Research article

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Evaluation of Serum Endocan Levels in Endometriosis: A case-control study

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Abstract

Objective. To evaluate the possible associations between serum endocan levels and endometriosis.

Study Design. A total of 60 women with histologically proven endometriosis and 40 women who underwent laparoscopy due to unexplained infertility without endometriosis were evaluated in a case-control study. Serum endocan, CA125, CA19.9, and CA15.3 levels were measured. Demographic, clinical, and laboratory parameters were compared.

Results There was no significant difference between the groups regarding age, body-mass-index, parity, and serum CRP and WBC levels. Serum endocan ($p<0.001$), CA125 ($p<0.001$), CA19.9 ($p=0.022$) and CA15.3 ($p=0.013$) levels were significantly higher in the endometriosis group compared to the control group. The correlation analysis showed that serum endocan level was positively correlated with the stage of the disease, CRP, and WBC, but not with remaining parameters, age, BMI, dysmenorrhea score, CA125, CA19.9, and CA15.3.

Serum CA125 can predict endometriosis (Cut off=26.2 IU/mL, AUC=0.955) with a sensitivity of 89% and specificity of 88%. Serum endocan can predict endometriosis (Cut off=454 ng/mL AUC=0.749) with a 93% sensitivity and 61% specificity.

Conclusion. The serum endocan levels were significantly elevated in women with endometriosis compared to the control group. Serum endocan can predict endometriosis with a sensitivity of 93% and specificity of 61%. ***Clin Ter 2020; 171 (6):e517-522. doi: 10.7417/CT.2020.2266***

Key words: *Angiogenesis, CA125, endometrioma*

Introduction

Endometriosis is one of the most important benign disorders in gynecology. It affects nearly one in every ten women of reproductive age and is associated with pelvic pain and sub- or infertility as well as reduced quality of life (1).

Endocan, also known as human endothelial cell-specific molecule 1, is a circulating proteoglycan mainly released by endothelial cells (2) and thought to be associated with the regulation of cell adhesion, inflammation, and tumor progression. Endocan is especially high in tumor endothelium (3). The serum concentrations of endocan are elevated in disorders characterized by endothelial hyperactivation or dysfunction. The studies have shown that endocan levels were increased (compared to the control group) in asthma (4), juvenile idiopathic arthritis (5), multiple myeloma (6), sarcoidosis (7), and hypertension (8). Moreover, in gynecological and obstetric points of view, elevated serum endocan levels were associated with polycystic ovary syndrome (7), endometrial and ovarian carcinoma (10), preeclampsia (11), and intrauterine growth retardation (12).

Angiogenesis is crucial for the implantation and development of ectopic endometriotic cells. An increasing number of studies suggest that multiple mechanisms contribute to the vascularization of endometriotic lesions, including angiogenesis, vasculogenesis, and inosculation. The transformation of tumors in the sleeping phase to rapidly growing tumors is shown to be correlated with endocan (13). Considering the central role of endocan in angiogenesis and inflammation, we aimed to evaluate the possible associations between serum endocan levels and endometriosis for the first time in the literature.

Materials and methods

Sixty women with endometriosis and forty women without endometriosis were evaluated in a cross-sectional, case-control study. Informed consent was obtained from all women. The study was approved by the Istanbul University, Cerrahpasa Faculty of Medicine Ethics Committee (01.10.2012/29095 and 16.11.2018/93785). All women were operated in Istanbul University Cerrahpasa Faculty of Medicine, Department of Obstetrics and Gynecology between 2015 and 2018.

The study group's inclusion criteria were consecutive women between 18 and 45 years old who had regular menstrual cycles with histologically proven endometriosis by laparoscopy. The endometriosis was diagnosed histologically by the samples taken during laparoscopy.

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The control group's inclusion criteria were consecutive women who underwent diagnostic laparoscopy due to unexplained primary or secondary infertility and did not have any macroscopic endometriotic lesion.

Exclusion criteria for both study and control groups included the history of ovarian surgery, systemic diseases; endocrine disorders; autoimmune disorders; malignancy; menopause, and hormonal treatment, including oral contraceptive pills in the last six months.

