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The Role of Serum Caspase 3 Levels in Prediction of Endometriosis Severity

Cihan Kaya[a] Ismail Alay[a] Hakan Guraslan[a] Asuman Gedikbasi[b] Murat Ekin[a] Sinem Ertaş Kaya[c] Engin Oral[d] Levent Yasar[a]

[a]Department of Obstetrics and Gynecology, University of Health Sciences, Bakirkoy Dr Sadi Konuk Training and Research Hospital, Istanbul, Turkey; [b]Department of Obstetrics and Gynecology, University of Health Sciences, Bakirkoy Dr Sadi Konuk Training and Research Hospital, Department of Biochemistry, Istanbul, Turkey; [c]Department of Obstetrics and Gynecology, VKV American Hospital, Istanbul, Turkey; [d]Department of Obstetrics and Gynecology, Istanbul University, Cerrahpaşa Faculty of Medicine, Istanbul, Turkey

Keywords
Apoptosis · Endometriosis · Tissue adhesions · Laparoscopic surgery

Abstract
Background/Aims: To identify the role of serum caspase 3, Annexin A2 (ANXA2), and Soluble Fas Ligand (sFasL) levels in the prediction of endometriosis severity. ***Methods:*** The study was performed on 90 women who were candidates for laparoscopic surgery due to endometrioma or any other benign ovarian cysts detected by ultrasound examination, pelvic pain, or infertility. The control group comprised 29 patients. The second group comprised 29 patients with stage I–II endometriosis and the third group comprised 30 patients with stage III–IV endometriosis. ***Results:*** Significant differences were detected between the control and stage III–IV endometriosis groups and between stage I–II and stage III–IV endometriosis groups in terms of caspase-3 levels (both, $p < 0.001$), ANXA2 levels ($p = 0.007$ and $p = 0.002$), and sFasL levels ($p = 0.022$ and $p = 0.044$). After receiver operating characteristic analysis, the area under curve was 93% (95% CI 57–82) at 10.7 ng/mL cut-off level for caspase-3 with 90% sensitivity and 87% specificity. ***Conclusion:*** Serum caspase-3 level may be a reliable predictor of endometriosis severity.

Introduction

Endometriosis is defined as abnormal growth of endometrial tissue outside the uterine cavity and affects about 10% women of reproductive age [1]. It can cause infertility, pelvic pain, dysmenorrhea, dysuria, dyspareunia, and dyschezia [2, 3]. It negatively affects the socioeconomic life of individuals and the society [4]. Patients mostly present with ovarian endometriomas, peritoneal lesions,

E-Mail karger@karger.com
www.karger.com/goi

Cihan Kaya, MD
Department of Obstetrics and Gynecology, University of Health Sciences, Tevfik Saglam, Str No 11, Zuhuratbaba
TR–34147 Bakirkoy, Istanbul (Turkey)
E-Mail drcihankaya@gmail.com

and/or endometrial infiltration into other pelvic organs, such as the bowel, bladder, and ureter [5]. According to the revised American Society of Reproductive Medicine (rASRM) classification system, the severity of endometriosis is staged as minimal, mild, moderate, and severe, depending on the invaded pelvic structures [6].

The pathogenesis of endometriosis is supported by the retrograde menstruation phenomenon [7], and several other factors may influence the peritoneal invasion of the disease [8].

Apoptosis is implicated in follicular atresia and cyclic shedding of the endometrium [9]. Moreover, endometrial cells apoptosis may promote the growth of fallopian tube cells growth at ectopic locations during menstrual shedding and may be associated with the pathogenesis of endometriosis [9]. Several pathways were reportedly responsible for cell apoptosis such as caspases, annexins, and the Fas/Fas ligand (FasL) system [9–11].

Caspases (cysteinyl aspartate-specific proteases) are a family of cell-signaling molecules and their activation is a marker of cellular damage [12]. These proteases have been associated with cell death cascade initiation and are an important marker for apoptotic signaling pathway [10].

Annexins are involved in various cellular processes such as endocytosis, exocytosis, and cellular adhesion [11]. These molecules were found associated with inflammation and other pain mediators [13]. Annexin A2 (ANXA2) is reportedly a key factor in adenomyosis development [14].

