THE VALUE OF CA-125, CA 19-9, INTERLEUKIN-6, INTERLEUKIN-8 AND HSCRP IN THE DIAGNOSIS OF ENDOMETRIOSIS

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ABSTRACT

Introduction*: Endometriosis is a pelvic inflammatory process involving a malfunction in immune-related mechanisms. The pathogenesis of endometriosis is complex. However, this complex structure leads to the identification of disease with several potential diagnostic markers. The aim of our study was to develop a practical non-invasive test for the diagnosis of endometriosis by evaluating the serum levels of cancer antigen-125 (CA-125), cancer antigen 19-9 (CA 19-9), interleukin 6 (IL-6), interleukin 8 (IL-8) and high-sensitivity C-reactive protein (hsCRP) in patients with endometriosis.*

Methods*: A prospective case-control study was designed in Akdeniz University, Faculty of Medicine, Department of Obstetrics and Gynecology, for 164 consenting women of reproductive age who underwent a laparoscopic infertility examination between November 2010 and January 2012. Serum levels of biomarkers and surgical staging of endometriosis were compared to develop a practical non-invasive test for the diagnosis of endometriosis.*

Results*: A total of 164 women who underwent a laparoscopic infertility examination were divided into two groups: controls and patients with endometriosis. The serum levels of CA-125 and CA 19-9 were significantly higher in all women with endometriosis. A logistic regression analysis of biomarker combinations indicated that the results of a four-marker panel of CA-125, CA 19-9, IL-6 and hsCRP were significantly associated with a positive diagnosis of patients with stage 3-4 endometriosis during the secretory phase.*

Conclusion*: This study evaluated the use of each biomarker singly and in combination for the diagnosis of endometriosis. CA-125 and CA 19-9 are reliable non-invasive biomarkers for determining the severity of endometriosis. During the secretory phase, an analysis of a combination of CA-125, CA 19-9, IL-6 and hsCRP allows the diagnosis of patients with stage 3-4 endometriosis.*

Key words*: Endometriosis, Serum Biomarkers, Non-Invasive Diagnosis, Laparoscopic Infertility Examination.*

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Introduction

Endometriosis is a common, benign, chronic, estrogen-dependent disorder. It can be associated with many distressing and debilitating symptoms, such as pelvic pain, severe dysmenorrhea, dyspareunia and infertility, but endometriosis can also be asymptomatic and only discovered incidentally during laparoscopy or laparotomy. Despite the use of several methods, the gold standard for the diagnosis of endometriosis is an endoscopic surgery. It is primarily a disease of the reproductive years.

The disease is estimated to affect 2%-18% of reproductive-age women and more than 40% women with infertility[(1)]; however, this number is widely disparate depending on the study. Unfortunately, the prevalence in the general population is not known. The high prevalence and severe outcomes of this disease have made it a major public health concern in last decades.

The pathogenesis of endometriosis has not been definitively established, but there are currently three main theories: implantation of endometrial tissue following retrograde menstruation, coelomic metaplasia and induction[(2)].

Recent studies also have presented evidence that alterations in humoral or cell-mediated immunity may make some women more susceptible to developing endometriosis[3,4]. At present, the definitive diagnosis of endometriosis requires surgery because imaging techniques, such as ultrasound and magnetic resonance imaging, have not been shown to be reliable in the diagnosis or staging of the disease[5]. Endometriosis induces local and systemic inflammation. Therefore, numerous studies have focused on the markers of inflammation in the peritoneal fluid and/or serum of women who have the disease.

The aim of our study was to develop a practical non-invasive test for the diagnosis of endometriosis by evaluating the serum levels of cancer antigen-125 (CA-125), cancer antigen 19-9 (CA 19-9), interleukin 6 (IL-6), interleukin 8 (IL-8) and high-sensitivity C-reactive protein (hsCRP).

Materials and methods

This study was conducted at the Obstetrics and Gynecology Department of Akdeniz University Hospital between November 2010 and January 2012. It was approved by the ethics committee of the Faculty of Medicine, Akdeniz University. Informed written consent was obtained from each patient prior to any procedure. Inclusion and exclusion criteria for the study were summarized in Table 1.