On admission, the demographic and clinical data were recorded, and the visual analog scale (VAS) score was calculated for each woman for the severity of pelvic pain and dysmenorrhea. A VAS score of 0 indicated no pain, and a VAS score of 10 indicated the worst pain ever experienced.

The severity of endometriosis was classified according to the recommendations of the American Society for Reproductive Medicine (ASRM) (14). The patients were classified as mild (stage 1-2) and moderate-to-severe (stage 3-4) endometriosis.

Samples were obtained in the operating theatre, and 5–10 ml of venous blood samples were collected using a peripheral venous catheter (PVC). In order to allow clotting, the blood samples were kept at room temperature for at least 30 minutes. The serum supernatants were separated following centrifugation at 5000g for 10 min. The supernatants were stored at -80°C until analysis. C-reactive protein (CRP) and white blood cell count (WBC) were measured. Serum CA125 and CA15.3 levels were measured with the electrochemiluminescence technique. CA19.9 was measured by immunometric assay. Enzyme-linked immunosorbent assay (ELISA) was used to measure the serum endocan levels (Aviscera Bioscience Inc, Cat. No: SK00318-01, CA, USA). The measurement steps were performed as instructed by the manufacturer.

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 18.0.

A preliminary study with ten women with endometriosis and ten women without endometriosis was performed using the serum endocan levels (613.2±178.0 ng/mL vs. 422.1±61.9 ng/mL, respectively). The preliminary results revealed a sample size of at least 32 cases in each group (alpha Error=0.05, power=99%)

The Kolmogorov-Smirnov test was performed in order to evaluate the homogeneity of variances. Parametric variables were presented as mean ± standard deviation (SD) and were evaluated using the T-test or ANOVA. Pearson's correlation test was used to evaluate the possible correlations between the parametric variables. The receiver operating characteristic (ROC) curve analysis was used to determine the markers' sensitivity and specificity for predicting endometriosis. P<0.05 was accepted as statistically significant.

Results

A total of 60 women with histologically proven endometriosis and 40 women without any macroscopic endometriotic lesions were included in statistical evaluation. Clinical and demographical features and laboratory parameters of endometriosis and non-endometriosis groups were presented in Table 1. All parameters were equally distributed. There was no significant difference between the groups regarding age, BMI, and parity. The dysmenorrhea rate was significantly higher in the endometriosis group (68.3% vs. 35%; p<0.001). Serum CRP and WBC levels were comparable between the groups.

Table 1. Demographic and clinical features of endometriosis and non-endometriosis groups

	Endometriosis (n=60) (mean±SD)	Control (n=40) (mean±SD)	p
Age (years)	32.5±8.0	30.8±7.8	0.295
BMI (kg/m^2)	24.3±4.5	24.7±4.5	0.760
Parity (n)	0.63±0.88	0.95±1.0	0.094
Dysmenorrhea rate (n,%)	41/60 (68.3%)	14/40 (35%)	**<0.001**
CA125 (IU/mL)	80.6±64.9	15.3±7.0	**<0.001**
CA15.3 (IU/mL)	19.1±10.9	14.4±5.2	**0.013**
CA19.9 (IU/mL)	20.9±16.5	12.8±15.8	**0.022**
CRP (mg/L)	3.4±4.4	2.2±2.1	0.07
WBC (n/µl)	7356±1592	6975±1778	0.08
Endocan (ng/mL)	590.8±206.0	442.3±93.8	**<0.001**

BMI, body mass index; CRP, C-reactive protein; SD, standard deviation; WBC, white blood cell.
p<0.05 is significant, the significant p-values are written in bold

In our study group, unilateral and bilateral endometrioma rates were 58.3% and 41.7%, respectively. Douglas pouch obliteration was not seen in 28 (46.6%) women, whereas 11 (18.3%) and 21 (35%) women had a partial and complete obliteration, respectively. Peritoneal endometriosis and deep infiltrating endometriosis were observed in 24 (40%) and 16 women (26.7%), respectively.

