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Investigation of diagnostic potentials of nine different biomarkers in endometriosis

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ABSTRACT

Objective: To investigate the diagnostic potentials of the serum levels of nine different biomarkers in endometriosis.
Study design: In this case-controlled, prospective clinical study, 80 women underwent laparoscopy or laparotomy with a preliminary diagnosis of chronic pelvic pain, severe secondary dysmenorrhea, infertility, pelvic endometriosis or pelvic mass. The 60 women with confirmed pelvic endometriosis constituted the endometriosis group, and the other 20 women without endometriosis constituted the control group.
Preoperative blood samples were obtained for serum biomarker measurements. Serum levels of nine different serum biomarkers including α-enolase, macrophage migration inhibitory factor, leptin, interleukin-8, anti-endometrial antibody, phosphoinositide dependent protein kinase 1, CA125, syntaxin-5, and laminin-1 were measured concurrently and compared between the control and endometriosis groups, and among control group and endometriosis subgroups including stage I, stage II, stage III and stage IV endometriosis.
Results: The serum levels of α-enolase, macrophage migration inhibitory factor, leptin, interleukin-8 and antiendometrial antibodies showed a statistically significant difference neither between control and endometriosis groups nor among control group and endometriosis subgroups. The serum levels of CA125, syntaxin-5 and laminin-1 showed a statistically significant difference both between the control and endometriosis groups ($p < 0.01$) and among control group and endometriosis subgroups ($p < 0.01$). Serum levels of laminin-1 in stage II and IV endometriosis; syntaxin-5 in stage I and II endometriosis; and CA125 in stage III and IV endometriosis were found to have the different levels compared to control group.
Conclusions: These findings show that the concurrent measurement of CA125, syntaxin-5 and laminin-1 might be a useful non-invasive test in strengthening the diagnosis of endometriosis and in predicting its severity.

Introduction

Endometriosis is a common gynecological disorder affecting 7–10% women in the reproductive years. Prevalence among those with infertility and chronic pelvic pain is reported high between 20 and 90%. Endometriotic patients often have symptoms such as dysmenorrhea, coital pain and infertility. These symptoms extremely disturb the quality of life of premenopausal women [1,2]. 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. Direct inspection of the abdominal cavity is recommended for the diagnosis of this disorder by means of laparoscopy or laparotomy [2,3]. The gold standard for the diagnosis of endometriosis is diagnostic laparoscopy; however, it is an invasive procedure that requires general anesthesia and surgical skill and is also not without hazards, which can include major vessel or bowel injury. Moreover, visual inspection of the pelvis has major limitations, particularly for the diagnosis of retroperitoneal and deep infiltrating lesions [4].

Endometriosis is a pelvic inflammatory condition involving a dysfunction in immune-related cells and macrophages within the

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peritoneum secreting a number of products, mainly cytokines and growth factors [5]. An intense inflammatory reaction with elevated pro-inflammatory cytokines and angiogenic factors, mobilization of fibroblasts and proliferation of connective tissue characterizes endometriosis but whether these phenomena are secondary to the disease remains elusive [6]. In an effort to find a less-invasive method for diagnosing endometriosis, and based on the fact that endometriosis induces a local, and also a likely systemic, inflammatory process, numerous studies have focused on markers of inflammation in the peritoneal fluid and/or serum of women who have the disease. It has been shown that many inflammatory and immunological biomarkers are provoked in patients with endometriosis [3,7].

In this case-controlled, prospective clinical study, we aimed to investigate the diagnostic potentials for endometriosis of nine different biomarkers. We performed biomarker measurements in serum because its procurement is truly noninvasive. Most studies have investigated these biomarkers individually; therefore, we measured concurrently the serum levels of the nine biomarkers in the study groups.

Materials and methods

This study was performed in the Department of Gynecology of University of Ondokuz Mayis in Turkey, over the course of 1 year. The women recruited in this study provided informed consent for a research protocol approved by the Ethics Committee on human research of the University. The study included the women undergoing laparoscopy or laparotomy with a preliminary diagnosis of chronic pelvic pain, severe secondary dysmenorrhea, infertility, pelvic endometriosis or pelvic mass. The women with laparoscopically or laparotomically confirmed pelvic endometriosis constituted the endometriosis group, and those without endometriosis constituted the control group. Endometriosis stages were determined according to the Revised American Society for Reproductive Medicine classification [8]. The subjects with autoimmune diseases, pelvic inflammatory disease, any malignancy, a history of delivery or abortion within the last six months, any endocrine disease, menopause, premature ovarian failure, menses, other pelvic masses out of endometrial adhesions or endometrioma, any anti-inflammatory or hormone medication within the last three months before operation, were excluded from the study.

