Non-invasive diagnosis of endometriosis based on a combined analysis of four plasma biomarkers

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Abstract

Purpose: The objective of the current study was to evaluate whether the analysis of different proinflammatory and angiogenesis-regulating cytokines in a well-defined patient population can be accurate for the diagnosis of endometriosis at different stages.

Material and methods: For this prospective study, plasma samples were collected from women undergoing laparoscopic surgery for subfertility. The stage of endometriosis was confirmed during laparoscopy.

Results: Plasma levels of interleukin 6 (IL-6), carbohydrate antigen (CA)-125 were significantly higher in women with endometriosis compared with controls (46.70 ±27.08 vs. 27.08 ±16.29, $p < 0.05$; 49.39 ±28.32 vs. 9.78 ±3.87, respectively). In women with minimal-mild endometriosis, only the plasma level of IL-6 was increased (56.45 ±23.21 vs. 27.08 ±16.29, $p < 0.05$). In women with moderate-severe endometriosis, plasma levels of IL-6 and CA-125 were significantly increased, and those of Epo and tumor necrosis factor α (TNF-α) were significantly decreased (44.53 ±31.07 vs. 27.08 ±16.29, $p < 0.05$; 57.84 ±61.44 vs. 9.78 ±3.87, $p < 0.05$; 26.60 ±10.19 vs. 30.32 ±7.94, $p < 0.05$; 58.61 ±7.93 vs. 65.40 ±9.86, $p < 0.05$, respectively). The area under curve (AUC) for Epo, TNF-α, IL-6 and CA-125 were 0.280, 0.322, 0.729 and 0.864, respectively.

Conclusions: The results of our study show that progression of endometriosis is associated with the elevated level of serum IL-6. Clearly, larger prospective studies are required to determine the diagnostic potential of measuring circulating inflammatory cytokine levels like IL-6 in endometriosis.

Key words: endometriosis, IL-6, CA-125, Epo, TNF-α.

Introduction

This perplexing disease affects 6-10% of women in the reproductive age (ages of 12-80 years) [1]. Laparoscopy is accepted as the "gold" standard for the diagnosis of endometriosis. The extent of endometriosis is generally staged by the American Society for Reproductive Medicine (ASRM) scoring system, which categorizes minimal and mild endometriosis as stage I and stage II, and moderate and severe endometriosis as stage III and stage IV [2].

A non-invasive diagnostic test for endometriosis might abolish the need for surgery. However, such a noninvasive diagnostic test is currently unavailable [3]. In the current practice, unexpected preoperative findings potentially can lead to under-treatment or unnecessary surgery. Accurate non-invasive diagnostic techniques are clearly necessary to manage women with endometriosis more effectively. In addition, a survey [4], which was taken by 7025 women with endometriosis, revealed that 65% of the women with endometriosis were initially diagnosed with another condition, which led to an average delay of 8 years between the start of symptoms and the actual diagnosis of endometriosis [5, 6].

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The immune deviations in endometriosis, including increased local production of some cytokines as well as elevated autoantibody production of local and systemic immunity, suggest that endometriosis may be an autoimmune disorder [7-11].

The objective of the current study was to evaluate whether the analysis of different proinflammatory and angiogenesis-regulating cytokines in a well-defined patient population can be accurate for the diagnosis of endometriosis at different stages.

Material and methods

For this prospective study, plasma samples were collected from women undergoing the laparoscopic surgery due to infertility at the gynecology clinic of the Department of Obstetrics and Gynecology, Istanbul University School of Medicine (Istanbul, Turkey). The study had received approval from the Ethics Committee of the Istanbul University School of Medicine and written consent from the participants before its initiation. Blood (4 ml) was drawn on the day of the surgery, centrifuged at 1000 rpm for 15 min, labeled and stored at –80˚C till analysis. The stage of endometriosis was confirmed during laparoscopy according to the revised American Fertility Society classification [12]. For each patient, relevant clinical information (age, stage of endometriosis, current medication and, number and type of previous operations) was recorded.

The first comparison was of controls (22 patients in which endometriosis was excluded laparoscopically by an experienced gynecologic surgeon) versus all stages of endometriosis (33 patients). Endometriosis patients were then divided into three groups according to the stage of endometriosis: controls (33 patients; no endometriosis), minimal-mild endometriosis (16 patients; stages I-II) and moderate-severe endometriosis (17 patients; stages III-IV).

Exclusion criteria were as follows: women (1) who were on hormonal medication; (2) who underwent an operation within 6 months; (3) who had other pelvic inflammatory disease.

Selection and measurement of biomarkers

After a widespread literature search, four plasma biomarkers were selected due to reported significant differences in the plasma concentrations between women with and without endometriosis. Erythropoietin (Epo), interleukin (IL)-6, tumor necrosis factor α (TNF-α) and carbohydrate antigen (CA)-125, are suggested to play a role in the development of endometriosis due to either their angiogenic potential or as autocrine/paracrine factors or by encouraging vascularization or survival and proliferation of ectopic endometrial cells [13-17].