Inclusion Criteria
Reproductive age (between 18 and 45 years)
Women who received a laparoscopic infertility examination
Exclusion Criteria
A suspected or ascertained diagnosis of malignancy
Pregnancy
Menopausal age
Any current infection (genital or systemic)
Pelvic inflammatory disease
Refusal to participate in the study

Table 1: Inclusion and exclusion criteria for the study.

Laparoscopic procedures were performed in the proliferative and secretory phase of the endometrial cycles. Blood samples were collected before the laparoscopy and centrifuged at 4000 rpm for 4 minutes. The serum samples were then aliquoted and stored at -80 0C until analysis.

During the laparoscopy, evidence of endometriosis was recorded and staged, according to the published revised American Fertility Society Classification System[6].

CA-125, CA 19-9, IL-6, IL-8 and hsCRP levels were measured in the Akdeniz University Hospital Biochemistry Laboratory. The quantitative detection of CA-125 and CA 19-9 was performed using a commercially available electrochemiluminescent immunoassay (ECLIA) kit provided by Roche Diagnostic GmbH (Germany). The concentrations of CA-125 and CA 19-9 were expressed as U/ml. The sensitivity for both CA-125 and CA 19-9 was 0,6 U/ml. The quantitative detection of IL-6 and IL-8 levels was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (DIAsource ImmunoAssays S.A. Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium). The concentrations of IL-6 and IL-8 were expressed as pg/ml. The sensitivity for both IL-6 and IL-8 was 1.0 pg/ml. The quantitative detection of hsCRP was performed using a monoclonal antibody and the nephelometric method (Siemens Healthcare Diagnostics Products GmbH, Germany).

The baseline characteristics of the two groups were compared with t-tests. Receiver operating characteristic (ROC) curves were constructed for each of the individual plasma markers to identify the discriminative power of each marker alone. Multivariate analysis was performed using logistic regression. The results are expressed as the median and range with 95% confidence intervals. Differences were considered statistically significant when the p-value was < 0.05.

Results

A total of 164 women who underwent laparoscopic infertility examination were divided into two groups: control cases (86 cases) with no pathologic findings, and patients with endometriosis (78 cases). The patients with endometriosis were subdivided into stage 1-2 (41 cases) and stage 3-4 (37 cases). The median age of the control cases was 31,4 years (min-max: 18-40). The median age of patients with stage 1-2 endometriosis was 28,9 years (min-max: 19-38), and the median age of patients with stage 3-4 endometriosis was 31,2 years (min-max: 19-43). The distribution of study samples according to the stage of endometriosis and menstrual cycle phase is shown in Table 2.

Cycle phase	Control cases	Patients with endometriosis	
	n(%)	**Stage 1-2 n(%)**	**Stage 3-4 n(%)**
Proliferative	45 (52.3)	22 (53.7)	20 (54)
Secretory	34 (39.6)	16 (39.0)	17 (46)
Unknown	7 (8.1)	3 (7.3)	0 (0.0)
Total	86 (100.0)	41 (100.0)	37 (100.0)

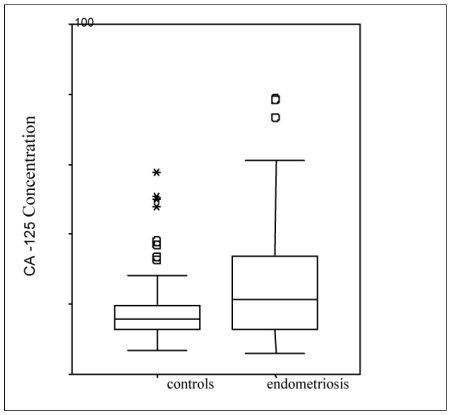
Table 2: Distribution of study samples according to the stage of endometriosis and menstrual cycle phase.

The distribution of study samples according to symptoms is shown in Table 3. There were no significant differences (p>0.05) between control cases and patients with endometriosis according to the age and symptoms except for dysmenorrhea. Dysmenorrhea was found to be significantly higher in patients with endometriosis (p=0.027).

