

Vascular endothelial growth factor (VEGF) and ovarian endometriosis: correlation between VEGF serum levels, VEGF cellular expression, and pelvic pain

Vascular endothelial growth factor (VEGF) serum levels and VEGF and cellular expression were prospectively analyzed in 60 patients (group A consisted of asymptomatic patients or patients presenting mild dysmenorrhea; 30 women comprised group B severe dysmenorrhea and/or chronic pelvic pain and/or dyspareunia) who underwent surgery for cystic ovarian endometriosis to assess whether a correlation exists among VEGF serum levels, VEGF cellular expression, and pelvic pain. No differences were found in VEGF serum levels and VEGF cellular expression between both groups. Therefore, we conclude that pain symptoms in ovarian endometriosis are not correlated with VEGF serum levels and VEGF cellular expression. (*Fertil Steril*® 2007;88:513–5. ©2007 by American Society for Reproductive Medicine.)

Endometriosis is a common gynecologic disease of unknown etiology. The most widely accepted hypothesis for the development of endometriosis is retrograde menstruation. However, some other factor renders certain women susceptible to the implantation and growth of this ectopic endometrium.

Angiogenesis appears as one of the processes involved in the pathogenesis of endometriosis (1, 2). Angiogenic factors are increased in the peritoneal fluid of patients with endometriosis (3, 4), in peritoneal implants (5), and in ovarian endometriomas (6, 7). On the other hand, some investigators have found that angiogenesis is related to pelvic pain (8).

We speculated that ovarian endometriomas in patients presenting with pelvic pain would have more angiogenesis than those in asymptomatic women and, therefore, their vascular features would be different (9).

One key mediator of angiogenesis is vascular endothelial growth factor (VEGF) homodimeric glycoprotein, which stimulates proliferation and migration of endothelial cells and enhances vascular permeability (10). Although VEGF is normally produced by various tissues in the female genital tract, such as the endometrium, ovary, and placenta, VEGF receptors are found exclusively in endothelial cells. Vascular endothelial growth factor is involved in normal endometrial turnover during the menstrual cycle, and is under the control of ovarian steroids (11). High concentrations of VEGF have been reported in peritoneal liquid in pelvic endometriosis and in cystic liquid in ovarian endometriomata (12).

The aim of our study was to further investigate the immunohistochemical expression of VEGF in the various cell pop-

ulations of ovarian endometriotic cysts and compare VEGF serum levels in asymptomatic patients and women with pelvic pain to determine whether this growth factor can be used as a serum marker of endometriosis activity.

In this prospective study, 65 premenopausal women (mean age: 34.38 ± 7.07) were enrolled from February 2003 to February 2005.

Patients were divided in two groups according to clinical complaints. Group A included asymptomatic patients or patients presenting mild or moderate dysmenorrhea, but without dyspareunia or chronic pelvic pain ($n = 33$). Group B included patients presenting severe dysmenorrhea (with no response to conventional analgesic, treatment such as antiprostaglandins, and requiring bed rest) and/or dyspareunia and/or chronic pelvic pain ($N = 32$). The degree of pain was established using a visual analogue scale (13).

All patients provided informed consent after the nature of the study was fully explained, and institutional review board approval (Clinica Universitaria de Navarra) was obtained before starting the study.

Blood samples were collected from all patients before anesthesia by venipuncture into 10-cc sterile tubes and were kept at room temperature until centrifugation at $400 \times g$ for 10 minutes. There were <2 hours allowed between blood collection and processing. Serum aliquots were then frozen at -80°C until measurement of VEGF serum levels.

Concentration of VEGF serum levels was measured using a commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instruction. Briefly, serum samples and standards were incubated for 2 hours in a microplate precoated with murine anti-VEGF monoclonal antibodies. After washing three times, enzyme-linked anti-VEGF polyclonal antibodies were added. Unbound antibodies were removed by washing. The intensity of the reaction was then revealed with tetramethyl-

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TABLE 1

Results.			
	Group A	Group B	P
Adherences ^b			.270
No	9 (30%)	14 (47%)	
Smooth	5 (17%)	6 (20%)	
Dense	16 (53%)	13 (33%)	
AFSr Stage ^b			.313
II	6 (20%)	4 (13%)	
III	19 (63%)	16 (53%)	
IV	5 (17%)	10 (33%)	
AFSr Score ^a	34.6 (28.9)	44.5 (30.08)	.199
Mean VEGF ^c	77.71 (127)	55.34 (61.48)	.65
VEGF immunoreactivity ^c	40.99 cells/mm ² (36.26)	37.63 cells/mm ² (36.19)	.67

^a Data expressed as mean (SD).

^b Tukey B test.

^c ANOVA test.

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benzidine, and optical density was measured at 450 nm. The sensitivity of the assay was <9.0 pg/mL. Inter- and intraassay variations were <10%.

All patients were surgically treated, and a definitive histologic diagnosis of ovarian endometriosis was obtained. The presence and type of pelvic adherence, revised American Fertility Society scores, and stages were recorded (14). Surgical samples were sent in formalin for histologic diagnosis.

