the necessity for novel biomarkers with superior discriminatory capacity.

ITIH4 is a type II acute-phase protein synthesised predominantly by the liver in response to inflammatory stimuli [9]. ITIH4 participates in extracellular matrix stabilisation through interactions with hyaluronic acid and has been implicated in tissue remodelling [10]. Emerging evidence has identified ITIH4 as a potential biomarker in malignancies, sepsis, and inflammatory conditions [11,12]. Its involvement in inflammatory cascades and extracellular matrix remodelling renders it a biologically plausible candidate biomarker for endometriosis.

Activated neutrophils swiftly secrete the NGAL, which is also known as lipocalin-2, an acute-phase glycoprotein, in reaction to inflammation and tissue injury [13]. NGAL is involved in the process of epithelial-mesenchymal transition, which is important for the development of endometriosis [14]. It also forms a complex with MMP-9, which helps break down extracellular matrix and allows cells to invade [15]. Endometriosis is a painful condition that may be diagnosed without invasive procedures, and recent research have shown that women with this condition have higher amounts of NGAL in their blood[16,17]. FNDC5 is a glycoprotein transmembrane that undergoes proteolytic cleavage to release irisin, a myokine with well-established properties anti-inflammatory [18]. Irisin attenuates pro-inflammatory cytokine release and modulates immune cell function [19]. Given that endometriosis is characterised by chronic peritoneal inflammation, alterations in FNDC5/irisin levels may reflect the inflammatory milieu of the disease [20]. Although the connection to disease severity is still not fully understood, preliminary research suggests that endometriosis patients have altered circulating irisin levels [21].

No prior research has investigated the diagnostic and staging usefulness of endometriosis using ITIH4, NGAL, and FNDC5 all at once as far as we are aware. Thus, the current research set out to compare the blood levels of these three biomarkers in women who had endometriosis surgically verified to those in healthy controls.

MATERIALS AND METHODS

Study Design

A case–control study was conducted at Babylon Teaching Hospital for maternity and Children, in the central Iraq Babylon Gouvernate from Desember 2025 to march 2026. One hundred women of reproductive age were recruited and allocated into two groups. The endometriosis group comprised 50 women with surgically confirmed endometriosis who underwent laparoscopic surgery at the aforementioned institution. Disease staging was performed intraoperatively according to the rASRM classification system [4], yielding 27 women with early-stage disease and 23 women with advanced-stage disease. The control group comprised 50 age-matched apparently healthy women attending the gynaecology outpatient clinic for routine examination, with no clinical symptoms.

criteria of participants

Women aged 18 to 45 years were eligible for inclusion. For the endometriosis group, eligibility required surgical confirmation of endometriosis via laparoscopy or laparotomy with histopathological verification. For the control group, eligibility required the absence of any clinical, ultrasonographic, pelvic inflammatory disease, or other pelvic pathology. Exclusion criteria applied to both groups comprised: current pregnancy or lactation; use of hormonal therapy within the preceding three months; presence of known malignancy; diagnosed autoimmune diseases; chronic inflammatory conditions hepatic or renal impairment; body mass index exceeding 35 kg/m²; acute infection or febrile illness at the time of sampling; and prior surgical treatment for endometriosis in the endometriosis group.

Sample Collection and Processing

All participants had 5 mL of peripheral venous blood drawn during the early proliferative phase of their menstrual cycles (days 2–5) in order to reduce the impact of cyclical hormonal fluctuation. Preoperative samples were collected from the endometriosis group on the morning of the planned surgical surgery. Under aseptic settings, blood was taken into simple serum separator tubes after an 8-hour overnight fast. Before biochemical analysis, samples were centrifuged at 3000 revolutions per minute for 15 minutes. Afterward, samples were kept at −80°C.

Biochemical Measurements

One commercially available kit, with the catalog number E2758Hu, was used to quantify serum ITIH4 concentrations. The sensitivity of the test was 0.13 ng/mL, while the detection range was 0.3-90 ng/mL. Both the intra- and inter-assay CVs were less than 8% and 10%, respectively. To find the amounts of NGAL in the serum, researchers used a double-antibody sandwich ELISA kit (MBS268158, catalog no.). The detection range of the test was 0.312-20 ng/mL, and it made use of a biotin-conjugated antibody that is specific for human NGAL. The amounts of serum FNDC5 were determined with the use of a sandwich ELISA kit (Cat No. E3206Hu). The sensitivity was 2.43 ng/mL, and the detection range was 5-1000 ng/mL. Both the intra- and inter-assay CVs were less than 8% and 10%, respectively. Complete adherence to the manufacturer's guidelines was maintained throughout all ELISA techniques. We used an automated hematology analyzer (sysmex xn-1000) to measure all of the blood count specs. The ELFA was used to ascertain hormonal parameters.