Blood samples were obtained 1–2 h before the operation. These samples were centrifuged at 5000 rpm for 5 min to separate the cell pellet and the supernatant. The sera were then stored at −41 °C until assayed. The measurements of serum biomarkers were performed using micro-ELISA method by the ELISA reader (awareness technology well model, USA) in the ELISA laboratory. Detection range, inter-assay and intra-assay coefficients of variation in addition to company addresses of the ELISA kits used for the biomarker measurements were shown in Table 1.

The serum levels of biomarkers including serum cancer antigen 125 (CA125), syntaxin5 (STX-5), laminin-1 (LN-1), α-enolase (enolase), macrophage migration inhibitory factor (MIF), leptin, interleukin-8 (IL-8), antiendometrial antibodies (AEA) and phosphoinositide dependent protein kinase 1 (PDPK1) were measured and compared both between control and endometriosis groups, and among control group and endometriosis subgroups including stage I, stage II, stage III and stage IV endometriosis.

Statistical analysis was performed using the SPSS 15.0 software (SPSS, Inc., Chicago, IL). The comparison of the demographic characteristics between the groups was performed using student *t* test. Kruskal–Wallis variance analysis and then a Mann–Whitney *U* test were used to compare the group differences of biomarker concentrations. To identify the group differences, an optimal cut-off value for each biomarker using receiver operator curves (ROC) was selected. The sensitivity and specificity for each biomarker were determined by using ROC curves. The effects of the biomarker combinations to distinguish between the endometriosis and control groups were investigated by multivariate logistic regression analysis. Nominal variables were evaluated using Pearson's Chi-square test. The levels of biomarkers were expressed as median and inter-quartile range (IQR), and a value of $p < 0.05$ was accepted statistically significant.

Results

The study included 60 women in the endometriosis group and 20 women in the control group. The mean age was 32.33 ± 7.01 in the endometriosis group and 34.20 ± 6.88 years in the control group. The mean body mass index (BMI) was 23.75 ± 4.39 in the endometriosis group and $24.89 \pm 5.10\ kg/m^2$ in the control group. The mean age and the mean BMI between the groups were not statistically different ($p > 0.05$, $p > 0.05$ respectively). The comparison of age and BMI between the control group and endometriosis subgroups was shown in the Table 2.

Any statistically significant difference was not found between enolase, MIF, leptin, IL-8, AEA and PDPK1 median levels of endometriosis and control groups. Also, there was not a statistically significant difference in terms of the levels of these six biomarkers among control group and endometriosis subgroups including stages I, II, III and IV endometriosis. However, CA125, STX-5 and LN-1 levels showed a statistically significant difference both between the control and endometriosis groups ($p < 0.01$) and among control group and endometriosis subgroups ($p < 0.01$) (Tables 3 and 4).

Table 1
Characteristics of ELISA kits used for the measurements of serum biomarkers.

Serum biomarkers	ELISA kits used for measurement	Detection range	Intra-assay precision	Inter-assay precision
Enolase	(ENO1/ENO1L1/MBPB1/MBPB1) ELISA kit, Cusabio, Belgium	1.25–80 ng/mL	CV < 8%	CV < 10%
MIF	Human MIF ELISA kit, Cusabio, Belgium	125–8000 pg/mL	CV < 8%	CV < 10%
Leptin	Leptin micro ELISA kit, Diasourge, Belgium	>0,04 ng/mL	–	–
IL-8	IL-8 micro ELISA kit, Diasourge, Belgium	>1,1 pg/mL	–	–
AEA	Human endometrium antibody (EMab) ELISA kit, Cusabio, Belgium	Absorbance optic density	CV < 15%	CV < 15%
PDPK1	Micro ELISA kit for phosphoinositide dependent protein kinase 1 (PDPK1), USCN, Belgium	0.156–10 ng/mL	CV < 10%	CV < 12%
CA125	Human carbonhydrate antigen 125 ELISA kit, Cusabio, Belgium	15–300 U/mL	CV < 15%	CV < 20%
STX-5	Human syntaxin-5 ELISA kit, Cusabio, Belgium	23.4–1500 ng/mL	CV < 8%	CV < 10%
LN-1	Human laminin micro ELISA kit, USCN, Belgium	78–5000 pg/mL	CV < 10%	CV < 12%

MIF: macrophage migration inhibitory factor, IL-8: interleukin-8, AEA: antiendometrial antibodies, PDPK1: phosphoinositide dependent protein kinase 1, STX-5: syntaxin5, LN-1: laminin-1.

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