Pathologic description of the cysts and histologic slides were retrieved from the archives. Representative areas of the cyst were selected for immunohistochemical investigations. Immunohistochemistry was performed on 5- μ m paraffin-embedded tissue sections. After heat drying, sections were deparaffinized in xylene and sequentially rehydrated in gradients of ethanol. After 3 washes with H₂O₂, the sections were incubated overnight at 4°C with the anti-VEGF (dil 1:100, AB-293-NA, R&D, Oxford, UK) and anti-CD-34 (dil:1/100, DAKO, Glostrup, Denmark). Positive reaction was revealed by the streptoavidin–biotin–peroxidase technique: sections were incubated with 3,3'-diaminobenzidine (0.05 diaminobenzidine in 0.05 M Tris buffer, pH 7.6 and 0.01% hydrogen peroxide), and counterstained with Mayer's hematoxylin. Positive controls for the reaction were represented by paraffin-embedded sections from renal carcinoma. Negative controls were made by substituting the primary antibody with a nonimmune serum.

The endothelial cell reactivity for VEGF was expressed as the mean of positive cells counted under a light microscope. The expression of VEGF was separately evaluated in the inner subepithelial specialized stroma and in the outer fibrous cap-

sule of the cysts. All counts were performed by two investigators. Both had to agree on the count of VEGF positive.

From 65 patients, five cases were ultimately excluded, because the histologic diagnosis was other than cystic ovarian endometriosis (two teratomas and three haemorrhagic cysts). The presence and type of pelvic adherence, mean revised American Fertility Society score, and revised American Fertility Society stages and sizes of endometriomas were not statistically different between groups (Table 1).

The mean VEGF serum levels in group A was 77.71 pg/mL \pm 127) and in group B was 55.34 pg/mL \pm 61.48) (P =.65). To verify whether this observation could have been biased by the lack of control for several possible confounders, the mean VEGF serum levels was adjusted with respect to gravidity, length of menses, infertility, and body mass index in a univariate general linear model (15). Using this model, no significant difference was observed in mean VEGF serum levels between the two groups.

The VEGF immunoreactivity was variable in the different cyst layers and was higher in the inner layer (33.20/mm²) than in the outer layer (12.20/mm²) when all cases were analyzed. In the asymptomatic group, VEGF cellular expression was 40.99 cells/mm² \pm 36.26, and in the asymptomatic group it was 37.63 cells/mm² \pm 36.19 (P =.67). No correlation was found between VEGF serum levels and VEGF cellular expression (P =.33, r = 0.128).

The presence of ovarian cystic endometriosis is associated with pelvic pain in women suffering this disease (8). On the other hand, angiogenic factors have been found increased in ovarian endometriomas (6). Angiogenesis is related to vascularization. Therefore, a correlation between

vascularization and the presence of pelvic pain might be assumed. Some studies assessing angiogenic activity in endometriosis have used either morphometric or immunohistochemical techniques in endometriotic tissue (6, 16–18). Other studies have evaluated vascular activity measuring serum (15, 19) or peritoneal fluid concentrations of angiogenic factors, such as VEGF (1, 3).

We evaluated VEGF serum levels and VEGF cellular expression in patients diagnosed with ovarian endometriomas. In the present study, we demonstrated for the first time that VEGF serum levels are not increased in patients presenting pelvic pain compared with those who are asymptomatic.

We conclude that although VEGF seems to play a key role in the local development of endometriotic lesions, the disease is not associated with a significant modulation in the levels of circulating VEGF, and the activity of endometriosis cannot be monitored using VEGF serum levels.

Previously, some investigators assessed that angiogenic factors are increased in the serum of patients with endometriosis (18) when compared with patients without endometriosis, and VEGF cellular expression is increased in patients diagnosed with ovarian endometriosis (7).

In the present study, we analyze for the first time whether the VEGF serum levels was higher in patients with pelvic pain than in asymptomatic patients, and whether VEGF levels are correlated with the VEGF tisular expression. Our study was also designed for seeking correlation among VEGF cellular expression, pelvic pain, and VEGF serum levels.

VEGF serum levels are not higher in symptomatic patients and the VEGF cellular expression is not correlated with serum levels. In agreement with Artini (20), we suppose that, although this factor plays an important role in endometriosis pathogenesis, in ovarian endometriosis, the angiogenetic processes are still present but probably not completely mediated by VEGF.

On the other hand, and in agreement with Gotieri (7), we found that the cells with the highest frequency of diffuse VEGF expression were macrophages, followed by capsular vessel and subepithelial stromal cells and by epithelial cells and capsular fibroblasts. Our results confirm the hypothesis that the VEGF expression is higher in the outer layer than the inner layer, and the outer angiogenesis would contribute to cyst growth, to the fibrosing process in the capsule, and to the formation of adhesions.

In conclusion, pelvic pain in ovarian endometriosis disease is not correlated with the VEGF serum levels and VEGF cellular expression. Further investigations should be done to evaluate the microvascular density in ovarian endometriosis and this correlation with VEGF cellular expression to get a better understanding of the role of this cytokine in the formation and growth of ovarian endometriotic cysts.

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