Statistical Analysis

A version of GraphPad Prism 9.0 was used to conduct the statistical analyzes. The Shapiro-Wilk test was used to determine whether the continuous data distribution was normal. The mean ± SD was used to compare groups for normally distributed continuous variables in the independent samples t-test. To assess the relationships between serum biomarker levels and clinical parameters, we used Spearman's rank (rho). The diagnostic and staging performance of each biomarker was evaluated using ROC curve analysis. Biomarkers and illness status were evaluated using binary logistic regression analysis for both univariate and multivariate relationships.

RESULTS

Within the age and body mass index categories, no statistically significant differences were found between the endometriosis and control groups (26.76 ± 7.11 vs. 25.50 ± 2.56 years, p = 0.247 and 24.41 ± 2.77 vs. 25.19 ± 2.24 kg/m², p = 0.119, respectively). In the endometriosis group, serum CA-125 and CRP levels were substantially higher than in the control group (48.56 ± 19.74 vs. 19.92 ± 4.60 U/mL and 6.10 ± 2.73 mg/L, respectively, with a p-value less than 0.001).

Table 1 shows that the endometriosis group had considerably higher NLR and PLR levels ($p < 0.001$ for both), and significantly lower hemoglobin and AMH levels ($p < 0.001$ for both).

Table 1. demographics, clinical presentations of the study groups.

Parameters	Endometriosis (n = 50)	Control (n = 50)	p-value
Age (years)	26.76 ± 7.11	25.50 ± 2.56	0.247
BMI (kg/m²)	24.41 ± 2.77	25.19 ± 2.24	0.119
CA-125 (U/mL)	48.56 ± 19.74	19.92 ± 4.60	< 0.001
CRP (mg/L)	6.10 ± 2.73	1.98 ± 0.87	< 0.001
Oestradiol (pg/mL)	172.65 ± 75.37	182.18 ± 72.26	0.522
NLR	3.32 ± 0.75	2.02 ± 0.42	< 0.001
WBC (×10³/µL)	8.44 ± 1.53	6.60 ± 1.08	< 0.001
PLR	174.42 ± 32.38	119.87 ± 20.80	< 0.001
Haemoglobin (g/dL)	11.83 ± 0.69	13.35 ± 0.79	< 0.001
AMH (ng/mL)	1.96 ± 0.86	3.14 ± 1.07	< 0.001
FSH (mIU/mL)	8.85 ± 2.26	6.55 ± 1.68	< 0.001
LH (mIU/mL)	7.06 ± 2.24	5.03 ± 1.52	< 0.001

Figure 1 and Table 2 show the serum levels of FNDC5, NGAL, and ITIH4 in the control group and the endometriosis group, respectively. Compared to the healthy group, the endometriosis group had substantially higher serum ITIH4 levels (43.60 ± 11.96 vs. 14.30 ± 2.74 ng/mL, $p < 0.001$; Cohen's $d = 3.378$). Likewise, the endometriosis group had noticeably elevated serum NGAL levels (135.69 ± 41.89 vs. 41.02 ± 5.82 ng/mL, $p < 0.001$; Cohen's $d = 3.166$). On the other hand, blood FNDC5 levels were noticeably lower in the endometriosis group as compared to the healthy group (4.17 ± 1.45 vs. 8.44 ± 1.23 ng/mL, $p < 0.001$; Cohen's $d = -3.163$).

Table 2. Serum biomarker levels in the study groups.

Biomarkers	Endometriosis (n = 50)	Control (n = 50)	p-value	Cohen's d
ITIH4 (ng/mL)	43.60 ± 11.96	14.30 ± 2.74	< 0.001	3.378
NGAL (ng/mL)	135.69 ± 41.89	41.02 ± 5.82	< 0.001	3.166
FNDC5 (ng/mL)	4.17 ± 1.45	8.44 ± 1.23	< 0.001	−3.163

Table 3 displays the results of comparing the serum biomarker levels of study groups in its early and advanced stages, according to the rASRM. In stage III-IV, the levels of serum ITIH4 were noticeably greater than in stage I-II (53.57 ± 9.57 vs. 35.10 ± 5.38 ng/mL, $p < 0.001$). Additionally, advanced-stage illness was accompanied by considerably higher serum NGAL levels (164.77 ± 45.02 vs. 110.92 ± 14.36 ng/mL, $p < 0.001$). While in stage I-II the serum FNDC5 levels were 5.34 ± 0.71 ng/mL, in stage III-IV they were considerably lower at 2.81 ± 0.70 ng/mL ($p < 0.001$).

