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SERUM AND ENDOMETRIAL TISSUE ENDOCAN LEVELS IN PATIENTS WITH ENDOMETRIAL POLYPS, HYPERPLASIA AND CANCER

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ABSTRACT

Introduction: Endocan is a proteoglycan which participates in inflammation and angiogenesis. This study aims to determine whether serum and endometrial tissue concentrations of endocan can be used to differentiate between benign and malignant endometrial pathologies. **Materials And Methods:** This is a cross-sectional review of 90 patients who underwent endometrial biopsy due to abnormal vaginal bleeding and who were diagnosed with endometrial polyp (n=65), endometrial hyperplasia (n=12) and endometrial cancer (n=13) at the study center. **Results:** When compared to the endometrial cancer patients, the patients with endometrial polyp had significantly younger age and longer height (p=0.001 and p=0.003 respectively). When compared to the endometrial cancer patients, the patients with endometrial hyperplasia had significantly younger age and less frequent menopause (p=0.01 and p=0.001 respectively). The patients with endometrial hyperplasia had significantly higher gravidity than the patients with endometrial polyp (p=0.020). The patients with endometrial polyp, endometrial hyperplasia and endometrial cancer had statistically similar concentrations of endocan in serum and endometrial tissue (p=0.249 and p=0.675 respectively). **Conclusion:** The patients with endometrial polyp, endometrial hyperplasia and cancer were statistically similar with respect to the endocan concentrations in serum and endometrial tissue.

KEYWORDS : endocan; endometrium; endometrial cancer; endometrial hyperplasia; polyps

INTRODUCTION

Endometrial polyps are benign pathologies which are characterized with the overgrowth of endometrial stroma and glands and the protrusion of this overgrowth into uterine cavity[1]. The majority of endometrial polyps are asymptomatic, their diagnosis is incidental in most cases[2]. However, endometrial polyps can also lead to abnormal vaginal bleeding in both premenopausal and postmenopausal women[3]. Endometrial polyps can be associated with endometrial hyperplasia and cancer[4-6]. It has been reported that the incidence of endometrial cancer changes between 0% and 4.8% in women with endometrial polyps[5,6].

Endometrial hyperplasia refers to the excessive proliferation of endometrial cells[7]. When accompanied with atypical cells and histopathological complexity, endometrial hyperplasia has been addressed as a precursor lesion for endometrial cancer[8]. It has been well established that endometrial hyperplasia and cancer share common predisposing factors[9]. Endometrial cancer is the most common gynecological malignancy in developed countries and the incidence of this gynecological malignancy is steadily increasing due to the expansion of elderly population and the rise in obesity rates[10]. The understanding of endometrial cancer has developed through the improvements in molecular biology and, thus, allowed the identification of target-specific treatment methods[11].

Endocan is a dermatan sulfate proteoglycan which is also named as endothelial cell specific molecule-1[12,13]. This proteoglycan is mainly synthesized in endothelial cells so that it plays a crucial role in inflammation and angiogenesis[14]. Moreover, endocan participates in the pathogenesis of vascular injury caused by organ specific pathologies. Therefore, serum concentrations of endocan increase in many inflammatory diseases and various malignancies[15,16].

This study aims to determine whether serum and endometrial tissue concentrations of endocan can be used to differentiate between benign and malignant endometrial pathologies.

MATERIALS AND METHODS

This is a cross-sectional review of 90 patients who underwent endometrial biopsy due to abnormal vaginal bleeding and who were diagnosed with benign and malignant endometrial pathologies at the study center between January 2018 and June 2018. The present study was approved by Institutional Review Board and local Ethical Committee (grant no: 2018/2-58). All participants were informed about the study protocol and written consent was obtained from each patient.

All patients who were diagnosed with benign and malignant endometrial pathologies due to endometrial biopsy results and who volunteered to participate in this study were included. The patients aged less than 25 years, the patients aged more than 90 years, the patients with obesity (body mass index more than 30 kg/m^2), the patients who had concurrent malignancy and the patients who smoked and/or consumed alcohol were excluded.

Data related with age, height, weight, gravidity, parity, menopausal state, concurrent diseases and oral contraceptive use were recorded for all patients. Body mass index (BMI) was calculated as follows: Body mass index (kg/m) =Weight (kg) / Height^2 (m^2)

The study cohort consisted of 65 patients who were diagnosed with endometrial polyp, 12 patients who were diagnosed with endometrial hyperplasia and 13 patients who were diagnosed with endometrial cancer according to the histopathological examination of endometrial biopsy specimens. The patients with endometrial polyp, endometrial hyperplasia and endometrial cancer were compared with respect to the levels of endocan in serum and endometrial tissue samples.