Plasma concentrations of Epo, IL-6 and TNF-α were determined by using commercially available ELISA kits (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. Plasma concentrations of the CA-125 level were measured using Microparticle Enzyme Immunoassay (MEIA) Abbott AxSYM instrument (Abbott Diagnostics, Abbott Park, IL, USA). Inter-assay coefficient of variation for Epo, IL-6, TNF-α and CA-125 was < 10, 6.4, 3.5 and < 10%, respectively. Intra-assay coefficient for Epo, IL-6, TNF-α and CA-125 was 5.9, 4.2, 1.8 and < 10%, respectively.

Statistical analysis

Data were expressed as mean ±SD. Statistical analysis was performed using the Student's *t*-test as appropriate, whereas means were compared among groups by a one-way analysis of variance (ANOVA). When homogeneity and normality of the samples were not appropriate, non-parametric statistical methods (Kruskal-Wallis and Mann-Whitney tests) were applied for comparison. If a significant overall difference was found in a one-way ANOVA or a Kruskal-Wallis test, post hoc Tukey or multiple comparison test were performed to identify any significant differences among individual groups. Receiver operating characteristic (ROC) curves were generated and confidence intervals for areas under ROC curves were calculated. Statistical significance was calculated using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) and was considered to be $P < 0.05$.

Results

Controls and endometriosis patients were matched for age (controls: 26.86 ±9.13, endometriosis patients: 31.24 ±7.24, $p > 0.05$). The plasma levels of IL-6, CA-125 were significantly higher, whereas the plasma levels of Epo and TNF-α were non-significantly decreased, in women with endometriosis compared with controls (IL-6: 46.70 ±27.08 vs. 27.08 ±16.29, $p < 0.05$; CA-125: 49.39 ±28.32 vs. 9.78 ±3.87, $p < 0.0001$; Epo: 26.26 ±9.31 vs. 30.32 ±7.94, $p > 0.05$; TNF-α: 60.22 ±9.11 vs. 65.40 ±9.86, $p > 0.05$, respectively) (Table 1).

Table 1. Comparison of serum erythropoietin (Epo), interleukin (IL)-6, tumor necrosis factor α (TNF-α) and carbohydrate antigen (CA)-125 values in women with and without endometriosis

	Endometriosis patients (*n* = 33)	Controls (*n* = 22)	*p*
IL-6, mIU/ml	46.70 ±27.08	27.08 ±16.29	0.016
CA-125, U/ml	49.39 ±28.32	9.78 ±3.87	0.0001
TNF-α, pg/ml	26.26 ±9.31	65.40 ±9.86	0.051
Epo, mIU/ml	60.22 ±9.11	30.32 ±7.94	0.099

Note: Student's t-test was used to evaluate statistically significant differences between the values.

Table 2. Comparison of serum erythropoietin (Epo), interleukin (IL)-6, tumor necrosis factor α (TNF-α) and carbohydrate antigen (CA)-125 values in women with and without endometriosis in relation to the stage of the disease

	Stage I-II endometriosis patients (*n* = 16)			Stage III-IV endometriosis patients (*n* = 17)			Controls (*n* = 22)			*p*
	Mean ±SD	Median	Min-Max	Mean ±SD	Median	Min-Max	Mean ±SD	Median	Min-Max	
IL-6, mIU/ml	56.45 ±23.21	29.2	18.9-205.2	44.53 ±31.07[b]	33.9	18.7-150.0	27.08 ±16.29	20.5	18.7-92.0	0.014
CA-125, U/ml	11.39 ±4.81	19.0	6.12-19.9	57.84 ±61.44[a,b]	39.8	8.7-309.3	9.78 ±3.87	15.9	3.56-18.47	0.000
TNF-α, pg/ml	67.48 ±11.29[a]	35.4	54.9-79.0	58.61 ±7.93[b]	21.6	48.0-75.3	65.40 ±9.86	33.9	49.8-80.2	0.013
Epo, mIU/ml	24.75 ±3.39	25.8	19.1-28.0	26.60 ±10.19[b]	22.6	18.0-60.0	30.32 ±7.94	35.3	21.1-46.5	0.021