Table 3. Serum biomarker levels and clinical parameters according to rASRM staging.

Parameters	Stage I–II (n = 27)	Stage III–IV (n = 23)	p-value
ITIH4 (ng/mL)	35.10 ± 5.38	53.57 ± 9.57	< 0.001
NGAL (ng/mL)	110.92 ± 14.36	164.77 ± 45.02	< 0.001
FNDC5 (ng/mL)	5.34 ± 0.71	2.81 ± 0.70	< 0.001
CA-125 (U/mL)	34.07 ± 7.66	65.57 ± 15.45	< 0.001
CRP (mg/L)	4.74 ± 2.19	7.70 ± 2.44	< 0.001
VAS pain score	5.00 ± 1.52	7.13 ± 1.42	< 0.001
Infertility duration (y)	3.81 ± 1.33	5.74 ± 1.81	< 0.001
Endometrioma diameter (cm)	4.15 ± 0.77	7.00 ± 2.07	< 0.001

Table 4 and Figure 1 show that there were substantial relationships between the new biomarkers and established clinical indicators within the endometriosis group, as demonstrated by Spearman's rank correlation analysis. The serum ITIH4 was correlated positively with CA-125 (rho = 0.678, p < 0.001), CRP (rho = 0.455, p = 0.001), VAS pain level (rho = 0.348, p = 0.013), infertility duration (rho = 0.446, p = 0.001), and endometrioma diameter (rho = 0.513, p < 0.001).

Table 4. The correlation coefficients between biomarkers and clinical parameters in the endometriosis group.

Clinical Parameters	ITIH4 (rho)	p-value	NGAL (rho)	p-value	FNDC5 (rho)	p-value
CA-125	0.678	< 0.001	0.537	< 0.001	−0.652	< 0.001
CRP	0.455	0.001	0.252	0.078	−0.445	0.001
VAS pain score	0.348	0.013	0.276	0.052	−0.556	< 0.001
Infertility duration	0.446	0.001	0.341	0.015	−0.387	0.005
Endometrioma diameter	0.513	< 0.001	0.423	0.002	−0.614	< 0.001
AMH	−0.188	0.191	−0.276	0.053	0.192	0.181
Age	−0.307	0.030	−0.185	0.199	0.088	0.545
BMI	−0.020	0.891	−0.049	0.733	0.118	0.415

