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Are cytokine levels in serum, endometrial tissue, and peritoneal fluid a promising predictor to diagnosis of endometriosis-adenomyosis?

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Summary

Aim: The basic aim was to find a non-invasive procedure to diagnose and monitor endometriosis-adenomyosis. *Materials and Methods:* A prospective study was carried out. The authors conducted a series of 60 consecutive patients who underwent diagnostic laparoscopy for benign gynecologic conditions. Endometrial, peripheral blood and peritoneal lavage samples were analyzed. IL-6, IL-16, TNF-alpha, and LIF levels were measured and compared. *Results:* The authors analyzed clinical data of 52 patients (26 endometriosis, 13 adenomyosis, and 13 control group). Peritoneal fluid IL-6 is significantly higher in stage IV endometriosis group than the control group ($p = 0.001$). In the endometriosis group, the levels of TNF-alpha in the peritoneal fluid was higher than the control group ($p = 0.008$). In the endometriosis and adenomyosis groups, the levels of IL-16 in the peritoneal fluid were significantly higher than the control group ($p = 0.000$ and $p = 0.002$). *Conclusions:* Significant immune-inflammatory changes were observed. When the underlying molecular mechanisms will be investigated, this will elicit studies on the immunotherapeutic treatment of endometriosis. Further studies are needed to assess various potential therapeutic interests for biomarkers in a large, well-defined patient population.

Key words: Cytokines; IL-6; Endometriosis; Peritoneal fluid; Tumor necrosis factor-alpha (TNF-α).

Introduction

Endometriosis is a condition where the functional and morphological endometrial glands and stromal structures are found outside the uterus. It a disease associated with pelvic pain and infertility and mainly affects women during their reproductive age. Hormonal influence could be the important predisposing factor as endometriosis is extremely uncommon before menarche and after menopause. It occurs most often in pelvis, peritoneum, ovaries, posterior cul-de-sac, and uterosacral ligaments. Retrograde menstruation, metaplasia, lymphatic and hematogenous outspread, mechanical transplantations, and some other theories have been introduced for its pathogenesis [1]. Histological examination is needed for a definitive diagnosis. Laparoscopy is currently considered to be the gold standard investigation in patients suspected to have endometriosis, but this is an invasive and relatively costly procedure and there may be significant delays in diagnosis [2]. Efforts to reduce this delay are required. Laboratory evaluation has a minor role in the diagnosis of endometriosis, although studies are underway investigating serum markers, genetics, and endometrial sampling [3]. The term of adenomyosis is the presence of endometrial glands and stroma inside the myometrium. Previously adenomyosis is also called endometriosis interna. The mechanism, clinical symptoms, and treatment of adenomyosis is completely different than endometriosis.

Despite high prevalence of endometriosis, little is known about the etiopathogenesis. Immune-inflammatory changes may be associated with its pathogenesis [4]. The inflammatory response to endometriosis, tissue repair, and revascularization is referred to macrophages and cytokines. Cytokines are proteins that play role in cell proliferation, activation, motility, adhesion, chemotaxis, and morphogenesis. The relation of some cytokines like interleukins (IL-1, IL-2, IL-6, IL-8, and IL-18) and tumor necrosis factor-alpha (TNF-alpha) to the pathogenesis of endometriosis has been previously studied [5]. Many studies focusing on this subject have the limitations of lack of peritoneal fluid sampling and lack of patients with adenomyosis. Mostly assessments are with only one biomarker or in small numbers of series. This present prospective study is designed to overcome these limitations. Cytokines were studied in endometrial tissue, peritoneal fluid, and serum of cases with endometriosis-adenomyosis. These were compared with control groups.

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Table 1. — *Patients' demographic features and the distributions of complaints.*

Groups	n	Median age ± sd (years)	BMI (kg/m2)	Dysmenorrhea n (%)	Dyspareunia n (%)	Pelvic pain n (%)	Primary infertility n (%)
Controls	13	34.8 ± 8.7	24.4 ± 2.1	4 (32%)	9 (72%)	4 (32%)	7 (56%)
Adenomyosis	26	49.1 ± 8.5	26.5 ± 1.9	6 (48%)	7 (56%)	10 (80%)	0
Endometriosis	13	32.4 ± 7.5	25.5 ± 2.9	10 (40%)	18 (73%)	24 (95%)	15 (60%)

BMI: body mass index; n: number of cases; sd: standard deviation.

The basic aim was to find a non-invasive procedure to diagnose and monitor endometriosis. Regarding this entity, in this study the authors analyzed the levels of IL-6, IL-16, leukemia inhibitory factor (LIF), and TNF- alpha in serum, endometrial tissue, and peritoneal fluid.

