Signs of reduced angiogenic activity after surgical removal of deeply infiltrating endometriosis

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Objective: To study the concentrations of vascular endothelial growth factor A (VEGF-A), soluble vascular endothelial growth factor receptors-1 and -2 (sVEGFR-1 and -2), angiogenin, and angiopoietin-2 (Ang-2) in serum and peritoneal fluid from healthy controls and women with advanced endometriosis. Further, we addressed the question of whether surgical removal of endometriotic lesions was associated with normalization of the serum concentrations of the same markers.

Design: Patients with endometriosis before and after surgery were compared with control patients.

Setting: University Hospital.

Patient(s): Twenty-one healthy controls and 32 women with advanced endometriosis.

Intervention(s): In women with endometriosis we performed surgical removal of endometriotic lesions using laparoscopy.

Main Outcome Measure(s): Data on serum and peritoneal fluid concentrations of selected markers in healthy controls and women with endometriosis before surgery and in serum 5 to 7 days after surgery.

Result(s): Women with endometriosis had elevated levels of VEGF-A, sVEGFR-1, and Ang-2 in serum and all studied markers in peritoneal fluid compared with healthy controls. Surgical removal of endometriotic lesions resulted in decreased serum levels of pro-angiogenic VEGF-A and increased levels of sVEGFR-2 that negatively regulates the action of VEGF.

Conclusion(s): Women with advanced endometriosis have serum and peritoneal fluid concentrations of several factors involved in the regulation of angiogenesis that differ from those in healthy women, and these changes at least partly normalize within a week after surgical removal of the endometriotic lesions. (Fertil Steril® 2010;94:52–7. ©2010 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, angiogenesis, VEGF, sVEGFR-1, sVEGFR-2, angiogenin, Ang-2

Endometriosis is a common gynecologic disease, occurring in about 10% of all women (1). Despite a number of theories concerning the origin of endometriosis (2), the precise pathogenetic mechanisms remain enigmatic. It is believed that peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities with respect to the pathophysiology and clinical significance of the disease (3). Deeply infiltrating endometriotic lesions often form adenomyoma-like structures with a surrounding fibromuscular hyperplasia (4). Endometriotic implants that penetrate >5 mm are defined as deeply invasive (5). Such endometriotic nodules are associated with clinical signs, symptoms, and prognosis that differ from those of the classical peritoneal lesions (6), and surgical removal is usually superior to medical treatment in attempts to relieve pain (7). Deeply infiltrating endometriosis located in the rectovaginal septum is quite common (8), and usually causes pelvic pain with particular characteristics such as severe deep dyspareunia and painful defecation (9). These lesions are difficult to diagnose, because they often remain unnoticed during laparoscopy.

It has been shown that endometriotic lesions recruit blood vessels by inducing angiogenesis (10). Peritoneal fluid in women with endometriosis contains increased amounts of macrophages and their secreted products, such as growth factors, cytokines, and angiogenic factors (11). Several angiogenic growth factors, such as vascular endothelial growth factor A (VEGF-A), are present in human ectopic endometrium (12, 13).

Vascular endothelial growth factor A has been detected in serum and peritoneal fluid from patients with endometriosis (13–17). Vascular endothelial growth factor A exerts its biologic effects by binding to either of its two tyrosine kinase receptors, vascular endothelial growth factor receptors 1 and 2 (VEGFR-1 and VEGFR-2). These receptors also exist in soluble forms, sVEGFR-1 and -2, which can be quantified in body fluids such as peritoneal fluid and blood (18–20). The soluble receptors are thought to act as negative regulators of VEGF availability for endothelial cells (20, 21), thereby reducing angiogenic activity.

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Angiogenin is a polypeptide that also is involved in stimulating angiogenesis by activating capillary endothelial cells (22). Elevated levels of angiogenin have been demonstrated in both serum and peritoneal fluid from women with endometriosis, and the levels correlated with the extent of the endometriosis (23, 24).

Angiopoietins constitute a family of growth factors including angiopoietin-1 and -2 (Ang-1 and Ang-2), both of which are ligands to the endothelium-specific receptor Tie2 (25). Ang-2 functions are context-dependent. Angiopoietin-2 facilitates angiogenesis if it functions in concert with VEGF, and it leads to vessel regression in the absence of VEGF (26). Angiopoietin-2 is thus a key regulator of vascular quiescence (27). It has also been shown that Ang-2 triggers an inflammatory response by activating the endothelium and inducing permeability (28, 29). Angiopoietin-2 mRNA and protein expression has been demonstrated in normal endometrium during the menstrual cycle (25, 30–32). Angiopoietin-2 can be assayed in serum (33), but to date, no comparisons of serum concentrations have been made between women with endometriosis and healthy controls.