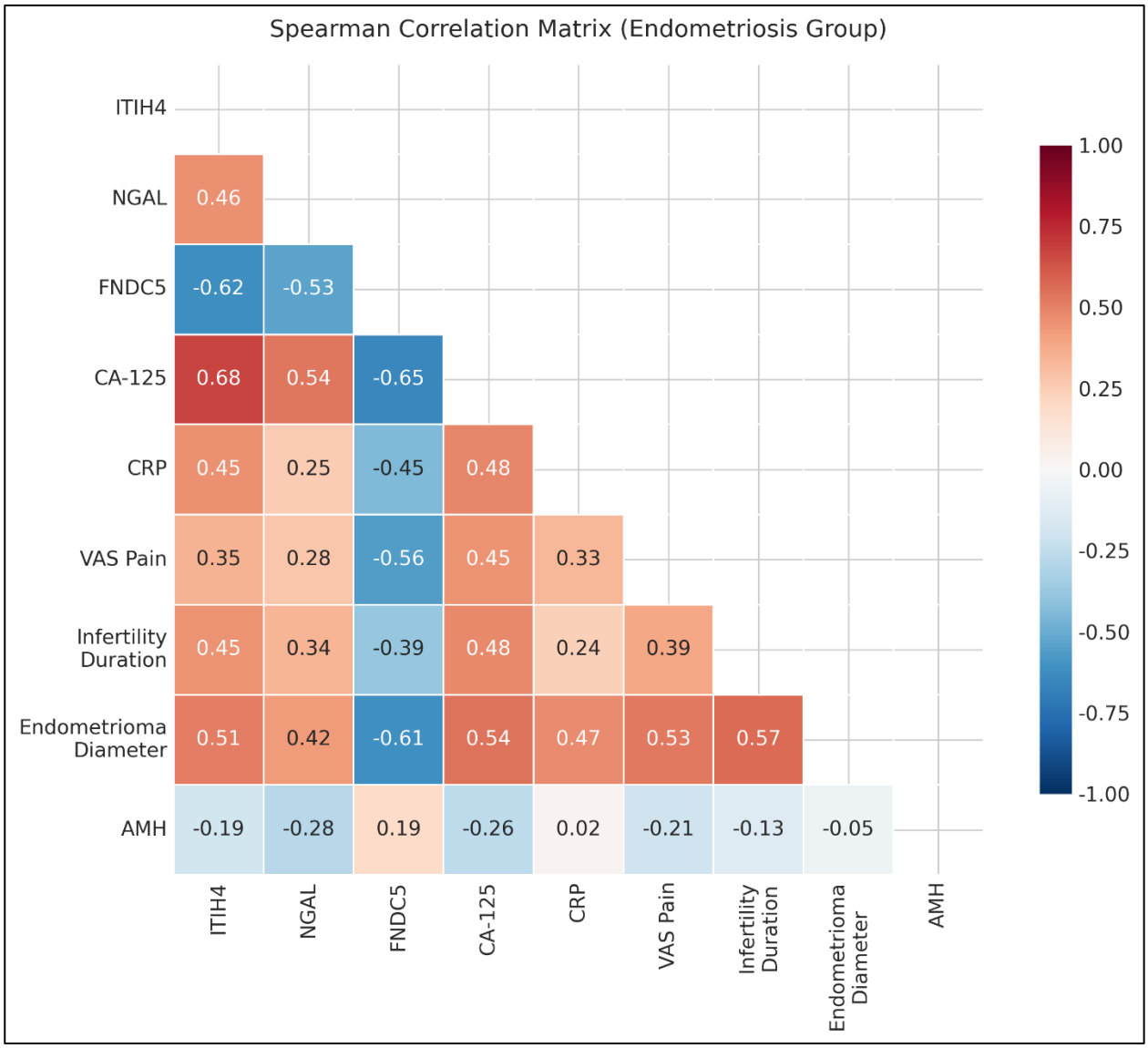

Figure 1. Spearman correlation matrix heatmap illustrating the inter-relationships between serum biomarkers and clinical parameters in the endometriosis group.

The diagnostic performance of serum ITIH4, NGAL, and FNDC5 in differentiating between healthy controls and endometriosis. The optimum cut-off for ITIH4 was 23.64 ng/mL, and the AUC was 0.998 (95% CI: 0.993-1.000) with a sensitivity of 100% and a specificity of 98.0%. Using a cut-off of 65.72 ng/mL, NGAL attained an AUC of 0.997 (95% CI: 0.990-1.000) (sensitivity 100%, specificity 100%). FNDC5 produced an AUC of 0.954 (95% CI: 0.912-0.996) at a cut-off of 6.48 ng/mL or less (84.0% sensitivity, 94.0% specificity). When compared, CA-125 had a sensitivity of 86.0% and a specificity of 100%, with a cut-off of 28.0 U/mL and an AUC of 0.984 (95% CI: 0.962-1.000). With an area under the curve (AUC) of 0.999 (95% CI: 0.997-1.000), the logistic regression-derived combination biomarker panel (ITIH4 + NGAL + FNDC5) was a success (Table 5 and Figure 2).

Table 5. ROC curve analysis for the diagnosis of endometriosis (endometriosis vs. control).

Biomarkers	AUC (95% CI)	Cut-off	Sensitivity (%)	Specificity (%)
ITIH4 (ng/mL)	0.998 (0.993–1.000)	≥ 23.64	100.0	98.0
NGAL (ng/mL)	0.997 (0.990–1.000)	≥ 65.72	100.0	100.0
FNDC5 (ng/mL)	0.954 (0.912–0.996)	≤ 6.48	84.0	94.0
CA-125 (U/mL)	0.984 (0.962–1.000)	≥ 28.0	86.0	100.0
Combined panel	0.999 (0.997–1.000)	—	100.0	98.0