Materials and Methods

A prospective study was carried out between April 2009 and August 2009 in the Department of Obstetrics and Gynecology, Selcuk University Meram Medical School. After approval by the Institutional Ethics Committee the authors conducted a series of 60 consecutive patients who underwent diagnostic laparoscopy for pelvic pain, primary infertility, dysmenorrhea, dyspareunia, and for benign gynecologic conditions. Informed consent was obtained from all patients. None of the patients had autoimmune diseases, pelvic inflammatory diseases, and the history of pregnancy in the last six months. They were diagnosed preoperatively by means of physical examination, transvaginal pelvic ultrasonography, and endometrial biopsy. The endometrial samples in heparinized tubes were studied on the same day by flow cytometry. In patients with suspicions of adenomiyosis, magnetic resonance imaging is also used. Peripheral blood samples are obtained before surgery. Blood samples were centrifuged at 400 g (20 minutes) and stored at -80 °C until assayed. Laparoscopy was performed with standard laparoscopic procedures in the follicular phase under general anesthesia in all. Primarily the peritoneal lavage sampling was obtained. These samples were centrifuged at 400 g (20 minutes) to separate the cells from the peritoneal fluid supernatants were removed and samples were stored at -80 °C until assayed for cytokine content. The diagnosis and staging of endometriosis was done by visual evaluation according to the classification of American Fertility Society. The location, size, and the stage of endometriosis was documented and noted to the operation findings. The tubes, ovaries, pouch of Douglas, and intestines were evaluated respectively. The assessment of benign gynecologic conditions confirmed with intraoperative frozen section. In subsequent follow-up some patients who were especially diagnosed with adenomyosis underwent hysterectomy for benign gynecological conditions. Therein definitive diagnosis with histological examination revealed adenomyosis on the hysterectomy specimen and these were included to the adenomyosis group. Patients with blood in the peritoneal fluid or pelvic infection or those who had been diagnosed with a gynecologic malignancy were excluded from the study. Patients were divided into two major groups according to histopathological diagnosis: group 1 - endometriosis, group II - adenomyosis. Patients not classified either in adenomyosis or in endometriosis groups were decided as control group. The demographic characteristics and complaints of patients were evaluated. IL-6, IL-16, TNF- alpha, and LIF levels were measured and compared.

Statistical analysis

Data of the study were analyzed with SPSS version 13.0. Results are expressed as median and range with 95% confidence intervals. To compare discontinuous variants Chi-square test was used. Mann Whitney U test or *t*-test was used to compare continuous variants. Bonferroni corrected Mann Whitney U test was used for double comparisons. A value of $p < 0.05$ was considered statistically significant.

Results

A total of 60 women who were underwent diagnostic laparoscopy included in this prospective study. Because of blood presence in abdominal cavity, four cases were excluded and four samples could not be used because of technical problems during the process of storing and melting. The authors therefore analyzed clinical data of 52 patients; 26 of these were diagnosed pelvic endometriosis. Adenomyosis was present in 13 cases. Thirteen cases who were not classified in adenomyosis or in endometriosis groups were generated as the control group. The results of clinical classification of endometriosis according to American Fertility Society (AFS) and Revised American Fertility Society (RAFS) were stage I endometriosis in six (12%) patients, stage II in four (8%) patients, stage III in three (5%) patients, and stage IV in 13 (25%) patients, respectively. Patients' demographic features and the distributions of complaints are demonstrated in Table 1. There were no significant differences between the groups with respect to demographic data (age-body mass index), the cytokine levels (IL-6, IL-16, TNF-alpha, and LIF) of blood and endometrial tissue and also LIF levels in the peritoneal fluid. According to this study there was no relation found between the complaints of patients and cytokine levels. The authors did find a statistically significant difference between the groups when comparing the levels of IL-6 in the peritoneal fluid. Peritoneal fluid IL-6 was significantly higher in stage IV endometriosis group than in the control group ($p = 0.001$). In the endometriosis group, the levels of TNF-alpha in the peritoneal fluid was higher than the control group ($p = 0.008$). In the endometriosis and adenomyosis groups, the levels of IL-16 in the peritoneal fluid were significantly higher than the control group ($p = 0.000$ and $p = 0.002$).

Discussion

Promising new therapies for the treatment of endometriosis are continuing to develop. Many studies have

been performed to investigate the underlying molecular mechanisms. The precise pathogenesis of endometriosis is still unclear but it is well-documented today and chronic pelvic inflammation is a common feature in affected women. Significant immune-inflammatory changes have been observed; the ectopic endometrial cells resist apoptosis and produce proinflammatory, angiogenic, growth, and tissue remodeling factors, which may contribute to the ectopic growth of endometrial tissue [6]. Activated peripheral mononuclear cells as well as endometriotic cells in situ are hypothesized to secrete various cytokines with pleiotropic biological activities [7]. It is likely that the growth regulation in vivo of endometrial and endometriotic cells is controlled by a complex combination of cytokine and growth factors [8]. Although benign in structure, endometriosis exhibit differential invasive, adhesive, and proliferative behavior.