The Fas/FasL system is also an important mediator of apoptosis. FasL is a type II membrane protein and belongs to the tumor necrosis factor family [15]. The dysregulation of the Fas/FasL system was reportedly responsible for abnormal elimination of regurgitated endometrial cells during normal menstruation; moreover, the cell membranes of Fas-bearing mononuclear cells may themselves become a target for getting phagocytized by FasL-expressing endometriotic cells [16].

Numerous markers, such as cancer antigen 125 (CA-125), cytokines, and angiogenic and growth factors, have been reported to have altered levels in endometriosis [17]; however, no biomarker or biomarker panel that is indicative of the disease severity has been identified [17, 18]. Routine bimanual gynecological examination alone may be insufficient to detect the disease severity before surgery [19]. Transvaginal ultrasound can be used to detect ovarian endometriomas but cannot reliably predict deep infiltrating endometriosis [20].

In this study, we aimed to evaluate the levels of 3 serum proteins representative of cell apoptosis–serum caspase-3, ANXA2, and the soluble FasL (sFasL) to preoperatively predict the diagnosis of endometriosis and the extent of endometriosis severity.

Materials and Methods

This prospective observational study was performed with 90 candidates (age, 15–55 years) for laparoscopic surgery due to (1) identification of a >4 cm endometrioma or other benign ovarian cysts or uterine pathologies on ultrasound examination, (2) chronic pelvic pain, or (3) infertility between August 1, 2016 and June 30, 2017. Informed consent was obtained from all participants and ethical approval was obtained from the institutional review board of Bakirkoy Dr Sadi Konuk Training and Research Hospital (approval no. 2016/237). The exclusion criteria were as follows: malignancy in the final pathology, chronic bowel disease, bowel surgery history, suspicion of pelvic inflammatory disease, any autoimmune disease history, any anti-inflammatory or hormonal or immunomodulatory medications use in the preceding 3 months, and other defined causes of infertility.

The following basic characteristics of the study population were recorded: age, gravidity, parity, body mass index, smoking habit, alcohol consumption, major presenting symptoms, visual analogue scale (VAS) scores, menstrual cycle phase at the time of serum sample acquisition, indications for surgery, performed surgical procedures, number of affected ovaries, ovarian cysts size, presence and location of deep infiltrating endometriosis, and the results of the final histopathology test. The presence of pouch of Douglas (POD) obliteration and ovarian and/or tubal adhesions was defined as per the rASRM classification [6]. According to this classification, POD obliteration was divided into none, partial, and complete. Bilateral or unilateral ovarian and/or tubal filmy or dense adhesions were scored as 1: <1/3 enclosure, 2: 1/3–2/3 enclosure, and 3: >2/3 enclosure.

Patients were divided into 3 groups of 30 patients. However, 1 patient in the control group and one patient in stage I–II endometriosis group were excluded due to the identification of malignancy in the final pathology results. Finally, the control group comprised 29 patients diagnosed only with benign ovarian cysts, such as dermoid, simple serous, or mucinous cysts, while the second group (stage I–II endometriosis group) and the third group (stage III–IV endometriosis group) comprised 29 patients and 30 patients diagnosed with stage I–II endometriosis and stage III–IV endometriosis, respectively, according to rASRM classification.

Sample Collection and Preparation

To analyze the serum levels of caspase-3, ANXA2, and sFasL, 2 cm^3 of venous blood was drawn preoperatively from the antecubital vein. The duration of the menstrual phase was not considered in this study. Blood samples were collected in heparin-containing tubes. The serum samples were isolated by centrifugation at 3,000 rpm for 10 min and maintained at –80 °C before performing assays.

