RESEARCH ARTICLE

Serum endocan levels in endometrial and ovarian cancers

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Background: Ovarian and endometrial carcinomas are the two most common malignancies of the female reproductive system. Endocan is a proteoglycan that is specific to vascular endothelial cells. Increased serum levels have been reported in some tumors. The aim of this study was to investigate serum endocan levels in cases of endometrial and ovarian cancer.

Methods: Levels of serum endocan were assessed in 27 patients with endometrial cancer and 20 with ovarian cancer, and in 38 control subjects with benign ovarian or endometrial disorders. Thirty-five healthy subjects were also included. Serum endocan levels were measured using a specific enzyme-linked immunosorbent assay. Serum CA-125 levels were also measured in the patient and control groups.

Results: All patients had detectable serum endocan levels among endometrial and ovarian cancer groups except six cases. However, in the benign and healthy control groups, all endocan levels were undetectable except for two cases in the benign group and three in the healthy control group. Serum endocan levels were significantly higher in the entire patient group than in the controls ($P < .0001$ for both). Serum endocan levels in cases of endometrial cancer and ovarian cancer were higher than in both the control groups ($P < .0001$ for both). Evaluation of all groups revealed a positive correlation between serum CA-125 and endocan levels ($r = .43$, $P < .0001$).

Conclusion: Although benign ovarian or endometrial disorders do not lead to expression of endocan, malignant cases can result in measurable endocan levels. This may be useful in differentiating benign and malign diseases of the endometrium or ovary.

KEYWORDS

cancer antigen 125, endocan, endometrial cancer, ESM-1, ovarian cancer

1 | INTRODUCTION

Ovarian and endometrial cancers are the most widely diagnosed malignant pathologies of the female reproductive system.¹ Epithelial ovarian cancer has the highest mortality levels among gynecological malignancies.² Six percent of all cancer-related mortalities in women result from ovarian cancer, yet survival rates have improved very little over the previous decade. Five-year survival is highly correlated with the stage of ovarian cancer at diagnosis. Five-year survival levels of more than 90% can be achieved if the disease is diagnosed and treated when still localized (stages I and II).^{3,4} Early diagnosis significantly improves outcomes. However, no noninvasive techniques are available for identifying early-stage ovarian cancer.

Endometrial cancer is the most common gynecological malignancy. It is also the fourth most prevalent form of cancer among women after breast, colorectal, and thyroid cancers.⁵ It derives from the inner lining of the uterus, and is responsible for approximately 90% of uterine cancers. Age at onset of endometrial cancer is decreasing.⁶ Prognosis in endometrial malignancies is generally good when diagnosed early and effectively treated by hysterectomy.⁷ However, there are no reliable serum biomarkers in clinical use for the identification of endometrial cancers.⁸ Delayed diagnosis generally results in poor prognosis and a low 5-year survival rate. Up to 70% of cases of endometrial cancer are diagnosed at stage I. The other 30% tend to be observed in various high-risk groups including patients with nonpolyposis colorectal cancer syndrome, obesity, diabetes, or breast cancer receiving

tamoxifen.^{9,10} Mortality levels worsen if the tumor extends beyond the uterine cavity, depending on the histological grade involved. Other factors reported to impact on the patient's outcome include lymphovascular vessel invasion, tumor dimensions, cervical involvement, and regional lymph node involvement.¹¹

Cancer antigen 125 (CA-125) has been shown to be overexpressed in 80% of ovarian cancers, depending on stage of tumor. It is therefore largely employed in determining the response to treatment in patients with epithelial ovarian cancer.¹² High levels of CA-125 may also be seen in endometrial, pancreatic, lung, breast, colorectal, and other gastrointestinal tumors, in addition to ovarian cancer. At a cutoff point of 35 U/ml, 78% sensitivity and 95% specificity predictive values have been determined in the context of malignant disease.¹³

