Relation of peritoneal fluid and serum vascular endothelial growth factor levels to endometriosis stage

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Summary

Objective: It is a well known fact that endometriosis is linked with apoptosis, extracellular matrix formation, and angiogenesis. In this study, the authors aim to investigate the relation between the extent of endometriosis and vascular endothelial growth factor (VEGF). *Materials and Methods:* Twenty-one patients who received laparoscopic intervention due to endometriosis constituted the patient group, whereas 19 patients who were operated due to extra-endometrial benign cyst were included in the control group. Following the laparoscopic pelvic assessment, peripheral blood samples and two cc of free peritoneal fluid from the Douglas pouch were obtained simultaneously. The samples were studied with regards to VEGF level via solid phase sandwich enzyme-linked immunosorbent assay (ELISA) method. *Results:* In the patient group, eight cases were diagnosed with Stages I and II endometriosis, while 13 cases were diagnosed with Stages III and IV endometriosis. Among the Stage I and II cases, serum VEGF levels were statistically significantly higher, as compared to the Stage III and IV cases, as well as the control group. *Discussion:* In conclusion, the authors found a relationship between elevated serum VEGF levels and early stage endometriosis.

Key words: Endometriosis; Vascular endothelial growth factor.

Introduction

Endometriosis is a polygenic multifactorial disease that is defined as the ectopic presence of functional endometrial tissues outside the uterine cavity, affecting 9-33% of women of reproductive age [1,2]. Although the etiology of the disease has not yet been clearly described, there are studies indicating the involvement of changes in the immune system [3]. Recent studies suggest that endometriosis is related to apoptosis, extracellular matrix formation, and angiogenesis [4-6]. Vascular endothelial growth factor (VEGF) is one of the factors that is studied in this regard which is a member of the platelet-derived growth factor family and plays a role in the angiogenesis. VEGF was first defined as a factor increasing the vascular permeability, however, it also triggers proliferation, migration, differentiation, and capillary formation in the endothelial cells, as well [7].

Endometriotic tissues demonstrate elevated levels of protein and VEGF mRNA expression, thus triggering vascular development in the peritoneal region and facilitating the implantation and viability of the endometrial cells [7-8]. In this study, based on these data, the authors aimed to investigate whether there is any relationship between VEGF levels and the extent of endometriosis.

Materials and Methods

The study was conducted in the Third Clinic of S. B. Izmir Ege Obstetrics and Gynecology Teaching and Research Hospital between September 2010 and September 2011. Twenty-one patients aged 19-51 years who presented to the present clinic because of adnexal mass, chronic pelvic pain or infertility, and who were scheduled for laparoscopic diagnosis and treatment subsequent to the final assessments, constituted the patient group, whereas 19 patients with extra-endometrial benign ovarian cyst were included in the control group. The staging of the endometriosis cases was performed based on the classification of the American Society for Reproductive Medicine (ASRM) including visual pelvic assessment, largeness and depth of lesions, and adhesive properties. The cases of ASRM Grades I and II were defined as early stage, whereas the ones with an ASRM Grades III and IV were deemed as advanced stage. Following the laparoscopic assessment and staging, peripheral blood and two cc of free peritoneal fluid, from the pouch of Douglas, samples were obtained simultaneously prior to the surgical procedure. The serum was separated.The serum and peritoneal samples were stored in Eppendorf tubes at 80°C until the date of analysis. The cases with blood in the peritoneal fluid (ruptured cyst), ectopic pregnancy, abscess in the fallopian tube, or pelvic infection, as well as those suspected of malignancy along with patients in whom a sample of two cc free peritoneal fluid could not be obtained, were excluded form the study. The patients were asked about their general and gynecologic medical history, clinical symptoms, menstruation period, and cause of hospital presentation, and the responses were noted in a pre-made information form. Blood and peritoneal VEGF levels were measured twice with solid phase sandwich enzyme-linked immunosorbent assay (ELISA) method. VEGF values were evaluated in pg/ml.