Human Caspase-3, ANXA2, and sFasL ELISA Assay

Samples were thawed and caspase-3 ELISA kit, ANXA2 ELISA kit, and sFasL ELISA kits (Elabscience Biotechnology, USA) were used for quantifying the serum levels of cas-

Table 1. Comparison of patient characteristics of the study population

	Control group (n = 29), mean ± SD	Stage I–II endometriosis (n = 29), mean ± SD	Stage III–IV endometriosis (n = 30), mean ± SD	p value
Age, years	35.07±15	40.83±7.78	36.93±9.21	0.138
BMI, kg/m^2	25.82±4.32	25.39±3.17	24.11±3.48	0.186
Gravidy	1.51±1.37	1.86±1.27	2.06±1.22	0.264
Parity	1.31±1.16	1.75±1.12	1.86±1.07	0.138
Smoking habit (cigarette per day)	1.55±2.7	1.72±3.61	2.23±3.56	0.714
Menstrual cycle phase, n (%)				0.99
Proliferative	22 (75.86)	22 (75.86)	23 (76.66)	
Secretory	7 (24.13)	77 (24.13)	7 (23.34)	
Alcohol consumption (glass per week)	0.2±0.49	0.2±.49	0.23±0.5	0.955
Major presenting symptom, n (%)				<0.001
Dismenorea	4 (13.79)	11 (37.93)	17 (56.66)[a, b]	
Dispareunia	0	2 (6.89)	8 (26.66)	
Dischesia	0	0	3 (10)	
Pelvic pain	8 (27.58)	3 (10.34)	1 (3.33)	
Urinary pain	0	0	1 (3.33)	
VAS score	1.55±1.95	2.1±1.98	7.3±1.11[a, b]	<0.001

[a] Control vs. stage III–IV endometriosis, $p < 0.001$.
[b] Stage I–II endometriosis vs. stage III–IV endometriosis, $p < 0.001$.
BMI, body mass index; VAS, visual analogue scale.

pase-3, ANXA2, and sFasL. The detection ranges of ELISA kits were as follows: caspase-3 ELISA kit, 0.313–20 ng/mL; ANXA2 ELISA kit, 0.625–40 ng/mL; and sFasL ELISA kit, 15.63–1,000 pg/mL.

Statistical Analysis

Data analysis was performed with SPSS (version 20.0; SPSS Inc., Chicago, IL, USA). All data were presented as mean ± SD. One-sample Kolmogorov-Smirnov test was performed to analyze the distribution of clinical and laboratory variables. Logarithmic transformations were used to normalize the distribution of variables. Student t test and Mann-Whitney U test were used for the comparison of parametric variables, and the chi-square test was used for the comparison of nonparametric variables. One-way analysis of variance was used for group comparisons of normally distributed variables, and post hoc Tukey's test was used for pairwise analysis of the study groups. Pearson and Spearman correlation analyses were used to determine the correlations among the laboratory measurements and the number of affected ovaries, endometrioma size, POD obliteration, and pelvic organ adhesion in stage III–IV endometriosis group. The area under the receiver operating characteristic (ROC) curve (AUC) was used for diagnostic performance of the measured laboratory variables, and the sensitivity and specificity of the appropriate cut-off levels for each blood sample were calculated for each group. Post hoc power analysis was performed to determine the power of the study results. For all calculations, p values <0.05 were considered statistically significant.

Results

The mean age was 35.07 ± 15 years for the control group, 40.83 ± 7.78 years for the stage I–II endometriosis group, and 36.93 ± 9.21 years for the stage III–IV endometriosis group. No significant differences were found among the groups in terms of age, gravidity, parity, smoking habit, alcohol consumption, menstrual cycle phase at the time of serum sample acquisition, and body mass index. Dysmenorrhea was significantly more prominent in the stage III–IV endometriosis group than in other groups ($p < 0.001$). The VAS score was 7.3 for the stage III–IV endometriosis group, which was significantly higher than the scores for other groups ($p < 0.001$). For further analysis, we divided patients into 2 groups: controls and endometriosis group. Dysmenorrhea was significantly more prominent and VAS score was significantly higher in the endometriosis group than in controls (both, $p < 0.001$; Table 1, 2). The indications for surgery, surgical procedures, and histopathology results of the study population are presented in Table 3. The mean caspase-3, ANXA2, and sFasL levels were presented in Table 4 (Fig. 1–3). Significant differences in caspase-3, ANXA2, and sFasL levels were found between control and the stage III–IV endometriosis group and between stage I–II and stage

Table 2. Comparison of patients characteristics in the control group and endometriosis group