Serum CA-125 can be used to identify late-stage ovarian cancers, but not endometrial cancers.⁸ CA-125 level elevation occurs in only 10%-20% of patients with early-stage endometrial cancer and in 25% of asymptomatic patients with recurrence.¹⁴ CA-125 has therefore been reported to be useful only as a guide to late-stage endometrial cancers.¹⁵

Due to these disadvantages associated with the CA-125, there has been considerable research aimed at identifying more reliable biomarkers to assist in early detection, as well as in treatment and general disease monitoring. Proteins studied in epithelial ovarian and endometrium carcinomas include human epididymis protein 4 (HE4), biglycan, vascular endothelial growth factor (VEGF), and YKL-40.¹⁵⁻¹⁸ To the best of our knowledge, no previous studies have investigated serum endocan levels in cases of ovarian or endometrial malignancies.

Endocan (endothelial cell-specific molecule-1, ESM-1) is a chondroitin-dermatan sulfate proteoglycan that is specific to the vascular endothelial cells and plays an important role in angiogenesis and inflammation.¹⁹ Increased serum endocan mRNA levels have been reported in some tumors, such as breast,²⁰ renal,²¹ and lung cancers,²² and elevation is associated with metastasis and poor prognosis.

Early diagnosis improves survival in both cancer types. No specific tumor markers have yet been identified for either endometrial or ovarian cancer. The aim of this study was to investigate serum endocan levels in cases of endometrial and ovarian cancer. We also measured serum CA-125 levels in the patient groups. Additionally, we analyzed the correlation between endocan and clinicopathological parameters in both types of cancer.

2 | MATERIALS AND METHODS

The study was performed at Obstetrics and Gynecology Department of Ataturk University Hospital, Erzurum, Turkey. Twenty-seven patients with endometrial cancer and 20 with ovarian cancer were included. Patients receiving preoperative chemotherapy, radiotherapy, or hormone therapy, with a history of another malignancy or who refused to participate were excluded. Thirty-eight subjects with pathologically confirmed benign ovarian (n=19) or endometrial disorders (n=19) were enrolled as the benign control group, and another 35 subjects as the healthy control group. Approval for the study was

granted by the local ethical committee. Participation in the study was voluntary, and written informed consent was obtained from all subjects. Preoperative blood samples were taken from all subjects after 10-12-hour fasting. The serum obtained was stored at -80°C until the time of analysis. Serum endocan levels were measured using a specific enzyme-linked immunosorbent assay. Additionally, serum CA-125 levels were measured in the patient and control groups. Serum CA-125 levels were measured using a Beckman-Coulter DXI device. Both endometrial and ovarian cancer patients were staged based on the International Federation of Gynecology and Obstetrics (FIGO) staging system and preoperative magnetic resonance imaging (MRI) findings.

2.1 | Analyte assay techniques

Endocan levels in serum samples were measured using ELISA with a "Human ESM-1 ELISA Kit" (Lunginnov, France). This kit employs a double-antibody sandwich enzyme-linked immunosorbent assay. Samples were pipetted into a 96-well microplate coated with a monoclonal antibody (also known as capture antibody) specific to the C-terminal of human endocan and incubated for 1 hour. Endocan present within a sample is bound by the capture antibody. Once any remaining unbound molecules had been washed away, a biotinylated secondary monoclonal antibody specific to the endocan N terminal was added to the wells and allowed to incubate for another 1 hour. Following a washing step, streptavidin-HRP (biotin-binding protein conjugated with polymers of horseradish peroxidase) was added and allowed to incubate in the dark for a further 30 minutes. Any remaining unbound material was again washed away. Chromogen solution was added and incubated in the dark for 10 minutes for the conversion of the colorless solution into a blue solution, the intensity of which was proportional to the amount of endocan in the sample. The color of the samples turned yellow due to the effect of the acidic stop solution. The colored reaction product was measured using an automated endocan reader at 450 nm. The results were expressed as picograms per milliliter (pg/mL).