Serum endocan levels were significantly higher in the endometriosis group compared to the control group (590.8±206.0 ng/mL vs. 442.3±93.8 ng/mL, p<0.001, alpha Error=0.05, power=98%). Serum CA125, CA 19.9, and CA 15.3 levels were also significantly higher in the endometriosis group compared to the control group (see Table 1).

Serum endocan and CA 125 levels were slightly elevated in the stage 3–4 group compared to the stage 1–2 group, although the differences both remained nonsignificant (p=0.122 and 0.171, respectively). CA 19-9 levels were significantly higher in the stage 3–4 group compared to the stage 1–2 group (p=0.040). There was no significant difference between the stage 1–2 and stage 3–4 groups regarding other parameters evaluated (see Table 2).

Table 2. Comparison of age, BMI, tumor markers and inflammatory markers between stage 1–2 and stage 3–4 endometriosis groups

	Stage 1-2 (n=33) (mean±SD)	Stage 3-4 (n=27) (mean±SD)	p
Age (years)	31.7±7.8	33.5±8.4	0.407
BMI (kg/m^2)	23.6±3.9	25.4±5.0	0.191
CA125 (IU/mL)	69.8±57.0	94.7±72.9	0.171
CA15.3 (IU/mL)	17.5±7.8	20.8±13.7	0.301
CA19.9 (IU/mL)	16.9±12.0	25.9±20.0	**0.040**
CRP (mg/L)	3.0±3.0	5.0±5.4	0.089
WBC (n/µl)	7647±1591	7000±1549	0.118
Endocan (ng/mL)	551.7±158.4	638.5±247.2	0.122

BMI, body mass index; CRP, C-reactive protein; SD, standard deviation; WBC, white blood cell.
p<0.05 is significant, the significant p-values are written in bold

Serum endocan values in the control group and according to endometriosis stages are also presented in Figure 1. There is a gradual increase with the advancing stage, although the differences both remained nonsignificant. The correlation analysis showed that serum endocan level was positively correlated with the stage of the disease (r=459; p<0.001), CRP (r=0.257, p=0.012), and WBC (r=0.251, p=0.012), but not with remaining parameters (Table 3). The box-plot representation of serum endocan levels according to the stages of endometriosis is shown in Fig. 1.

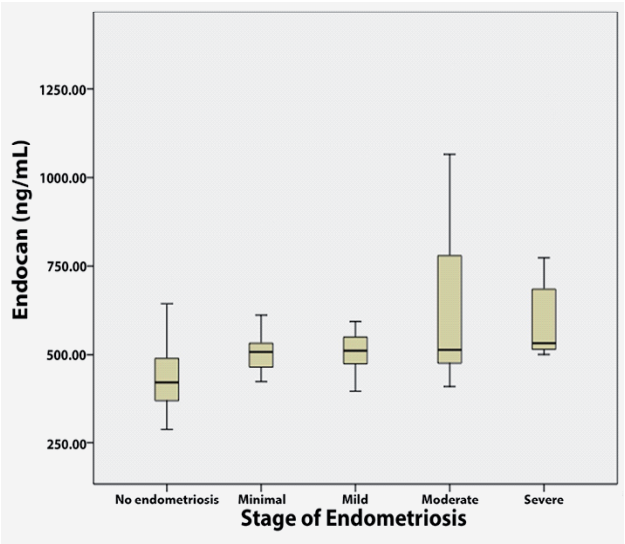

Fig. 1. The box-plot representation of serum endocan levels according to the stages of endometriosis.

Table 3. Correlation analysis of serum endocan with demographic and clinical parameters

	Endocan	
	r	p
Age	0.119	0.237
BMI	-0.163	0.140
Dysmenorrhea score	0.256	0.052
CA125	0.194	0.063
CA15.3	0.200	0.069
CA19.9	0.168	0.115
CRP	0.257	**0.012**
WBC	0.251	**0.012**
Endometriosis stage	0.459	**<0.001**

BMI, body mass index; CRP, C-reactive protein; WBC, white blood cell.
Pearson's correlation analysis
P<0.05 is significant, the significant p-values are written in bold.