The study data were analyzed with SPSS (Statistical Package for the Social Sciences) program. The distribution of the quantitative data was analyzed with the Kolmogorov-Smirnov test, and homogeneity was evaluated by the Levene's test. The parameters with normal distribution and homogenous variance were analyzed via parametric methods, while the parameters with non-normal distribution and heterogeneity were analyzed with non-parametric methods. Regarding the parametric methods, the intergroup comparisons were carried out with the Independent t-test. Regarding the non-parametric methods, intergroup comparisons were performed with

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Table 1. — *Demographic characteristics of the study groups.*

	Patient			Control			
	Mean	SD	Median	Mean	SD	Median	*p*
Age	30.38	7.58	28	33.15	10.41	34	0.342
Pregnancy	1.05	1.32	1	2.16	2.32	1	0.105
Parity	0.62	0.74	0	1.47	1.47	1	0.051

Table 2. — *Distribution of the cases relative to the symptoms.*

Symptom	Patient group n (%)	Control group n (%)	*p*
Dysmenorrhea	21 (100)	3 (15.8)	0.001
Dyspareunia	16 (76.2)	2 (12.5)	0.001
Non-cyclic pelvic pain	16 (76.2)	8 (42.1)	0.028
Irregular menstruation	13 (16.9)	5 (26.3)	0.024
Infertility	9 (42.8)	0 (0)	0.001

Table 3. — *Distribution of the cases relative to the endometriosis stage.*

	n	%
Early stage (Grade I-II)	8	38.1
Advanced stage (Grade III-IV)	13	61.9
Total	21	100

Table 4.— *Blood and peritoneal VEGF levels in the study population.*

	Patient			Control			
	Mean	SD	Median	Mean	SD	Median	P
Blood VEGF	133.71	89.15	122	101	105.02	75	0.101
Peritoneal VEGF	98.67	228.04	26	73.21	139.33	10	0.186

Table 5. — *Comparison of the patients and the control groups relative to the blood VEGF levels.*

		(A) Early stage subgroup n: 8	(B) Advanced stage subgroup n:13	(C) Control group n:19
Blood VEGF $p = 0.045$	Mean	192.86	104.14	101
	Standard deviation	37,02	18,85	24,9
	Median	176	87	75
	Maximum	400	233	360
	Minimum	108	16	12
	p	*p* (A-B): 0.047	*p* (A-C): 0.032	*p* (B-C):0.924

Table 6. — *Comparison of peritoneal VEGF levels between the patient subgroups and the control group.*

		(A) Early stage subgroup n: 8	(B) Advanced stage subgroup n:13	(C) Control group n:19
Peritoneal VEGF $p = 0.257$	Mean	179.14	58.43	73.21
	Standard Deviation	146.24	20.14	31.96
	Median	28	22.50	10
	Maximum	1056	270	564
	Minimum	18	10	10
	p	*p* (A-B): 0.176	*p* (A-C): 0.213	*p* (B-C):0.825

Mann-Whitney U test and Moses test. The relationships between non-categorical parameters were analyzed by the Kendall's Tau-b and Spearman's rho test. The categorical parameters were compared using Chi-square, continuity correction, and Fisher's exact tests. The data were studied using 95% confidence interval and *p* values < 0.05 were recognized as statistically significant.

Results

The patient and control groups had an age range of 19-51 years and the mean age was 30.38 ±7.58 and 33.15±10.41 years, respectively. Both groups were compared with regard to age, pregnancy, and parity, revealing that pregnancy and parity were higher in the control group, though there was no statistically significant difference (Table 1). Infertility history, irregular menstruation, non-cyclic pelvic pain, dysmenorrhea, and dyspareunia were more common in the patient group than in the control group ($p < 0.05$) (Table 2). Regarding the endometriosis staging, eight patients (38.1%) were early stage (Grades I-II), whereas 12 patients (61.9%) were advanced stage (Grades III-IV) (Table 3). There was no statistically significant difference between the patient and control groups with regards to blood and peritoneal VEGF levels ($p > 0.05$) (Table 4).