The performance characteristics of this kit as listed by the manufacturer are as follows: limit of quantification 300 pg/mL, detection from 300 pg/mL to 10000 pg/mL, intra-assay precision 4.8%, and interassay precision 7.6%. No interference was observed with hemolyzed or hyperlipidemic plasma or serum. Values beneath the detection limit of 300 pg/mL were regarded as "0".

2.2 | Statistical analysis

Data recording and analysis was performed on "SPSS 20.0 for Windows" (SPSS Inc., Chicago, IL, USA) software. Descriptive data were expressed as number and % for categorical variables and median (minimum and maximum) or mean±standard deviation for numeric variables. Differences among groups were analyzed using the Kruskal-Wallis test. The Mann-Whitney *U* test was used for binary comparisons. Correlations between results were assessed using Spearman's rank correlation analysis. The ROC curve, an expression of a particular technique's predictive power, was used to determine

sensitivity, specificity, and cut-off values for serum endocan. A value of $P < .05$ was regarded as significant.

3 | RESULTS

Mean age at diagnosis was 52.3 ± 14.5 years in the patients with ovarian cancer, 53.7 ± 12.2 in those with endometrial cancer, 49.4 ± 7.9 in the benign controls, and 49.2 ± 7.4 in the healthy controls. No statistically significant difference was determined in terms of age between the controls and the disease group. Histopathological findings in the ovarian cancer and endometrial cancer cases are shown in Table 1.

With six exceptions, all cases in the endometrial and ovarian cancer groups had detectable serum endocan values. However, in the benign and healthy control groups, all cases had undetectable endocan values except for two cases in the benign group and three in the healthy control group. Serum endocan levels in the entire patient group were significantly higher than in the benign and healthy controls ($P < .0001$ for both).

TABLE 1 Distribution of patients according to histopathology of ovarian and endometrial cancer

Histopathology	Ovarian cancer n (%)	Endometrial cancer n (%)
Serousadenocarcinoma	13 (65%)	—
Granulosa cell tumor	5 (25%)	—
Neuroendocrine tumor	1 (5%)	—
Sertoli-Leydig tumor	1 (5%)	—
Adenocarcinoma	—	25 (96.2%)
Carcinosarcoma	—	1 (3.8%)

Variables were expressed as number (n) and %.

Median (minimum-maximum) serum levels were 560.4 (0-4036.68) pg/mL in cases of endometrial cancer, 670.2 (0-6693) pg/mL in the ovarian cancer group, 0.0 (0-661.65) pg/mL in the benign control group, and 0.0 (0-551) pg/mL in the healthy control group (Figure 1). Serum endocan levels in the endometrial cancer and ovarian cancer groups were higher than those in the benign control group ($P < .0001$ for both) and those in the healthy subjects ($P < .0001$). There was no significant difference between the endometrial and ovarian cancer groups in terms of serum endocan ($P = .10$). Additionally, the endocan findings in the benign and healthy control groups were similar.

Statistically significant differences in CA-125 levels were observed between the ovarian cancer group (64.5 [23-4969] mU/L) and both the control groups (27.2 [5-70] mU/L and 28.3 [4.5-50.8] mU/L; Figure 2). CA-125 levels were higher in the patients with ovarian cancer compared with the benign and healthy controls ($P < .0001$ for both). No statistically significant difference was determined between either of the control groups and the patients with endometrial cancer (37 [8-313] mU/L). Serum CA-125 results were similar between the two. CA-125 levels were higher in the ovarian cancer cases than in the endometrial cancer group ($P = .002$).

Patients with endometrial cancer were evaluated in terms of preoperative MRI findings and FIGO staging (Table 2). When patients with stage I or II cancer were classified as early stage ($n = 12$, 60%) and those with stage III or IV cancer as late stage ($n = 8$, 40%), no difference between endocan or CA-125 levels was observed between the two groups.