	Control group (n = 29), mean ± SD	Endometriosis group (n = 59), mean ± SD	p value
Age, years	35.07±15	38.85±8.69	0.261
BMI, kg/m^2	25.82±4.32	24.74±3.37	0.33
Gravidy	1.51±1.37	1.96±1.24	0.146
Parity	1.31±1.16	1.81±1.09	0.055
Smoking habit (cigarette per day)	1.55±2.7	1.98±3.56	0.79
Menstrual cycle phase, n (%)			0.58
Proliferative	22 (78.86)	45 (76.27)	
Secretory	7 (24.13)	14 (23.72)	
Alcohol consumption (glass per week)	0.21±0.5	0.22±0.49	0.87
Major presenting pain symtom, n (%)			<0.001
Dismenorea	4 (13.79)	28 (47.45)	
Dispareunia	0	10 (16.94)	
Dischesia	0	3 (5.08)	
Pelvic pain	8 (27.58)	4 (6.77)	
Urinary pain	0	1 (1.69)	
Major VAS score	1.55±1.95	4.74±3.06	<0.001

BMI, body mass index; VAS, visual analogue scale.

Table 3. Surgery indications, surgical procedures, and histopathology results of the study population

	Control group (n = 29)	Stage I–II endometriosis (n = 29)	Stage III–IV endometriosis (n = 30)
Surgery indication, n (%)			
Endometrioma	0	0	30 (100)
Dermoid cyst	0	2 (6.89)	0
Infertility	0	6 (20.68)	0
Myoma	0	6 (20.68)	0
Adnexal mass	29 (100)	15 (51.72)	0
Surgical procedures, n (%)			
Cystectomy	15 (51.72)	9 (3.03)	18 (60)
Oophorectomy	10 (34.48)	6 (20.68)	5 (16.6)
Hysterectomy + oophorectomy	4 (13.79)	0	7 (23.33)
Diagnostic laparoscopy	0	14 (48.27)	0
Histopathology results, n (%)			
Endometriosis	0	29 (100)	30 (100)
Dermoid cyst	7 (24.13)	2 (6.89)	0
Simple serous cyst	16 (55.17)	10 (34.48)	0
Myoma	0	6 (20.68)	0
Mucinous cyst	6 (20.68)	5 (17.24)	0

III–IV endometriosis groups (Table 4). Significant differences were detected between control and endometriosis groups in terms of caspase-3 and ANXA2 levels ($p < 0.001$ and 0.046; Table 5). After ROC analysis, AUC was 93% (95% CI 57–82) at 10.7 ng/mL cut-off level for caspase-3 with 90% sensitivity and 87% specificity (Fig. 4). In further comparison of control and endometriosis groups, AUC was 79% (95% CI 68–88) at 9.04 ng/mL cut-off level for caspase-3 with 70% sensitivity and 72% specificity (Tables 6, 7). In the stage III–IV endometriosis group,

Table 4. Comparison of serum caspase 3, Annexin A2, sFasL levels of study population

	Control group (n = 29), mean ± SD	Stage I–II endometriosis (n = 29), mean ± SD	Stage III–IV endometriosis (n = 30), mean ± SD	p value
Caspase 3, ng/mL	6.75±3.84	8.4±2.77	14.84±4.17[a, b]	<0.001
Annexin A2, ng/mL	13.4±6.43	14.21±7.36	21.53±12.12[c, d]	0.001
sFasL, pg/mL	107.19±58.57	115.45±68.79	158.68±88.18[e, f]	0.018

[a] Stage I–II endometriosis vs. stage III–IV endometriosis, $p < 0.001$.
[b] Control vs. stage III–IV endometriosis, $p < 0.001$.
[c] Stage I–II endometriosis vs. stage III–IV endometriosis, $p = 0.007$.
[d] Control vs. stage III–IV endometriosis, $p = 0.002$.
[e] Stage I–II endometriosis vs. stage III–IV endometriosis, $p = 0.044$.
[f] Control vs. stage III–IV endometriosis, $p = 0.02$.
sFasL, soluble Fas ligand.