We hypothesized that the serum and peritoneal concentrations of VEGF-A, sVEGFR-1 and -2, angiogenin, and Ang-2 differ between women with advanced endometriosis and healthy controls, and that these values normalize after surgical removal of the endometriotic lesions. We therefore studied the concentrations of VEGF-A, sVEGFR-1 and -2, angiogenin, and Ang-2 in serum and peritoneal fluid from healthy controls and women with grade III–IV deeply infiltrating endometriosis alone or in combination with ovarian endometriomas. Further, we addressed the question of whether surgical removal of endometriotic lesions was associated with normalization of the serum concentrations of the same markers.

MATERIALS AND METHODS

The study group consists of 32 women of reproductive age with advanced peritoneal and deeply infiltrating endometriosis. All 32 women had extensive pelvic endometriosis and growth of endometriotic nodules in the rectovaginal septum. Of these 32 women, the rectal wall adjacent to the rectovaginal septum was partly destroyed by endometriosis in 4 women, the rectal wall was affected but not destroyed by rectovaginal endometriotic nodules in 23 women, and rectovaginal endometriotic nodules were combined with ovarian endometriomas in 5 women. The extent of the endometriosis was scored according to the revised American Society for Reproductive Medicine Classification (34), and was equivalent to stage III–IV in all cases. Repeated attempts with conservative treatment using GnRH-analogues, gestagens, or combined contraceptive pills combined with nonsteroid anti-inflammatory drugs and other pain killers did not relieve the severe pelvic pain that was their main symptom. The remaining treatment option was surgical intervention, and the treatment of these women consisted of laparoscopic hysterectomy and resection of endometriotic nodules but without removal of adnexa in 11 women, laparoscopic hysterectomy combined with resection of endometriotic nodules in the rectovaginal septum and removal of adnexa in 12 women, and laparoscopic hysterectomy with resection of endometriotic nodules in the rectovaginal septum combined with removal of adnexa and partial resection of the anterior rectal wall in 9 women. All known and recognizable endometriotic lesions were removed. Histopathologically, all samples of suspected endometriotic lesions contained foci of endometrial glandular epithelium surrounded by stromal or muscular tissue. Of these 32 women, 11 had regular menstrual cycle and they were all in the secretory phase of the menstrual cycle at the time of surgery, as confirmed both by endometrial histopathology (35) and serum progesterone levels. The remaining 21 patients had oligomenorrea, metrorrhagia, or menometrorrhagia, and according to endometrial histopathology and serum progesterone levels they had not ovulated and were thus anovulatory or in the proliferative phase. Women with other diseases than endometriosis and those who had received hormonal treatment such as combined contraceptive pills, gestagens, or GnRH-analogues within the last 3 months were not included in the study.

Twenty-one healthy reproductive-aged women without endometriosis were recruited as controls. Venous blood samples were collected in the morning before surgery. They had no symptoms of endometriosis, and no endometriotic lesions were found during the laparoscopic sterilization that they all underwent. Of these 21 women, 10 were assessed as being in the proliferative phase and 11 in the secretory phase of the menstrual cycle, as confirmed both by endometrial histopathology (35) and serum progesterone levels.

Venous blood samples were collected from the 32 patients with endometriosis in the morning before surgery and 5 to 7 days after surgery. Peritoneal fluid samples that not were contaminated by blood were collected by aspiration during surgery. Both the serum and peritoneal fluid samples were cooled on ice water and centrifuged at 1,500 $\times$ g for 10 minutes in a refrigerated centrifuge. Serum and peritoneal fluid samples were stored at -70°C until analyzed.

The Ethics Committee at the Research Centre for Obstetrics, Gynaecology and Perinatology in Moscow approved the study protocol and informed consent was obtained from all patients.

Analysis of Serum and Peritoneal Fluid

Samples were analyzed for VEGF-A, its soluble receptors sVEGFR-1 and -2, angiogenin, and Ang-2, using commercially available ELISA kits (DVE00, DVR100B, DVR200, DAN00, and DANG20, R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. All analyses were performed in duplicates. The color development was measured in a SpectraMax 250 (Molecular Devices, Sunnyvale, CA).