Still the diagnosis and staging of endometriosis can only be established by invasive procedures: laparoscopy or open surgery [2, 9]. CA-125 has been widely used for detection of endometriosis and monitoring of progressive disease. However, the sensitivity of this biomarker alone is unsatisfactory [9,10]. In the study of Patacchiola *et al.*, they did not succeed in identifying a clinically useful noninvasive diagnostic biomarker or panel of biomarkers [10].

In the present prospective study IL-6, IL-16, LIF, and TNF-alpha cytokines in the blood, peritoneal fluid, and endometrial tissue were compared. With some aspects this study includes many preliminary assessments. With regards to this topic, this is the first study to evaluate the levels of TNF-alpha and IL-16 in the endometrial tissue of patients in all stages of endometriosis and adenomyosis. As a consequence, the comparison of the present results with literature is restricted.

There were no significant differences between the groups with respect to demographic data (age-body mass index), the cytokine levels (IL-6, IL-16, TNF-alpha, and LIF) of blood and endometrial tissue and also LIF levels in the peritoneal fluid. In this study peritoneal fluid levels of interleukin-6 with endometriosis stage IV were significantly higher than controls ($p = 0.001$). The stage of disease may considerably alter the cytokine levels. Increased peritoneal fluid levels of interleukin-6 in patients with active red endometriosis may be related to endometriosis-associated infertility and to the pathogenesis of endometriosis [11]. IL-6 is a pro-inflammatory cytokine involved in the activation of T cells. IL-6 in the endometrial tissue and serum of patients with adenomyosis and endometriosis was not statistically different than the control group. To the present authors' knowledge, this is the first report in the literature of patients with adenomyosis that compared IL-6 levels in endometrial tissue and serum between controls.

Mihalyi *et al.* conducted a case–control study in 294 infertile women. In this study plasma levels of IL-6, IL-8, and CA-125 were increased in all women with endometriosis and in those with minimal–mild endometriosis, compared with controls [12]. In contrast to the present findings, significantly higher levels of interleukin-6 were observed in the serum of subjects with endometriosis in the report of Othman *et al.* [13].

No statistically significant results were obtained when comparing the level of LIF in the serum, peritoneal fluid, and endometrial sampling of patients with endometriosis (all stages), adenomyosis, and control group ($p > 0.005$). This is the first study to evaluate the levels of LIF in the serum of patients with all stages of endometriosis and also adenomyosis.

In the endometriosis group, the levels of TNF-alpha in the peritoneal fluid was higher than the control group ($p = 0.008$). The primary role of TNF-alpha is in the regulation of immune cells and known to be critically involved in the regulation of infectious, inflammatory, and autoimmune phenomena. By promoting the growth of endometriotic cells, TNF-alpha in the peritoneal fluid may be an essential factor in the pathogenesis of endometriosis [7]. TNF-alpha cytokine with pro-inflammatory and pro-angiogenic roles could potentially be involved in the diagnosis of endometriosis. The diagnostic accuracy of serum IL-6 and peritoneal fluid TNF-alpha levels in endometriosis showed a 90-100% sensitivity) and a 67-89% specificity in the study of Bedaiwy *et al.* [14].

In the endometriosis and adenomyosis groups, the levels of IL-16 in the peritoneal fluid were significantly higher than the control group ($p = 0.000$ and $p = 0.002$). In the study of Koga *et al.*, significantly higher concentrations of IL-16 in the peritoneal fluid of women were observed with only advanced endometriosis (stages III/IV) [14]. On the other hand, no relation was found between serum levels of IL-16 and endometriosis in the study of Zhang *et al.* [15]. IL-16 in peritoneal fluid may play a role in the pathogenesis of endometriosis by initiating or sustaining inflammatory responses in the peritoneal cavity [16].

Some factors have undoubtedly contributed to the conflicting results in literature. Phase of the menstrual cycle and stress factors before surgery might have affected plasma biomarker levels. None of the measured cytokines showed significant correlation with the complaints of patients. To detect and monitor endometriosis, a group of biomarkers may be more useful rather than a single indicator. In combination with imaging techniques, such a panel of biomarkers may indicate which women necessitate a laparoscopy and eliminate countless unnecessary operations [17].

In attempt to find a non-invasive procedure to diagnose and monitor endometriosis and adenomyosis, the present authors designed a prospective study. When the underlying molecular mechanisms will be investigated, this will elicit studies that will provide immunotherapeutic treatment of endometriosis. Further studies are needed to analyze various potential therapeutic interests for biomarkers in a large, well-defined patient population.

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