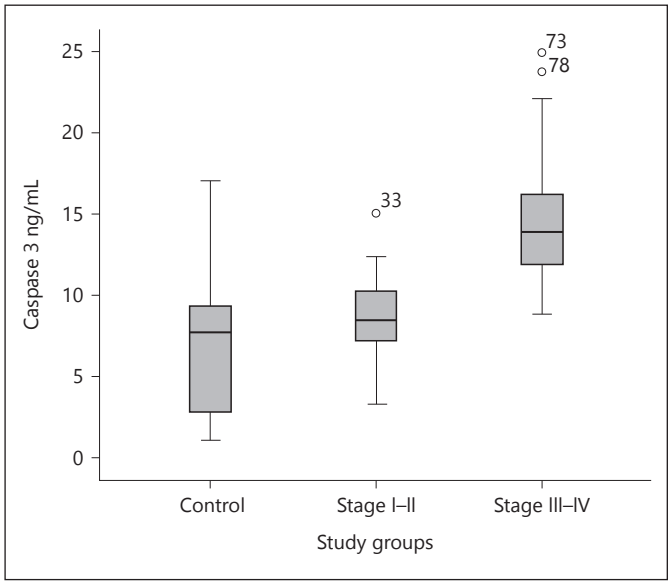

Fig. 1. The boxplot analysis of Caspase 3 levels between study groups.

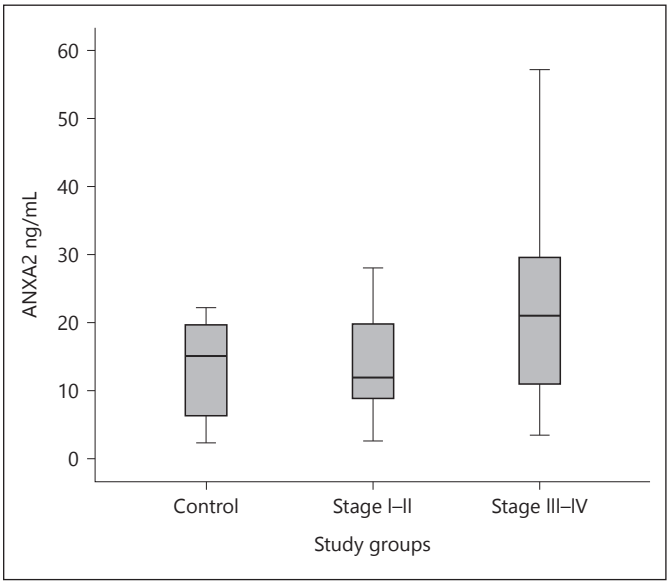

Fig. 2. The boxplot analysis of Annexin A2 (ANXA2) levels between study groups.

pelvic organ adhesions were evaluated; consequently, 8 (26.6%) patients had <1/3 enclosure, 5 (16.6%) patients had 1/3–2/3 enclosure, and 17 (56.6%) patients had >2/3 enclosure; 13 (43.3%) of these patients had bilateral endometrioma, and 17 (56.6%) had unilateral endometrioma. Nine (30%) patients had no POD obliteration, 12 (40%) had partial POD obliteration, and 9 (30%) had total POD obliteration. In the stage III–IV endometriosis group, 21 (70%) patients had deep infiltrating endometriosis. As for the location, 15 (50%) of them had peritoneal surface involvement, 4 (13.33%) has a sacrouterine ligament nodule, and 2 (6.66%) presented rectal serosal involvement. Seven (23.33%) patients had concomitant adenomyosis and endometriosis. The mean endometrioma size was 61 ± 20.56 mm (30–120 mm) in the stage III–IV endometriosis group. Endometrioma size positively correlated with ANXA2 and sFasL levels; however, the results were not statistically significant (Table 8). Post hoc power analysis showed 100% power for caspase-3 levels between control and stage III–IV groups and between stage I–II and stage III–IV groups. A power value of 89.9% was calculated for ANXA2 levels between control and stage III–IV groups and 80.4% power for ANXA2 levels between stage I–II and stage III–IV groups.

Table 5. Comparison of serum caspase 3, Annexin A2, sFasL levels of the control group and endometriosis group

	Control group (n = 29), mean ± SD	Endometriosis group (n = 59), mean ± SD	p value
Caspase 3, ng/mL	6.75±3.84	11.68±4.79	<0.001
Annexin A2, ng/mL	13.4±6.43	17.93±10.64	0.046
sFasL, pg/mL	107.19±58.57	137.43±81.53	0.098

sFasL, soluble Fas ligand.