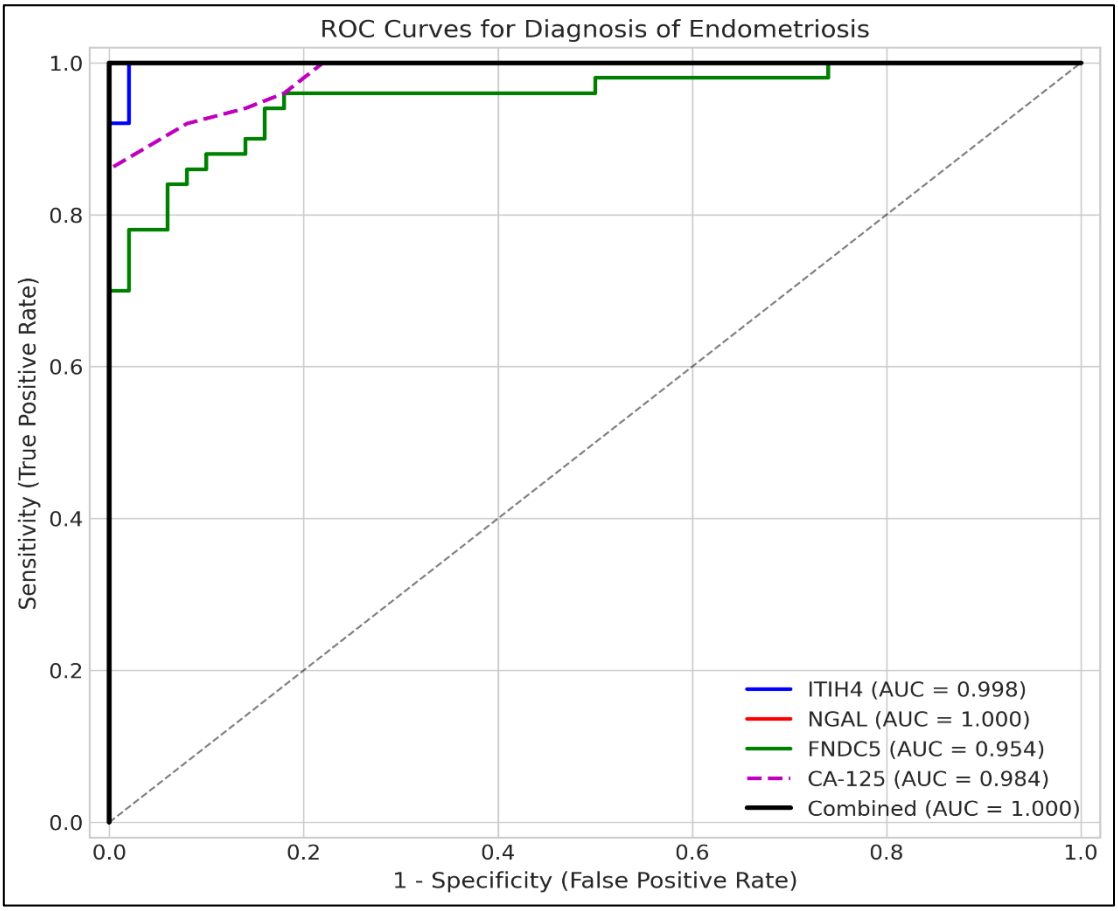

Figure 2. ROC curves for the diagnosis of endometriosis. The combined panel (ITIH4 + NGAL + FNDC5) achieved the highest diagnostic accuracy.

With a 95% CI of 0.920-1.000; a cut-off of 4.13 ng/mL or less; 100% sensitivity; and 85.2% specificity, FNDC5 showed the best individual staging accuracy. With a cut-off of 45.0 U/mL, sensitivity of 91.3%, and specificity of 96.3%, CA-125 attained an AUC of 0.957 (95% CI: 0.908-1.000). AUC = 0.916 (95% CI: 0.838-0.994; cut-off > 44.62 ng/mL; sensitivity = 82.6%, specificity = 92.6%), was produced using ITIH4. With a cut-off of 126.83 ng/mL, sensitivity of 69.6%, and specificity of 85.2%, NGAL showed an AUC of 0.794 (95% CI: 0.667-0.921). According to Table 6 and Figure 3, the combined panel produced an AUC for staging of 0.995 (95% CI: 0.982-1.000).

Table 6. ROC curve analysis for the staging of endometriosis.

Biomarkers	AUC (95% CI)	Cut-off	Sensitivity (%)	Specificity (%)
ITIH4 (ng/mL)	0.916 (0.838–0.994)	≥ 44.62	82.6	92.6
NGAL (ng/mL)	0.794 (0.667–0.921)	≥ 126.83	69.6	85.2
FNDC5 (ng/mL)	0.965 (0.920–1.000)	≤ 4.13	100.0	85.2
CA-125 (U/mL)	0.957 (0.908–1.000)	≥ 45.0	91.3	96.3
Combined panel	0.995 (0.982–1.000)	—	100.0	96.3