Serum endocan and the three tumor markers were evaluated for their ability to predict endometriosis (Table 4). ROC analysis revealed that CA125 had the highest AUC of 0.955 for predicting endometriosis followed by endocan, CA15.3, and CA19.9 in descending order (0.749, 0.673 and 0.646; respectively) (Fig. 2). All four parameters were significant to predict endometriosis.

Table 4. ROC Analysis of CA125, endocan, CA15.3 and CA19.9 for prediction of endometriosis

	AUC (95% CI)	Cut-off value	Sensitivity	Specificity	p
CA125	0.955 (0.915-0.995)	26.2 IU/mL	89%	88%	**<0.001**
Endocan	0.749 (0.633-0.865)	454 ng/mL	93%	61%	**<0.001**
CA19.9	0.673 (0.555-0.791)	11.5 IU/mL	66%	66%	**0.007**
CA15.3	0.646 (0.527-0.765)	15.0 IU/mL	68%	66%	**0.023**

AUC, area under curve; CI, confidence interval; ROC, receiver operator characteristics
P<0.05 is significant, the significant p-values are written in bold.

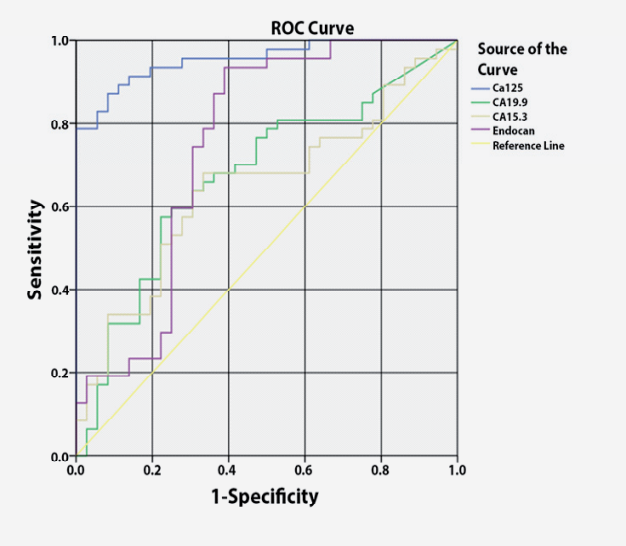

Fig. 2. ROC Analysis of CA125, endocan, CA15.3, and CA19.9 for prediction of endometriosis.

Serum endocan has significant positive correlations with endometriosis, but there is no correlation with age, BMI, and tumor markers, CA125, CA15.3, and CA19.9.

Discussion

According to our search in Medline and Google Scholar with the keywords "endocan AND endometriosis", "endocan AND endometrioma", "endocan AND endometrium", this is the first study to evaluate the association between serum endocan and endometriosis. In our study, the serum endocan, CA125, CA19.9, and CA15.3 levels were significantly elevated in the endometriosis group compared to the control group. Serum endocan and CA125 levels were slightly higher in the stage 3–4 group compared to the stage 1–2 group, although the difference remained nonsignificant.

Endocan, a novel endothelial cell dysfunction marker, is a soluble proteoglycan released from the vascular endothelium. It may play a central role in the pathogenesis of endothelial dysfunction (15,16) and organ-specific inflammation (17). Endocan is known to be associated with endometrial and ovarian cancer, inflammation, and diseases characterized by endothelial dysfunction such as preeclampsia and sepsis (15,18,19). Its secretion is mediated by various cytokines and growth factors, such as the tumor necrosis factor-a (TNF-a) and the vascular endothelial growth factor (VEGF) (20,21). In accordance with the growing evidence suggesting a link between endocan and inflammation, we observed a significant positive correlation between serum endocan and CRP and WBC.