Discussion

The present study demonstrated that serum caspase-3, ANXA2, and sFasL levels are higher in patients with stage III–IV endometriosis than in controls and patients with stage I–II endometriosis.

In daily practice, most gynecologists are not able to differentiate between ovarian endometrioma and endometriosis with a deep infiltrating disease using currently available diagnostic modalities, such as vaginal speculum examination, clinical rectovaginal wall examination, imaging technologies, and laboratory tests [17, 21].

The "immunoescape" theory of endometriosis connects the disease pathogenesis to the failure of apoptotic pathways to remove endometriotic cells. These pathways are described as caspases that act as protease on several cellular proteins [22]. Two main classes of caspases were described: caspases-9, known as initiator caspases, and caspases-3, known as effector caspases. Apoptosis may be triggered by the interaction among Fas ligand (FasL/CD95L) [16], tumor necrosis factor α (TNF-α) [23], transforming growth factor β (TGF-β), cytokines and overexpression or inappropriate expression of c-MYC and p53 [24].

Women with and without endometriosis have been previously reported to have comparable serum interleukin-6 (IL-6) [25, 26], IL-8 [25], TNF-α, and IL-1 [7, 25–27] levels. However, some studies reported higher peripheral levels of IL-6 [27], IL-8 [28], TNF-α [29], and interferon-γ [27] in patients with endometriosis than in controls. A study evaluating the role of proinflammatory cytokines in endometriosis found that soluble TNF-α values were decreased from minimal stages to severe stages of the disease; however, membrane TNF-α levels increased as the disease worsened [23]. In another study evaluating TNF-α, TGF-β, IL-8, and monocyte chemoattractant protein-1 levels in patients with endometriosis, the serum and peritoneal fluid levels of TNF-α

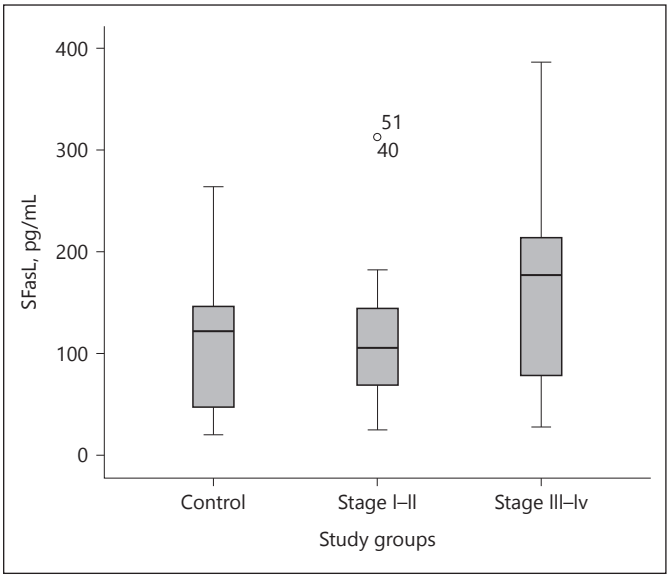

Fig. 3. The boxplot analysis of soluble Fas Ligand (sFasL) levels between study groups.

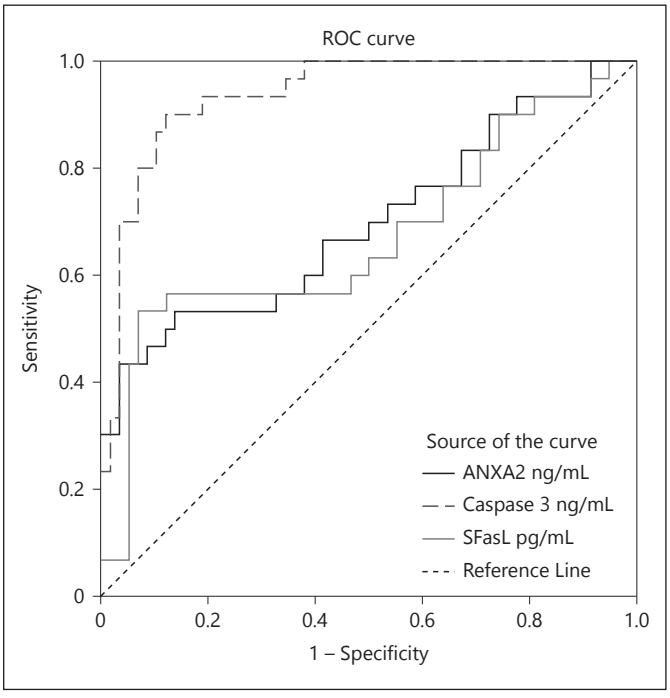

Fig. 4. The receiver operating characteristic (ROC) curve analysis of Caspase 3, Annexin A2 (ANXA2) and soluble Fas Ligand (sFasL) levels for control group.