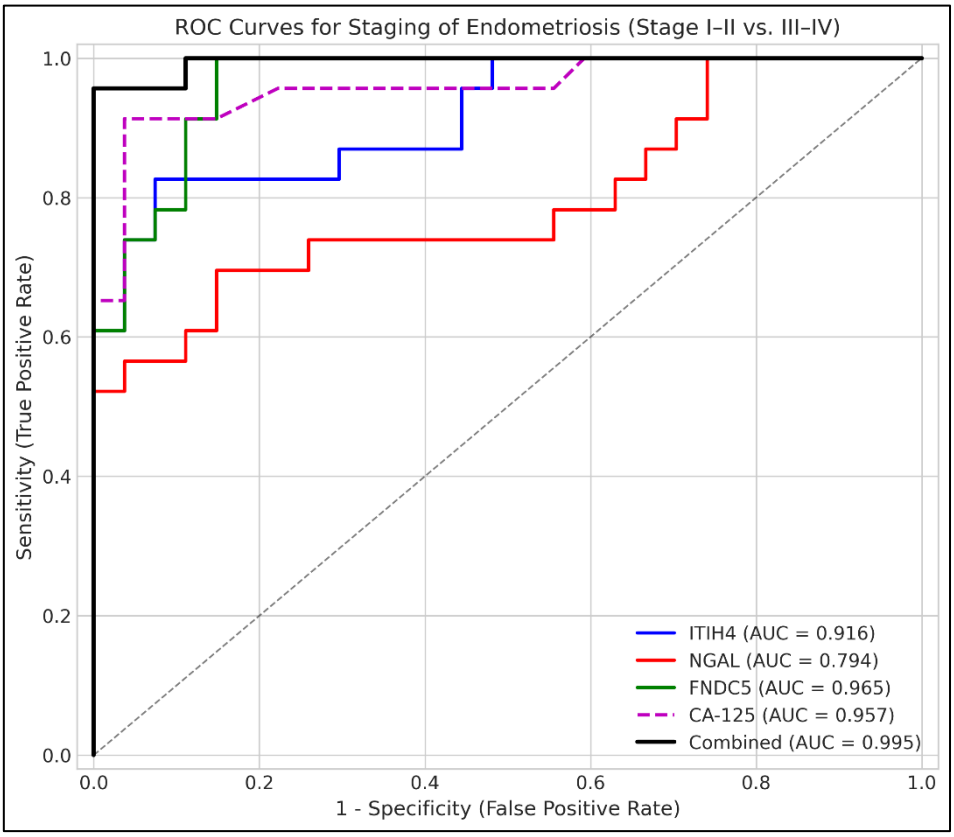

Figure 3. ROC curves for the staging of endometriosis stage.

In the univariate model, ITIH4 demonstrated an odds ratio (OR) of 1.414 (95% CI: 1.22–1.64, $p < 0.001$), NGAL demonstrated an OR of 1.406 (95% CI: 1.21–1.63, $p < 0.001$), and FNDC5 demonstrated an OR of 0.712 (95% CI: 0.59–0.86, $p < 0.001$). In the multivariate logistic regression model incorporating all three biomarkers simultaneously, each retained independent significance: ITIH4 (adjusted OR = 1.305, $p < 0.001$), NGAL (adjusted OR = 1.299, $p < 0.001$), and FNDC5 (adjusted OR = 0.771, $p < 0.001$). For staging prediction (Stage I–II vs. III–IV), univariate logistic regression yielded OR values of 2.492 ($p < 0.001$) for ITIH4, 2.134 ($p < 0.001$) for NGAL, and 0.351 ($p < 0.001$) for FNDC5 (Table 7).

Table 7. Binary logistic regression analysis for the diagnosis of endometriosis.

Models	Biomarkers	B	OR (95% CI)	p-value
Univariate	ITIH4	0.347	1.414 (1.22–1.64)	< 0.001
Univariate	NGAL	0.341	1.406 (1.21–1.63)	< 0.001
Univariate	FNDC5	−0.340	0.712 (0.59–0.86)	< 0.001
Multivariate	ITIH4	0.266	1.305 (1.14–1.49)	< 0.001
Multivariate	NGAL	0.261	1.299 (1.13–1.49)	< 0.001
Multivariate	FNDC5	−0.260	0.771 (0.65–0.91)	< 0.001

OR = odds ratio

The predictive association between the three new biomarkers and serum CA-125 levels in the endometriosis group was evaluated using multiple linear regression analysis (Table 8). The overall model demonstrated statistical significance ($F(3,46) = 20.369$, $p < 0.001$) and accounted for 57.1% of the variance in CA-125 levels ($R^2 = 0.571$, adjusted $R^2 = 0.543$). FNDC5 emerged as the most significant independent predictor ($B = −5.117$, $SE = 1.857$, $t = −2.756$, $p = 0.008$), with NGAL following closely ($B = 0.114$, $SE = 0.056$, $t = 2.047$, $p = 0.046$) and ITIH4 also contributing ($B = 0.441$, $SE = 0.225$, $t = 1.958$, $p = 0.056$).