Patients with ovarian cancer were evaluated in terms of preoperative MRI results and FIGO staging (Table 2). When patients with stage I or II cancer were classified as early stage ($n = 12$, 60%) and those with stage III or IV cancer as late stage ($n = 8$, 40%), a statistically significant difference was observed in preoperative serum CA-125 values between the two groups ($P = .001$). However, no significant difference was observed in serum endocan levels between these groups. When

FIGURE 1 Serum levels of endocan. Results were given as median. * $P < .0001$ compared with both control groups

FIGURE 2 Serum (mU/L) levels of CA-125. Results were given as median. * $P=.002$ compared with ovarian cancer group. ** $P<.0001$ compared with both control groups

TABLE 2 Distribution of patients according to stage of endometrial and ovarian cancer

Stage	Endometrial cancer n (%)	Ovarian cancer n (%)
I	22 (84.6%)	12 (60%)
II	1 (3.8%)	—
III	3 (11.6%)	7 (35%)
IV	—	1 (5%)

Variables were given as number (n) and %.

the entire patient group, subjects with either ovarian or endometrial cancer, was analyzed, no statistically significant difference was observed in CA-125 levels between patients with tumor size >2 cm ($n=14$, 53.8%) and those with tumor size ≤ 2 cm ($n=12$, 46.2%). No significant difference was also observed in serum endocan levels between the two tumor size groups.

When all the groups were evaluated together, a positive correlation was determined between serum CA-125 and endocan levels ($r=.43$, $P<.0001$). While there was no correlation between endocan and CA-125 in endometrial cancer, a significant positive correlation was observed in the ovarian cancer group ($r=.54$, $P=.015$).

Serum CA-125 levels were 80% sensitive and 62.5% specific in differentiating ovarian cancer cases from patients with benign diseases. The cut-off value was set at 35 U/mL.

Serum CA-125 levels were 76.9% sensitive and 45% specific in differentiating endometrial cancer cases from patients with benign diseases. The cut-off value was set at 25 U/mL.

Sensitivity and specificity for serum endocan were 90% and 97% in ovarian cancer and 85% and 97% in endometrial cancer. Among the entire patient group, sensitivity and specificity for serum endocan were 87% and 97%. The cut-off values for endometrial and ovarian cancers were set at 185.76 pg/mL and 192.06 pg/mL, respectively.

4 | DISCUSSION

The findings of this study show that endometrial and ovarian cancer can cause an increase in serum endocan levels. Endocan and CA-125 were correlated in these patients. Although one previous study investigated ovarian tissue endocan expression in ovarian cancer,²³ none have investigated serum levels of endocan in benign or malign gynecological disorders. This is therefore the first study to investigate serum endocan levels in cases of ovarian and endometrial cancer.

Numerous studies have demonstrated an increase in serum levels of several tumor markers in cases of endometrial and ovarian cancer. HE4 is a novel biomarker that is expressed in epithelial ovarian cancers but not in normal surface epithelium. While elevation in serum HE4 levels in patients with epithelial ovarian cancer has been reported in over 80% of cases, in Moore et al.'s study,²⁴ serum HE4 levels were less commonly elevated than CA-125 levels in women with benign gynecological disorders. Another study by Kalogera et al.²⁵ reported HE4 elevation in patients with endometrial cancer and determined a correlation with primary tumor diameter and myometrial invasion. Other authors have suggested that HE4 is a more sensitive marker than CA-125 in endometrial cancer.^{26,27}

Both ovarian and endometrial tissues develop from the Müllerian system, and cancers arising from the two tissues share similarities in terms of etiological factors, gene expression profiles, tumorigenic mechanisms, pathological changes, and metastatic characteristics.^{28,29} It is not surprising that a single marker or marker panel may be capable of recognizing both conditions. CA-125 has been found to be overexpressed in both ovarian cancer and endometrium cancer tissues, and increased CA-125 serum levels have been proposed as a biomarker for the detection and monitoring of both malignancies. However, the unsatisfactory sensitivity and specificity of the CA-125 assay has prompted intensive efforts in the search for superior biomarkers.^{12,27}