Recent studies suggest that endocan expression is associated with the vascular transformation of stem cells and endothelium to mesenchyme shift processes such as arterial wall remodeling (22-25). El Behery et al. (26) have shown that endocan levels were elevated in ovarian cancer compared to the controls, and endocan was an independent prognostic marker in overall survival in epithelial ovarian cancer. Laloglu et al. (10) evaluated 27 women with endometrial cancer, 20 women with ovarian cancer, 19 women with benign ovarian pathology, and 19 women with benign endometrial pathology and healthy controls. They have shown that serum endocan levels in women with endometrial and ovarian cancer were significantly higher than those of women with benign disorders and healthy women. Median serum levels were 560.4 pg/mL in endometrial cancer cases, and 0.0 pg/mL in the benign disorder group and healthy controls. Since all the values were given only as a median, it is not possible to compare our results with the results from the study of Laloglu et al. (10). Moreover, they reported that the endocan levels in the benign disorder group and healthy women were comparable. Unfortunately, the authors did not define the distribution of benign endometrial lesions; therefore, it is not clear if they evaluated women with simple or complex endometrial hyperplasia. They evaluated all endometrial and ovarian benign lesions as a group and compared this single group with healthy controls or the malignancy group. For that reason, it is hard to interpret their results relating to our study. Besides, in our study, we did not evaluate women with endometrial hyperplasia. The pathogeneses of endometrial hyperplasia and endometriosis are quite different. It may be possible that endocan is increased in endometriosis but not in endometrial hyperplasia, but this finding must be evaluated in the same study group.

Endometrioma is a common pathology among women with endometriosis and affects 17%-44% of patients with endometriosis. (27) Alcázar and García-Manero (28) evaluated 65 women with endometrioma by transvaginal color Doppler and microvessel density and concluded that the vascularization of ovarian endometriomas was higher in women with pelvic pain compared to the asymptomatic women. In our study, the correlation analysis between endocan and dysmenorrhea showed a borderline p-value of 0.052, which may be evaluated in greater scale studies.

The results from studies about endocan in bacteremia and sepsis are inconsistent. Scherpereel et al. (19) suggested that the serum endocan was significantly elevated in patients admitted to the intensive care unit with sepsis compared to healthy donors and patients with systemic inflammatory response syndrome. Adekola et al. (11) have observed that pregnancies complicated by acute pyelonephritis with bacteremia were associated with reduced endocan levels.

Considering the molecular associations of endocan in endothelial dysfunction, inflammation, and angiogenesis, it is difficult to explain which one is more dominant in which disorder. Preeclampsia and IUGR are conditions mainly associated with endothelial dysfunction and hypoxia. Endometrial and ovarian cancers are mainly associated with neoangiogenesis and inflammation. Endometriosis is a combination of all three mechanisms.

In our study, we observed that serum endocan was independent of age and BMI. For that reason, endocan may be used in all age and BMI groups and needs no correction for other parameters.

In our study, the ROC analysis for the prediction of endometriosis revealed that CA125 had the highest AUC for predicting endometriosis compared to endocan, CA15.3, and CA19.9 in descending order. The sensitivity and specificity for the prediction of endometriosis were 89% and 88% for CA125 and 93% and 61% for endocan. The sensitivity of

endocan was higher than CA125. Furthermore, endocan was not correlated with the other tumor markers, indicating that it is elevated in a different patient group than CA125. The sensitivity and the specificity rates were not given to suggest endocan as a diagnostic marker for endometriosis alone and by itself, rather to define where it stands in comparison with the other well-known markers and potential use as panel marker in combination with other relevant markers.

The main limitation of our study and many other marker studies about endometriosis is that the control group does not consist of completely healthy women. The diagnosis of endometriosis is proved and excluded only with laparoscopy, and it is not ethical to perform laparoscopy in completely healthy women without any indication. Our study's main strength is that it is the first study evaluating the possible associations between serum endocan levels and endometriosis, which is also crucial for the understanding of the pathophysiology of endometriosis.

Conclusion

Our study is the first study to evaluate the associations between serum endocan and endometriosis. The serum endocan levels were significantly higher in the endometriosis group compared to the control (unexplained infertility) group. Serum endocan levels were slightly higher in the stage 3–4 group compared to the stage 1–2 group, although the difference remained nonsignificant. Serum endocan can predict endometriosis with a sensitivity of 93%, however a low specificity of 61%.

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Conflict of Interest
None.

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