Table 6. ROC curve analysis of caspase 3, Annexin A2, and sFasL levels of the study population

	Cut-off levels	AUC, %	Specivity, %	Sensitivity, %	95% CI
Control					
Caspase 3, ng/mL	7.72	21	20	51	0.11–0.31
Annexin A2, ng/mL	12.62	36	41	55	0.25–0.48
sFasL, pg/mL	114.32	39	50	55	0.26–0.51
Stage I–II endometriosis					
Caspase 3, ng/mL	8.32	34	30	55	0.30–0.55
Annexin A2, ng/mL	11.86	42	38	51	0.22–0.45
sFasL, pg/mL	104.93	43	41	51	0.31–0.55
Stage III–IV endometriosis					
Caspase 3, ng/mL	10.7	93	87	90	0.57–0.82
Annexin A2, ng/mL	13.96	69	50	70	0.88–0.98
sFasL, pg/mL	107.76	67	50	63	0.54–0.80

AUC, area under curve; sFasL, soluble Fas ligand.

Table 7. ROC curve analysis of caspase 3, Annexin A2, and sFasL levels of the control group and endometriosis group

	Cut-off levels	AUC, %	Specivity, %	Sensitivity, %	95% CI
Control					
Caspase 3, ng/mL	7.72	21	20	51	0.11–0.31
Annexin A2, ng/mL	12.62	36	41	55	0.25–0.48
sFasL, pg/mL	114.32	39	50	55	0.26–0.51
Endometriosis group					
Caspase 3, ng/mL	9.04	79	72	70	0.68–0.88
Annexin A2, ng/mL	15.21	63	54	55	0.51–0.74
sFasL, pg/mL	122.7	60	52	47	0.48–0.73

AUC, area under curve; sFasL, soluble Fas ligand.

Table 8. Correlation analysis of caspase 3, Annexin A2, and sFasL levels and number of affected ovaries, endometrioma size, pouche of Douglas obliteration and pelvic organ adhesions in the stage III–IV endometriosis group

	Caspase 3		Annexin A2		sFasL	
	r	*p* value	*r*	*p* value	*r*	*p* value
Number of affected ovaries	–0.16	0.38	0.27	0.13	0.34	0.06
Endometrioma size	–0.3	0.87	0.34	0.06	0.32	0.08
Pouche of douglas obliteration	–0.1	0.56	0.1	0.58	0.25	0.17
Pelvic organ adhesions	–0.06	0.74	0.21	0.24	0.28	0.12

sFasL, soluble Fas ligand.

were not detectable in controls but were very high in early stages and decreased with the severity of the endometriosis. TGF-β levels were significantly higher in patients than in controls and increased with the severity of the disease. Serum levels of IL-8 and monocyte chemoattractant protein-1 were significantly higher in early stages and decreased with the severity of the disease [30].

CA-125 is the most extensively investigated and widely used peripheral biomarker of endometriosis [31].

Vascular endothelial growth factor (VEGF), which has a critical role in angiogenesis, may contribute to the development of endometriotic lesions [32]. However, no consensus on the significance of VEGF as a biomarker of endometriosis is currently available, because it has been reported to be either increased [6] or comparable [33] in women with endometriosis when compared to controls.

In a study evaluating 28 biomarkers in women without detectable disease by ultrasound, multivariate analysis of annexin V, VEGF, CA-125, soluble intercellular adhesion molecule-1, and glycodelin levels in plasma samples enabled the diagnosis of endometriosis with a sensitivity of 81–90% and a specificity of 63–81% compared with controls [18].

In an experimental study, the effect of selective cyclooxygenase-2 inhibition on autologous endometrial grafts was evaluated, and consequently, a regression of endometrial grafts was observed [34]. The authors concluded that these results may be associated with the downregulation of VEGF-mediated angiogenesis [34].