Table 8. Multiple linear regression analysis predicting CA-125 from serum biomarkers in the endometriosis group.

Predictors	B	SE	t	p-value	95% CI for B
(Constant)	35.283	17.154	2.057	0.045	0.76–69.81
ITIH4	0.441	0.225	1.958	0.056	−0.01–0.89
NGAL	0.114	0.056	2.047	0.046	0.002–0.225
FNDC5	−5.117	1.857	−2.756	0.008	−8.85–(−1.38)

Model summary: $R^2 = 0.571$, Adjusted $R^2 = 0.543$, $F(3,46) = 20.369$, $p < 0.001$.

DISCUSSION

The current investigation reveals, for the first time, the concurrent assessment of serum ITIH4, NGAL, and FNDC5 as non-invasive indicators for diagnosing and staging endometriosis. Our findings demonstrate that blood concentrations of ITIH4 and NGAL are markedly elevated, but FNDC5 levels are decreased significantly in women with endometriosis relative to healthy controls. Moreover, these biomarkers show a notable correlation with disease severity as categorized by the rASRM staging system, with more advanced stages of the disease displaying more significant changes. The diagnostic performance of all three biomarkers, both individually and in combination, was outstanding, indicating their potential clinical usefulness as non-invasive supplements to existing diagnostic methods.

The marked increase of serum ITIH4 in endometriosis patients in the present study (43.60 ± 11.96 vs. 14.30 ± 2.74 ng/mL, p <0.001) is in line with its known function as a acute-phase protein that is upregulated under conditions of inflammatory [9]. The plays a key part in extracellular matrix stabilisation by covalent modification with hyaluronan [10].

The pathophysiology of endometriosis entails chronic peritoneal inflammation, abnormal tissue remodeling, and degradation of the extracellular matrix [22], all of which could induce ITIH4 expression. Our results were consistent with the Wölfler et al findings. [23], ITIH4 fragments were discovered by two-dimensional gel electrophoresis in the peritoneal fluid of women with endometriosis. Additionally, Lo Turco et al. [24] identified ITIH4 in the follicular fluid of women with infertility. In addition, Ma et al. [11] revealed that ITIH4 act as a vital inflammatory marker associated with the degree of systemic inflammatory responses, which were in line with the increasing ITIH4 expression in the late-stage tumours (53.57 ± 9.57 in stage III–IV vs. 35.10 ± 5. 38 ng/mL in stage I–II). The strong positive correlation of ITIH4 with CA-125 (rho = 0.678; $p < 0.001$) also supports, in line with other evidence, an inflammatory pathogenesis for the raised ITIH4 in endometriosis, as CA-125 is a known inflammation-related glycoprotein. Remarkably, the ROC curve determined that ITIH4 (AUC=0.998) had better performance than CA-125 (AUC=0.984) for diagnosis, indicating its higher sensitivity.

The elevated serum NGAL levels in our endometriosis group (135.69 ± 41.89 vs. 41.02 ± 5.82 ng/mL, $p < 0.001$) are consistent with the findings of Guney et al. [16], who reported significantly increased serum NGAL levels in women with endometrioma compared with controls and proposed NGAL as a potential endometriosis non-invasive biomarker. Similarly, Krizanac et al. [17] reported significantly increased circulating lipocalin-2 (NGAL) levels in endometriosis patients in their comprehensive review of NGAL expression patterns in the reproductive system. The biological plausibility of NGAL elevation in endometriosis is well-established: NGAL is promptly released by activated neutrophils in response to tissue damage and inflammation, is involved in the epithelial–mesenchymal transition process that plays a key role in the pathogenesis of endometriosis, and forms a complex with MMP-9 that promotes extracellular matrix degradation and cellular invasion [14,15]. Our finding of significantly elevated NGAL levels in stage III–IV disease compared to other stage (164.77 ± 45.02 vs. 110.92 ± 14.36 ng/mL) supplements the results of Guney et al. [16] by demonstrating a stage-related increase, suggesting that NGAL may be a marker of disease burden and activity. Nevertheless, our findings do not agree with those of Durmus et al.[25] which showed no difference in urinary NGAL levels between study groups and HCs. This discrepancy may be attributable to the different biological compartments assessed (serum versus urine), as serum NGAL may more accurately reflect systemic inflammatory activation in endometriosis. The moderate staging AUC of NGAL (0.794) compared with ITIH4 (0.916) and FNDC5 (0.965) suggests that whilst NGAL is an excellent diagnostic marker, its staging utility may be enhanced when combined with other biomarkers.