[a]*p < 0.05: stage I-II vs. stage III-IV*

[b]*p < 0.05: stage III-IV vs. controls*

Note: Kruskal-Wallis test was used to calculate the overall p-values; Mann-Whitney test was used to perform the pairwise comparisons.
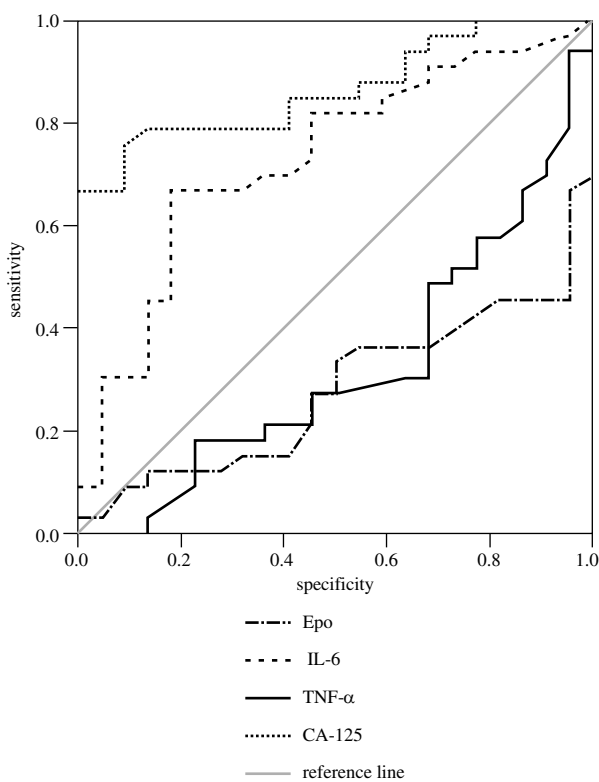

Fig. 1. Receiver operating characteristic (ROC) curve for the identification of endometriosis for serum erythropoietin (Epo), interleukin (IL)-6, tumor necrosis factor α (TNF-α) and carbohydrate antigen (CA)-125

In women with minimal-mild endometriosis, the only significant difference was the increased plasma level of IL-6 in comparison to the controls (IL-6: 56.45 ±23.21 vs. 27.08 ±16.29, *p* < 0.05). In women with moderate-severe endometriosis, plasma levels of IL-6 and CA-125 were significantly increased, and those of Epo and TNF-α were significantly decreased, when compared with controls (IL-6: 44.53 ±31.07 vs. 27.08 ±16.29, *p* < 0.05; CA-125: 57.84 ±61.44 vs. 9.78 ±3.87, *p* < 0.05; Epo: 26.60 ±10.19 vs. 30.32 ±7.94, *p* < 0.05; TNF-α: 58.61 ±7.93 vs. 65.40 ±9.86, *p* < 0.05, respectively) (Table 2).

Receiver operating characteristic curve analysis was used to examine the diagnostic test performance of Epo, TNF-α, IL-6 and CA-125. The area under curve (AUC) for Epo, TNF-α, IL-6 and CA-125 were 0.280, 0.322, 0.729 and 0.864, respectively (Fig. 1).

Discussion

The results of this study show that minimal-mild endometriosis patients displayed higher serum IL-6 levels, while moderate-severe endometriosis patients displayed higher serum Epo, TNF-α, IL-6 and CA-125 levels than other patients.

Increased serum IL-6 levels observed in patients with minimal-mild endometriosis in our study are in line with findings of some reports [18-20] but depart from others that found no value for serum IL-6 in the diagnosis of endometriosis at any stage [21, 22] and from yet others that reported slightly elevated serum IL-6 levels in controls [23-25]. Additionally, it is worthwhile to note that serum IL-6 was the only marker that was constantly elevated in all our comparison groups (endometriosis group, minimal-mild endometriosis group and moderate-severe endometriosis group).

Inflammatory markers such as TNF-α and Epo were slightly higher in the controls than in the endometriosis group. This observation may be explained by the possibility that controls with adhesions, etc. had increased plasma concentrations of inflammatory cytokines. Comparable TNF-α [20, 23-25] levels were previously reported in women with and without endometriosis. However, other investigators reported elevated levels of TNF-α and Epo in endometriosis patients compared with controls [13, 18, 26]. These discrepancies may be explained by differences in the study design (i.e. different inclusion criteria and different

menstrual cycle phases) as well as genetic diversity. It is important to note that although CA-125 is the most extensively investigated and used biomarker of endometriosis [27], it is well established that CA-125 levels lack diagnostic power as a single biomarker of endometriosis [28].

Regarding the role of cytokines in the pathogenesis of endometriosis, our findings are consistent with previous reports [8-10, 29, 30], which state that endometriosis is associated with the elevated level of serum IL-6 and the elevated level of this cytokine correlates with a more advanced stage of the disease. However, we were unable to reveal any statistically significant differences in the case of the other tested cytokines (Epo and TNF-α), which is also consistent with some studies [29, 30].

Conclusions

Establishing a non-invasive diagnostic test for endometriosis can have a radical impact on the patients' quality of life and the accuracy of the treatment by potentially reducing the number of unnecessary laparoscopies. The results of our study show that progression of endometriosis is associated with the elevated level of serum IL-6. Undoubtedly, larger well-designed prospective studies are urgently needed to determine the diagnostic potential of cytokines like IL-6 in endometriosis.

We certify that there is no conflict of interest with any financial organization regarding the material discussed in this manuscript.

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