Measurement Of Serum Endocan Levels

Serum samples for the measurement of endocan levels were

centrifuged for 15 minutes at 4000 xg at + 4°C. The serum samples obtained were divided into aliquots and stored in a deep freeze at - 80°C until analysis. Endocan levels in serum samples were measured using ELISA with a "Human ESM-1 ELISA Kit" (Elabscience Biotechnology Co. Ltd., Lot: AK0016DEC07042, Wuhan, China). It uses a double-antibody sandwich enzyme-linked immunosorbent assay. Samples like serum and standards were put into the 96 well microplate being coated on with a monoclonal antibody (also known as Capture Antibody that is specific for C terminal of human endocan), and incubated for 90 minutes. Endocan molecules present within a sample are bound by the Capture Antibody. After washing away of any unbound molecules, a secondary monoclonal antibody specific for N terminal of endocan that has been biotinylated, was added to the wells to incubate for another 1 hour. After a washing step, streptavidin-HRP (biotin-binding protein conjugated with polymers of horseradish peroxidase) was added and allowed to incubate for 30 minutes. Unbound material was washed away. Chromogen solution was added and incubated for 15 minutes (protected from light) for the conversion of the colorless solution to a blue solution, the intensity of which was proportional to the amount of endocan in the sample. As an effect of the acidic stop solution, the color has become yellow. The colored reaction product was measured using an automated ELISA reader at 450 nm. The results were expressed as picograms per milliliter (pg/mL). The intra-assay and inter-assay coefficients of variation were less than 8% and 10% respectively.

Measurement Of Endometrial Tissue Endocan Levels

Endometrial tissues were homogenized and then put in lysis buffer (50 mM HEPES, 0.1 M NaCl, 10 mM EDTA, 4 mM sodium pyrophosphate, 10 mM sodium fluoride, 2 mM sodium orthovanadate (pH 7.5); 1 mM phenylmethylsulfonylfluoride, 1% Triton X-100, 5 g/mL leupeptin, 5 g/mL aprotinin). Supernatants were acquired after centrifugation for 20 minutes at 12000 ×g at 4°C. Subsequently, the extracted protein concentration was detected by the BCA Protein Assay Kit (Thermo Scientific, Hudson, NH, USA). Protein sample was mixed with loading buffer and boiled for 10 minutes. Total 60 g protein was separated on 10% SDS-PAGE gel per well, and then transferred to polyvinylidenedifluoride membranes (Roche Diagnostics, Indianapolis, IN, USA). After blocked with 5% defatted milk at room temperature for 1 h, the membranes were incubated overnight at 4°C with goat anti-human endocan polyclonal antibody (1:1000; R&D Systems, Minneapolis, MN, USA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:10000; Abmart, Shanghai, China) monoclonal antibody, respectively. GAPDH was served as an endogenous loading control. Next, the membranes were washed for three times with TBST, then incubated with rabbit anti-goat IgG (H+L)-HRP (1:5000, Abmart) for 2 hours at 25°C, respectively. Ultimately, proteins were visualized using enhanced chemiluminescence reagents (Thermo Scientific), and the relative expression of endocan protein was analyzed by densitometry using the Image-J imaging analysis software (NIH, Bethesda, MD). The results were expressed as nanograms per milliliter (ng/mL). The intra-assay and inter-assay coefficients of variation were less than 8% and 10% respectively.

Statistical Analysis

Collected data were analyzed by Statistical package for Social Sciences version 22.0 (SPSS IBM, Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation whereas categorical variables were denoted as numbers or percentages where appropriate. Normality of data distribution was assessed by Kolmogorov-Smirnov test. One way analysis of variance, Kruskal-Wallis test and Pearson correlation test were used for the analyses. In order to avoid the risk of Type 1 error by multiple comparisons, alpha levels were adjusted using the conservative Bonferroni correction method. Two-tailed p values less than 0.05 were accepted to be statistically significant.

RESULTS

Table 1 shows the sociodemographic and clinical characteristics of the participants. When compared to the endometrial cancer patients, the patients with endometrial polyp had significantly younger age and longer height (p=0.001 and p=0.003 respectively). Gravidity was significantly lower, parity was significantly lower and menopause was significantly less frequent in the patients with endometrial polyp than the patients with endometrial cancer (p=0.023, p=0.045 and p=0.001 respectively). When compared to the endometrial cancer patients, the patients with endometrial hyperplasia had significantly younger age and less frequent menopause (p=0.01 and p=0.001 respectively). The patients with endometrial hyperplasia had significantly higher gravidity than the patients with endometrial polyp (p=0.020).