The notable decrease in serum FNDC5 levels noted in our endometriosis cohort (4.17 ± 1.45 vs. 8.44 ± 1.23 ng/mL, $p < 0.001$) signifies a new and clinically important discovery. FNDC5 functions as the precursor of irisin, a myokine celebrated for its significant anti-inflammatory actions, which lower the pro-inflammatory cytokines production [18,19]. The diminished FNDC5 levels in endometriosis may reflect the consumption or suppression of this anti-inflammatory mediator in the context of chronic peritoneal inflammation. Slate-Romano et al. [30] demonstrated that irisin reduces inflammatory pathways signalling by attenuating activation NF-κB and reducing production of IL-6 and TNF-α, mechanisms directly relevant to endometriosis pathophysiology. Our findings appear to contrast with those of Kaya Sezginer et al. [21], who reported elevated serum irisin levels in

endometriosis patients. However, several methodological differences might explain this discrepancy, such as the determination of FNDC5 (the membrane-bound precursor) rather than irisin (the cleaved circulating fragment), differences in assay specificity, size of the sample (our study: n = 100 vs. their study: n = 30), and staging of different diseases.

It is biologically believable that although circulating irisin could be transiently increased as a compensatory anti-inflammatory effect, the membrane-bound FNDC5 precursor could be depleted due to enhanced cleavage, hence reduced serum levels of FNDC5. In addition, the inverse and significant correlations of FNDC5 with markers of disease severity (endometrioma diameter: rho = −0.614; VAS pain score: rho = −0.556; CA-125: rho = −0.652) further corroborate the expectation that the loss of FNDC5 correlates to an inflammatory disease severity. Khan et al. [28] and Barbagallo et al.[29] have likewise outlined the intricate function of irisin/FNDC5 in reproductive diseases including that its anti-inflammatory actions may not be able to counter in conditions of chronic inflammation.

The logistic regression analysis added further confirmation to our results. In the multivariate analysis, all three markers maintained independent predictive value for endometriosis (ITIH4: OR = 1.305; NGAL: OR = 1.299; FNDC5: OR = 0.771; all $p < 0.001$), suggesting that each one of them provides unique information. Multivariate linear regression analysis also revealed that these three biomarkers accounted for 57.1% of the variance of CA-125 ($R^2 = 0.571$, $F = 20.369$, $p < 0.001$), with FNDC5 being the most powerful solitary predictor (B = -5.117, p = 0.008).This is of clinical relevance because it implies that these novel markers not only use similar pathophysiological tracks of CA-125, but also include additional diagnostic information.

The ROC curve analysis showed excellent diagnostic performance, with AUC values of 0.998 for ITIH4, 0.997 for NGAL and 0.954 for FNDC5. FNDC5 showed the best individual accuracy for staging (AUC = 0.965), followed by CA-125 (AUC = 0.957), ITIH4 (AUC = 0.916) and NGAL (AUC = 0.794). The combined panel showed an AUC of 0.995 for staging, indicating that the use of several biomarkers is more beneficial in the clinic than single markers. These results are in agreement with the recent trend in the biomarker literature showing that panels of complementary markers are superior to single biomarkers for complex diseases (e.g., endometriosis) [26]. Foda and Aal [27] also recommended the use of multi-biomarker panels including inflammatory markers.

Several limitations of the present study should be acknowledged. The results of our study may be limited by the sample of 100 people which could affect generalizability. The the study precludes assessment of changes in biomarker concentrations, or their response to therapy, over time. In addition, the control group was composed of healthy women rather than women with other pelvic diseases (eg, ovarian cysts or pelvic inflammatory disease), which could potentially overestimate the diagnostic specificity in clinical practice. The high AUC values are although statistically correct need for external validation in independent cohorts before clinical use. Further studies with larger samples as well as inclusion of disease controls, longitudinal follow-up.

CONCLUSION

The current research shows that serum ITIH4, NGAL, and FNDC5 are potential non-invasive biomarkers for endometriosis staging and diagnosis. Females diagnosed with endometriosis had markedly elevated blood concentrations of ITIH4 and NGAL, with dramatically decreased levels of FNDC5, with progressive alterations that aligned with the advancement of the condition. The integrated biomarker panel outperformed CA-125 alone in

both staging (AUC = 0.995) and diagnosis (AUC = 0.999). According to these results, adding ITIH4, NGAL, and FNDC5 to a multi-biomarker panel may help with correct staging and early non-invasive diagnosis, which might shorten the existing diagnostic delay. Prior to clinical deployment, validation in larger, multi-center cohorts is necessary.

Declarations

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