Symptom	**Control cases n(%)**	**Patients with endometriosis n(%)**	**Total n(%)**	**p***
Dysmenorrhea 0.027	39 (45.3)	48 (61.5)	87 (53)	0.027
Dyspareunia 0.102	24 (27.9)	30 (38.5)	54 (33)	0.102
Pelvic pain 0.135	24 (27.9)	29 (37.2)	53 (32.3)	0.135
Primary infertility 0.208	51 (5.3)	52 (66.7)	103 (62.8)	0.208
Secondary infertility 0.208	35 (40.7)	26 (33.3)	61 (37.2)	0.208

Table 3: Distribution of study samples according to symptoms.
**t-test was used*

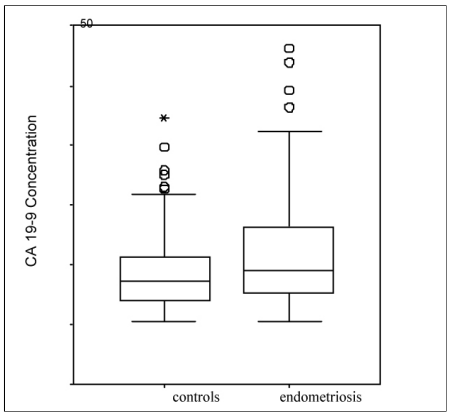
The serum levels of CA-125 were significantly higher in women with endometriosis compared with controls (p<0.05) (Figure 1).



18.4 ± 9.7 25.7± 17.0 (P=0.001)

Fig. 1: CA-125 levels in controls and endometriosis patients.

Serum CA-125 had a sensitivity of 70 % and a specificity of 79 % when discriminating between patients with stage 3-4 endometriosis and controls at a cut-off value of 21.7 U/ml. The serum levels of CA 19-9 were significantly higher in women with endometriosis and in women with stage 3-4 endometriosis compared with controls (p<0.05) (Figure 2).



8.9 ± 47.4 11.84 ± 10.2 (P=0.04)

Fig. 2: CA 19-9 levels in controls and endometriosis patients.

The sensitivity and specificity for CA 19-9 in the diagnosis of stage 3-4 endometriosis, using a 9.41 U/ml cut-off value, were 62% and 68%, respectively.

There were no statistically significant differences between cases with endometriosis and controls in the serum levels of IL-6, IL-8 and hsCRP (p>0.05) (Table 4).

Serum	**Patients with endometriosis (median)**	**Controls (median)**	**P value**
CA -125	25,74 +/- 17	18,4 +/- 9,7	0,001
CA 19-9	11,8 +/- 10,2	8,9 +/- 7,4	0,04
IL-6	12 +/- 9,1	9,3 +/- 10,8	0,086
IL-8	17,1 +/- 10,2	20,5 +/- 13,6	0,072
hsCRP	0,17 +/- 0,2	0,21+/- 0,27	0,271

Table 4: Levels of serum biomarkers.

However, serum IL-6 had a sensitivity of 82% and a specificity of 48% when discriminating between patients with endometriosis and controls at a cut-off value of 6.215 pg/ml. When a combination of biomarkers was evaluated using logistic regression, the results of a four-marker panel of

CA-125, CA 19-9, IL-6 and hsCRP were found to be significantly associated with a positive stage 3-4 endometriosis diagnosis during the secretory phase.

Discussion

In our study, when combinations of markers were evaluated using logistic regression analysis, the levels of a four-marker panel of CA-125, CA 19-9, IL-6 and hsCRP were found to be significantly associated with a positive diagnosis of patients with stage 3-4 endometriosis during the secretory phase.

The diagnosis of endometriosis is difficult because of non-specific symptoms, late presentation and the required use of laparoscopy, which involves risks and human error, including missing non-specific lesions[9]. Laparoscopic surgery is considered indispensable in assessing the presence, severity and recurrence of the disease. In recent years, significant effort have been expended in an attempt to identify an easy way to diagnose endometriosis[7].