Endocan is synthesized by endothelial cells and regulated by tumor cell-derived factors, including VEGF.³⁰ Various cytokines, such as interleukin-1 β (IL-1 β) and tumor necrosis factor alpha (TNF- α), also play a role in the regulation of endocan synthesis.³¹

Angiogenesis, defined as the emergence of new blood vessels, is an important factor both in tumor growth and in spread and metastasis. Endocan synthesis has been shown to be induced by proangiogenic factors, such as VEGF-A and VEGF-C, involved in the progression of cancer lymphoangiogenesis and angiogenesis.³² Studies have also investigated levels of endothelial markers involved in angiogenesis, tumor tissues, blood circulation, and levels in urine.^{33,34}

Zhang et al.³⁵ showed that endocan is expressed in neogenically active tissue and cell types. They also suggested that endocan may be an important marker in angiogenesis and neogenesis.

Studies have shown that serum endocan concentrations increase under septic conditions.³⁶⁻³⁸ Higher endocan levels have also been determined in severe sepsis than in mild sepsis. It has therefore been suggested that endocan can be used as a marker in detecting and determining the severity of sepsis.³⁶

However, increased secretion of endocan has been observed in several malignant diseases, such as hepatocellular carcinoma, bladder cancer, and renal clear cell carcinoma.³⁹⁻⁴¹

Nault et al.³⁹ measured serum levels of VEGF, endocan, syndecan-1, and glypican-3 molecules in patients with early or advanced hepatocellular cancer and reported higher serum endocan levels in cases of cancer compared to patients with noncancerous alcoholic cirrhosis. They reported that serum ESM-1 levels in cancer patients were correlated with survival and invasion. They also suggested that since ESM-1 is synthesized for tumor endothelial cells it may represent a good marker of angiogenesis and may even be a potential target in the treatment angiogenesis.³⁹

In Roudnicky et al.'s study,⁴⁰ plasma endocan levels in cases of invasive bladder cancer were also higher than those in healthy individuals. In addition, using real-time PCR, endocan expression was shown to be 1000-100 000 times higher in cancerous tissue. Due to the increases in plasma endocan levels, those authors suggested that these could be used as a prognostic marker in cases of invasive bladder cancer. Additionally, they determined that interaction between the endocan molecule and VEGF-A on the cell surface facilitates binding to VEGFR-2. On the basis of these findings, they concluded that increased synthesis of endocan, in association with tumor-related VEGF-A elevation, may stimulate vascularization. In other words, endocan molecules may be involved in tumor spread by increasing angiogenesis.⁴⁰

Leroy et al.⁴¹ measured serum endocan levels in cases of renal "clear cell" tumor and reported 3-10-times higher levels in patients with renal papillary cancer compared to healthy individuals. They suggested that endocan may represent a potential test for use in assessing tumor response to antiangiogenic treatments.⁴¹

Matano et al.⁴² investigated the relation between tumor invasion and endocan expression in patients with pituitary adenoma and reported that increasing endocan is involved in tumor angiogenesis and can be used to show pituitary adenoma invasion of the neighboring cavernous sinus. They suggested that it may therefore be useful in

showing invasion and angiogenic status of pituitary adenomas.⁴² One study also reported increased endocan expression in tissue samples from patients with ovarian cancer.²³

In our study, serum endocan levels were greater in endometrial and ovarian cancer cases with high sensitivity and specificity. Although benign ovarian or endometrial disorders do not generally lead to expression of endocan, malignant disorders can result in measurable endocan levels. This may be useful in differentiating between benign and malign diseases of endometrium or ovary.

In conclusion, serum endocan levels may increase in cases of both ovarian and endometrial cancer. Further studies involving larger patient numbers are now needed to confirm the role of CA-125 and endocan in the diagnosis of these diseases.

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