In our study, we observed that caspase-3 levels were higher in stage III–IV endometriosis group than in control and stage I–II endometriosis groups. ROC analysis revealed a sensitivity of 90% and specificity of 87% for caspase-3 level in stage III–IV endometriosis group. In further analysis with controls and sum of patients with endometriosis independent of the stage of the disease, we found 70% sensitivity and 72% specificity of serum caspase-3 levels. Further, we checked for the presence of any correlation between caspase-3 levels and endometrioma size, POD obliteration, pelvic organ adhesions, and the number of affected ovaries in the stage III-IV endometriosis group. A negative correlation was detected, possibly indicating that increased caspase-3 levels may induce apoptosis and reduce ovarian endometrioma size and endometriosis adherence. The lower caspase-3 levels in control and stage I–II disease groups may be explained by the insufficient inflammatory stimuli required for secreting caspase-3. Considering the effect of apoptosis on endometriosis development, lower caspase-3 levels may result in the survival of endometriotic cells, and immunoescape of endometriotic cells may occur due to apoptotic pathway failure.

The overexpression of ANXA2 was validated in ectopic lesions of human adenomyosis and highly correlated with the severity of dysmenorrhea in patients with adenomyosis [35]. In another study evaluating the effects of prostaglandin E2 and COX inhibitors on the reduction of ANXA2 levels in peritoneal macrophages isolated from women with or without endometriosis, ANXA2 levels were markedly reduced in women with endometriosis [14]. Considering the same speculated pathophysiology of adenomyosis and endometriosis, we evaluated ANXA2 levels in our study to determine the severity of disease while drawing comparisons with controls. We observed that the levels of ANXA2 were significantly higher in stage III–IV endometriosis group than in controls and stage I–II endometriosis group, which is in agreement with the results of previous reports on adenomyosis [14, 35].

In a study analyzing the FasL expression in tissue specimens derived from 2 groups of women with severe endometriosis and without endometriosis, the higher expression of FasL in these cells suggests a possible immune privilege of endometrial tissues and may be associated with an explanation of endometriosis [36]. In another study, the authors concluded that fallopian tube epithelium can induce apoptosis in human endometrial cells and the development of endometriosis may be explained by the failure of FasL/FasR regulatory mechanisms [9]. In our study, we evaluated sFasL levels, which were significantly higher in stage III–IV endometriosis group than in other study groups. Our results are also in agreement with those of the study by Illanes et al. [13] who suggested that the failure of FasL/FasR regulatory mechanisms plays a role in pathogenesis of endometriosis [9].

Although a well-trained sonographer or a radiologist with specific expertise in MRI findings of endometriosis may help in endometriosis diagnosis [37], the preoperative evaluation of endometriosis with POD obliteration using a noninvasive technique in stage III–IV endometriosis is otherwise still a major challenge.

Although there are conflicting reports about the relationship between the validity of the blood samples drawn and the menstrual phase at the time of blood acquisition, we collected blood samples without considering the menstrual phase because caspase-3, ANXA2, and sFasL levels were found not affected by menstrual phase [14, 34–36, 38].

Our study has some limitations. Our results are derived from a small subset of patients with endometriosis, and control and stage I–II groups had various pelvic masses, such as dermoid cysts, simple serous or mucinous cysts, or uterine fibroids; this may have interfered with the results. Another limitation was the lack of confirmation of our results in the peritoneal fluid samples or tissue specimens. A further study with

healthy controls and stage I–II endometriosis-only groups with serum, peritoneal, and tissue samples may be designed.

In summary, deciding to opt for an extensive surgery, such as low anterior resection of the bowel, or extensive ureteric dissection or resections, may be distressing for a woman of reproductive age who probably requires only a simple ovarian cystectomy. Serum caspase-3 levels may be a reliable predictor of endometriosis severity with high specificity and sensitivity rates.

Acknowledgment

None.

Disclosure Statement

The authors declare that they have no conflicts of interest to disclose.

Condensation

Serum caspase-3 levels may be a predictor of endometriosis severity. sFasL levels may be used for investigation of ethiopathogenesis of endometriosis.

Clinical Trials Registration Number

CT03020108.

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