Table 1: Sociodemographic And Clinical Characteristics Of The Participants

	Endometrial polyp (n=65)	Endometrial hyperplasia (n=12)	Endometrial cancer (n=13)	p
Age (years)	45.9±3.7	46.8±5.6	63.2±9.1	0.001*, 0.01**
Height (m)	1.63±0.91	1.61±0.84	1.59±0.75	0.003*
Weight (kg)	71.8±5.7	74.5±6.4	73.9±8.3	0.491
Body mass index (kg/m^2)	28.2±3.6	30.7±5.2	30.0±4.1	0.184
Gravidity	3.2±1.4	4.3±2.2	4.0±2.1	0.023*, 0.020***
Parity	2.6±1.1	3.1±1.9	3.1±1.8	0.045*
Menopause	17 (26.2%)	4 (33.3%)	12 (92.3%)	0.001*, 0.01**
Hypertensive disease	14 (21.5%)	4 (33.3%)	6 (46.2%)	0.160
Diabetes mellitus	15 (23.1%)	2 (16.7%)	5 (38.5%)	0.398
Oral contraceptive use	6 (9.2%)	1 (8.2%)	0 (0.0%)	0.524

*There is statistical significance between polyp and cancer groups.

**There is statistical significance between hyperplasia and cancer groups.

***There is statistical significance between polyp and hyperplasia groups.

Table 2: Serum And Endometrial Tissue Endocan Levels Of The Participants

	Endometrial polyp (n=65)	Endometrial hyperplasia (n=12)	Endometrial cancer (n=13)	p
Serum endocan (pg/ml)	322.5±128.4	430.1±230.5	330.9±144.2	0.249
Endometrial tissue endocan (ng/g)	3.3±1.7	4.2±1.9	3.4±1.9	0.675

Table 2 demonstrates the serum and endometrial tissue concentrations of endocan in patients with endometrial polyp, endometrial hyperplasia and endometrial cancer. The patients with endometrial polyp, endometrial hyperplasia and endometrial cancer had statistically similar concentrations of endocan in serum and endometrial tissue (p=0.249 and p=0.675 respectively).

Table 3 displays the sociodemographic and clinical characteristics of the patients with endometrial cancer. Serum concentrations of endocan do not significantly correlate with age

(r=0.293, p=0.331), BMI (r=0.043, p=0.890), gravidity (r=0.092, p=0.765), parity (r=0.246, p=0.418), disease stage (r=0.244, p=0.422), tumor grade (r=0.238, p=0.433) and endometrial tissue concentrations of endocan (r=0.214, p=0.483) in patients with endometrial cancer. Similarly, endometrial tissue concentrations of endocan do not significantly correlate with age (r=0.438, p=0.134), BMI (r=0.497, p=0.084), gravidity (r=0.535, p=0.060), parity (r=0.520, p=0.069), disease stage (r=0.484, p=0.094) and tumor grade (r=0.368, p=0.216) in patients with endometrial cancer.

Table 3: Sociodemographic And Clinical Characteristics Of The Patients With Endometrial Cancer

Patient no	Age (years)	BMI (kg/m^2)	Gravidity	Parity	Stage	Grade	Serum endocan (pg/ml)	Tissue endocan (ng/g)
1	71	30.0	5	3	2	2	300.64	3.07
2	77	29.0	6	5	3	3	105.33	3.58
3	45	27.0	2	2	2	3	115.26	2.77
4	74	32.0	4	4	3	3	125.73	4.56
5	51	25.0	2	2	2	2	171.70	2.23
6	77	29.0	3	2	3	3	181.27	3.49
7	55	26.0	4	3	2	2	213.83	2.76
8	57	29.0	3	3	2	2	232.97	2.87
9	72	42.0	6	4	3	3	246.38	3.63
10	54	30.0	3	3	2	2	247.02	3.36
11	52	31.0	5	3	2	2	300.44	4.61
12	61	30.0	5	3	2	3	593.70	3.61
13	76	30.0	4	4	3	3	1767.52	3.83

DISCUSSION

Angiogenesis refers to the formation of vessels from pre-existing vasculature[17]. This is an essential procedure for tissue repair and remodeling in chronic diseases[18]. Angiogenesis is also required for the emergence and growth of tumors and it has been well established that this procedure correlates with metastasis and poor prognosis[19]. Angiogenesis related with the development of tumors indicates the impairment in the maturation and functioning of blood vessels[20]. That is, endothelial cells in tumors are so disorganized and loosely connected that they fail to cover the vascular walls uniformly. This impairment might hinder the infiltration of immune cells and enhance the dissemination of tumor cells[20]. Vascular formation during the development of tumors involves the proliferation and migration of endothelial cells[21]. These alterations related with endothelial cells are under the regulation of several cytokines such as transforming growth factor, vascular endothelial growth factor (VEGF) and platelet derived growth factor. Amongst them, VEGF has a major role in angiogenesis as it triggers endothelial mitosis and vascular permeability[22].