The development of a simple blood test to screen patients at risk for endometriosis would reduce the number of unnecessary interventions. The concentration of serum CA-125, a 200 kDa glycoprotein, has been associated with the precense of many gynecologic disorders, including endometriosis[11]. The CA-125 antigen is expressed in many normal tissues, such as the endometrium, endocervix and peritoneum. CA-125 has been known for some time to be elevated in endometriosis, especially in advanced cases, and Mol et al. found that CA-125 may be more useful for diagnosing stage 3-4 disease than stage 1-2 disease[12-13]. CA-125 levels were found higher in patients with endometriosis[14] and also were found a correlation with disease stage in various studies[15]. Maiorana et al. indicated that CA-125 is more accurate for diagnosing women with later stages of the disease, which is in concordance with a review by Mol[16]. Kafali et al. looked at fluctuations in CA-125 levels across the menstrual cycle and found that alterations in this flux could be used as a possible diagnostic tool[17]. Some studies have assessed the value of CA-125 measurements during treatment. Chen et al. found that CA-125 levels fell significantly after 3 months of treatment with danazol[18]. Matalliotakis et al. studied the effect of leuprolide acetate and danazol on CA-125 levels. In addition, reduced CA-125 levels have been observed during treatment with both drugs[19]. Pittaway et al.concluded that the preoperative and postoperative values of this marker could be used to guide therapy, but that CA-125 levels cannot be used, in and of themselves, as a diagnostic test[20].

In our study, the serum levels of CA-125 were significantly elevated in all women with endometriosis and in women with stage 3-4 endometriosis compared with controls ($p<0.05$). Serum CA-125 had a sensitivity of 70% and a specificity of 79% when discriminating between patients with stage 3-4 endometriosis and controls at a cut-off value of 21.7 U/ml.

CA 19-9 was initially detected as an antigen related to colorectal carcinoma and can serve as a marker of potential metastasis and malignancy and also endometriotic tissue also expresses CA 19-9[21]. In the first study to assess CA 19-9, Panidis et al. reported that the baseline levels of CA 19-9 were elevated above the usual normal range in five of eight women with endometriosis, although no control group was studied[22]. The levels dropped significantly during treatment with danazol. Matalliotakis et al. found similar results[23]. Harada et al. found that the serum CA 19-9 levels in patients at any stage of endometriosis were significantly higher than in patients without endometriosis, and CA-19 serum levels correlated to surgical staging score[21]. But in some studies, there was no association between elevated CA 19-9 levels and endometriosis[12,24].

In our study, the serum levels of CA 19-9 were significantly higher in all women with endometriosis and in those with stage 3-4 endometriosis compared with controls ($p<0.05$). The sensitivity and specificity for CA 19-9 in the diagnosis of stage 3-4 endometriosis, using 9.41 U/ml as the cut-off value, were 62% and 68%, respectively.

IL-6 is a pleotropic cytokine that is produced by a variety of cell types, including monocytes, lymphocytes, fibroblasts, endothelial cells and keratinocytes. It is also produced by both eutopic and ectopic endometrium[25]. The cytokine is important in mediating acute phase reaction, inflammation and angiogenesis during folliculogenesis and the formation of decidua. Martinez et al. detected significantly elevated IL-6 levels in women with minimal-mild endometriosis, and this finding allowed them to discriminate between minimal-mild endometriosis and moderate-severe endometriosis

(mainly endometriomas) and other benign ovarian tumors[26]. Bedaiwy et al. indicated that serum IL-6 levels could be used to discriminate between patients with or without endometriosis[27]. In their study, serum IL-6 levels were not significantly affected by the menstrual cycle phase and disease stage. Therefore, IL-6 concentration should be viewed as a qualitative rather than a quantitative test of severity. They have emphasized the diagnostic value of serum IL-6 in detecting endometriosis and found that it achieved a sensitivity of 90% and a specificity of 67% when using a serum threshold of 2 pg/ml[27].

Othman et al. compared a group of serum cytokines individually and in combination. They found significantly higher levels of IL-6 in women with endometriosis than in controls. The researchers found that IL-6 provided a sensitivity of 71% and a specificity of 66% for discriminating between patients with endometriosis and controls at a cut-off value of 1.9 pg/ml[28]. IL-6 serum concentrations was found higher in patients with endometriomas and decrease after laparoscopic surgery or treatment with a GnRH agonist[29]. Mihalyi et al. found higher levels in women with both stage 1-2 and stage 3-4 disease[30]. A study has indicated that both eutopic and ectopic endometrium produce IL-6, and this may limit the value of serum IL-6 as an independent tool for predicting the presence of endometriosis[31]. Jee et al. demonstrated no significant differences in serum IL-6 levels between patients with endometriosis and controls[32].