The comparison of blood VEGF levels among the patient subgroups revealed that early stage patients had significantly higher VEGF levels ($p = 0.047$). The comparison of early stage patient subgroup and the control group with regard to blood VEGF levels showed that the early stage patient subgroup had significantly higher blood VEGF levels as compared to the control group ($p = 0.032$). However, there was no significant relationship between the advanced stage patient subgroup and the control group in terms of blood VEGF levels ($p = 0.924$) (Table 5). There was no statistically significant difference between the early stage and advanced stage patient subgroups ($p = 0.176$), between early stage patient subgroup and the control group ($p = 0.213$), and between advanced stage patient subgroup and the control group ($p = 0.825$) (Table 6).

Discussion

Endometriosis is a disease of unclear etiology with high morbidity that affects 9-33% of women of reproductive age which reduces quality of life by accompanying infertility and pelvic adhesion, as well as chronic pelvic pain symptoms [1, 9, 10]. The definitive diagnosis including the extent of the disease, is achieved by laparoscopic pelvic examination, lesion excision, and histopathologic examination [11, 12]. Since the diagnosis is established with an invasive method, investigators have been looking for new markers that can improve the non-invasive diagnostic methods [13, 14]. To date, many of the studies aiming to unveil the etiopathogenesis of endometriosis have focused on macrophages in the peritoneal cavity, cytokines secreted from those macrophages, and growth factors. VEGF is one of those cytokines and growth factors which is studied by *in vivo* and *in vitro* researches [7, 15].

Although VEGF is a platelet-derived growth factor, it is also an important mediator of local angiogenesis [16]. Angiogenesis is one of the factors playing a key role in the occurrence, development, and progression of endometriotic lesions [17]. Endometriotic lesions have been shown to have different vascular patterns depending on the localization and stage (early or advanced) [18]. In peritoneal endometriosis, vascularization is intense in early active (red) lesions and these lesions include many angiogenic vessels that are indicative of high mitotic index. However, dark endometriotic foci contain mature vessels. Ovarian and deep infiltrative endometriotic foci are highly vascularized and have vessels with no immature pericytes [19]. Therefore, angiogenetic factors are observed to increase in patients with endometriosis, peritoneal implants, as well as in the peritoneal fluid of patients with ovarian endometriosis. The presence of elevated VEGF levels has been shown in the peritoneal fluid of ovarian endometrioma cases [20].

Matalliotakis *et al.* and Goter *et al.* showed increased serum angiogenetic factor levels in endometriosis patients as compared to the non-endometriotic patients [21, 22].

In the present study, blood and peritoneal VEGF levels were higher in the patient group than in the control group, however, the difference was not statistically significant. Gagne *et al.* found no relationship between endometriosis and blood-peritoneal VEGF levels. In their study, the exact time of sample collection during menstruation was not noted [20]. However, it is reported that VEGF levels may differ at different times of menstruation. In a study supportive of this opinion, Pupo-Nogueira *et al.* compared peritoneal VEGF concentrations at proliferative phase and early secretion phase in women with definitive diagnosis of endometriosis. Late secretion phase demonstrated significantly higher VEGF levels in the peritoneal fluid [23]. The authors believe that using no specific time point for the collection of blood and peritoneal samples and the balance generated by the elevated blood VEGF levels in patients with early stage disease and the blood VEGF levels at advanced stages, may have influenced their results.

The most significant result of this study was the evidences indicating a relation of VEGF level with early and advanced stage endometriosis. The authors determined significantly higher blood VEGF levels in the early stage endometriosis subgroup than in the advanced stage endometriosis subgroup and the control group. Fujishita *et al.* found higher blood VEGF levels in early stage endometriosis than in advanced stage endometriosis [24]. It is known that early stage endometriotic foci have higher amount of highly vascularized red lesions as compared to scar lesions [25]. This result helps us to see why VEGF level is higher in early stage endometriosis cases with rich vascularization.

In conclusion, VEGF level was found to be elevated in the early stages of endometriosis with rich vascularization. This result should be further supported by large-scale studies including exact timing of sample collection during various endometriotic stages. Thus, the authors believe that high VEGF levels can be useful in predicting the disease stage preoperatively and in establishing a differential diagnosis of endometriosis in combination with the other radiologic and serologic markers.

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