The aforementioned angiogenic growth factors induce the expression of endocan within endothelium[23]. After being secreted from endothelial cells, endocan regulates the interactions between the leukocyte function associated antigen-1 and intercellular adhesion molecule-1[24]. Animal models have shown that the overexpression of endocan has been associated with tumor progression[25,26].

Since endocan participates in inflammation, cell adhesion and carcinogenesis, it has been reported that endocan might be used as a novel endothelial marker for malignant disease[23-26]. As endocan expression has been detected in endothelial cells of tumors, it would be prudent to expect the presence of endocan in serum and tissue samples of the patients diagnosed with cancers. It has been reported that the increase in endocan mRNA concentrations designates poor prognosis in patients with breast, kidney, bladder, pancreas, stomach, colon, liver and brain tumors[27].

El Behery et al. were the first to assess the expression of endocan in ovarian cancer. In their study, there was positive expression of endocan in endothelium of all ovarian cancer specimens while there was no expression of endocan in endothelium of all normal ovarian tissues. Moreover, VEGF was expressed in endothelium of all ovarian cancer specimens and 70% of normal ovarian tissues. A significant and negative association was also found between overall survival and tissue endocan expression in patients with ovarian tumors. Therefore, tissue endocan expression was defined as an independent prognostic marker for overall survival in epithelial ovarian cancer[15].

Laloglu et al. compared the serum endocan concentrations of patients with ovarian cancer (n=20), endometrial cancer (n=27), benign ovarian diseases (n=19), benign endometrial pathologies (n=19) and healthy controls (n=35). Forty one out of 47 patients (87.2%) diagnosed with ovarian and endometrial cancers had detectable serum endocan levels. On the other hand, only 5% of the patients with benign ovarian and endometrial pathologies and 6% of healthy controls except three individuals had detectable serum concentrations of endocan. Serum endocan levels in the ovarian cancer and endometrial cancer groups were significantly higher than those in the benign ovarian and endometrial pathology groups and in the healthy control group. Endocan values correlated strongly with tumor size, tumor grade and disease stage and, thus, serum endocan levels were addressed as one of the most accurate indicators for the postoperative recurrence in patients with ovarian cancer. Additionally, serum concentrations of endocan were statistically similar in healthy controls and patients with benign ovarian and endometrial diseases. In case the cut-off value for serum endocan level was set at 192.06 pg/ml, its sensitivity and specificity for ovarian cancer were 90% and 97% respectively. When the cut-off value for serum endocan level was accepted as 185.76 pg/ml, its sensitivity and specificity for endometrial cancer were 85% and 97% respectively[16].

CONCLUSIONS

To the best of our knowledge, this is the first study to investigate how serum and endometrial tissue concentrations of endocan are altered in benign and malignant endometrial pathologies. This study points out that the patients with endometrial polyp, endometrial hyperplasia and endometrial cancer had statistically similar endocan concentrations in serum and endometrial tissue. This unexpected and contradictory finding can be attributed to the relatively small cohort size and variations in the techniques adopted for the measurement of serum and endometrial tissue endocan levels. The power of the present study was also limited by the lack of randomization and absence of data related with VEGF levels in serum and endometrial tissue. Another power limiting factor was the inability to draw receiver operating characteristic curves and assign cut-off values for serum and endometrial tisuue endocan concentrations due to the small cohort size.

Further research is warranted to clarify the role of serum and

endometrial tissue endocan levels in differential diagnosis of benign and malignant endometrial diseases.

Author Contribution

MB and FC- Protocol/project development, Data collection and management, Data analysis, Manuscript writing/editing, Manuscript conceptualization; MKP- Data analysis, Manuscript writing/editing, Manuscript conceptualization; DTA- made substantial contributions to conception, acquisition, or interpretation of data.

Conflict Of Interest

The authors declare no conflict of interest.

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Ethics Approval And Consent To Participate

Ethical approval was obtained for this study from the Afyonkarahisar Health Sciences University Clinical Research Ethics Committee (2030-KAEK-2).

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