In our study, there was no significant difference in serum IL-6 level between cases with endometriosis and controls, but serum IL-6 had a sensitivity of 82% and a specificity of 48% in discrimination of patients with endometriosis and controls at a cut-off value of 6.215 pg/ml. Our cut-off value was different from the other cited studies. The use of different commercially available kits may influence the results.

IL-8 is a monocyte/macrophage-derived chemokine that is capable of attracting and activating neutrophils[30]. In a study, no significant differences were found between patients with endometriosis and controls about serum IL-8 level[33]. On the other hand, Pizzo and Gomes-Torres et al, demonstrated significantly elevated serum IL-8 levels in women with endometriosis versus controls, and the levels were higher in stage 1-2 disease than in stage 3-4 disease (34-35). In our study, there was no significant serum IL-8 level difference between cases with endometriosis and controls.

Endometriosis can be considered an inflammatory disease. C-reactive protein (CRP) is an acute phase protein and inflammatory reaction marker that could serve as a potential non-invasive biomarker of endometriosis[30]. Abro et al. reported increased levels of CRP in women with stage 3-4 endometriosis[36]. However, Xavier et al. found no significant differences in serum CRP levels between patients with endometriosis and controls[37]. Lermann et al. found no significant differences between the levels of CRP and hsCRP in women with endometriosis and controls. There was no association between hsCRP and CRP levels and the disease stage. On the other hand, hsCRP was significantly lower than CRP in women without endometriosis. Therefore, the researchers indicated that hsCRP might be able to serve as a marker for the absence of endometriosis[38]. In our study, there was no statistically significant difference between cases with endometriosis and controls in the serum levels of hsCRP.

Seeber et al. evaluated the combined use of putative serum markers for the diagnosis of endometriosis, rather than the use of each marker in isolation. The researchers found that when using the serum concentration of four markers [(CA-125, leptin, macrophage migration inhibitory factor (MIF), macrophage chemotactic protein-1 (MCP-1)] and a two-tiered decision rule, nearly half of the subjects in this population would have been diagnosed with 93% accuracy[14]. Mihalyi et al. measured the plasma concentrations of IL-6, IL-8, tumor necrosis factor alpha (TNF-alpha), hsCRP, CA 19-9 and CA-125. The researchers stated that advanced statistical analysis of a panel of six selected biomarkers on samples obtained during the secretory phase or during menstruation allows the diagnosis of both minimal-mild and moderate-severe endometriosis with high sensitivity and clinically acceptable specificity[30]. In our study, when combinations of markers were evaluated using logistic regression analysis, the levels of a four-marker panel of CA-125, CA 19-9, IL-6 and hsCRP were found to be significantly associated with a positive diagnosis of patients with stage 3-4 endometriosis during the secretory phase.

One of the limitation of our study is that we did not use a heterogeneous control group. We evaluated an infertile group, and microscopic evi-

dence of endometriosis in normal-appearing peritoneum is common in asymptomatic infertile women. The other limitation of our study is that stress factors directly before surgery might affected serum biomarker levels, such as blood drawn just prior to anesthesia. Therefore, blood drawn for endometriosis biomarker analysis should be drawn independently of any surgery.

Conclusion

This study evaluated the use of each biomarker singly and in combination for the diagnosis of endometriosis. CA-125 and CA 19-9 were identified as reliable non-invasive biomarkers for determining the severity of endometriosis. IL-6 may contribute to the diagnosis of early stage endometriosis. During the secretory phase a combination of CA-125, CA 19-9, IL-6 and hsCRP allows the diagnosis of patients with stage 3-4 disease. In our study, we sought a non-invasive, non-surgical way to diagnose endometriosis. We found that biomarkers might be useful in the diagnosis of advanced stage disease. Additional studies focused on the early, non-invasive diagnosis of endometriosis are warranted.

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