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ENDOMETRIOSIS

Serum YKL-40 levels are altered in endometriosis

Abdullah Tuten[1], Mine Kucur[2], Metehan Imamoglu[1], Mahmut Oncul[1], Abdullah Serdar Acikgoz[1], Nigar Sofiyeva[1], Zeynep Ozturk[2], Baris Kaya[3], and Engin Oral[1]

[1]Department of Obstetrics & Gynecology, [2]Department of Biochemistry, Cerrahpasa School of Medicine, Istanbul University, Istanbul, Turkey, and [3]Department of Obstetrics & Gynecology, Near East University School of Medicine, Lefkosa Mersin, Turkey

Abstract

Endometriosis is traditionally defined as the presence of endometrial glands and stroma in ectopic locations, especially the pelvic peritoneum, ovaries and rectovaginal septum. YKL-40, a new biomarker of inflammation, is secreted by activated macrophages and neutrophils in different tissues with inflammation. Serum concentrations of YKL-40 are elevated in patients with diseases characterized by inflammation. We aimed to investigate the possible association between serum YKL-40 levels and endometriosis. A total number of 88 women were recruited for this case–control study. About 53 patients with surgically proven endometriosis were included, while 35 patients without endometriosis comprised the control group. Patients were classified as having minimal, mild, moderate and severe disease in accordance with the severity. Two new groups were formed by combining patients with minimal and mild disease (Stage 1–2) and with moderate and severe disease (Stage 3–4). Serum YKL-40 levels were statistically higher in the endometriotic group compared to control group (*p*:0.001). YKL-40 levels were significantly higher in Stage 3–4 group compared to Stage 1–2 group (*p* values 0.001) as well. Correlation analysis revealed a positive correlation between serum YKL-40 levels and the stage of the disease. YKL-40 may be utilized as a marker for determining the severity of endometriosis.

Keywords

Endometriosis, inflammation, staging, YKL-40

History

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Introduction

Endometriosis is traditionally defined as the presence of endometrial glands and stroma in ectopic locations, especially the pelvic peritoneum, ovaries and rectovaginal septum. Clinical features of endometriosis include dysmenorrhea, dyspareunia, chronic pelvic pain, irregular uterine bleeding, infertility and it is estimated to be affecting 6–10% of women of reproductive age [1]. The gold standard for exact diagnosis of endometriosis is laparoscopic visualization of lesions and histological confirmation. Thus, pathway to the exact diagnosis and then treatment is often delayed. It is evident that new non-invasive methods are needed for diagnosis in earlier time period of endometriosis.

Although one of the most frequently recognized gynecological diseases, the pathophysiology of endometriosis remains controversial. However, endometriosis is a pelvic inflammatory condition involving a dysfunction in immune-related cells and macrophages within the peritoneum secreting a number of products, mainly cytokines and growth factors [2,3]. Moreover, at present, there is no consensus regarding the value of inflammatory factors as non invasive biomarkers of endometriosis.

YKL-40 is a 40 kDa plasma glycoprotein and a member of the ''mammalian chitinase-like proteins'' [4]. It is named after its three N-terminal amino acids and its molecular mass of 40 kDa [5]. YKL-40, a new biomarker of inflammation, is secreted by activated macrophages and neutrophils in different tissues with inflammation [6,7] Serum concentrations of YKL-40 are elevated (compared to healthy subjects) in patients with diseases characterized by inflammation, increased extracellular remodeling and ongoing fibrosis such as bacterial infections [8], rheumatoid arthritis [9], osteoarthritis [10], hepatic fibrosis [11] and hepatitis [12,13]; asthma and chronic obstructive pulmonary diseases [14], neuroinflammation [15] and inflammatory bowel disease [16].

To the best of our knowledge, this is the first study so far to evaluate whether serum YKL-40 levels are altered in women with endometriosis or YKL-40 may be utilized in non-invasive screening and staging of the disease.

Address for correspondence: Mine Kucur, Assoc Prof, M.D., Department of Biochemistry, Cerrahpasa School of Medicine, Istanbul University, Fatih, Istanbul 34015, Turkey. Tel: +90 532 654 35 67. E-mail: mkucur@hotmail.com

Materials and methods

About 94 women who had undergone surgery either laparoscopic or laparotomic due to suspected ovarian endometriosis, infertility and chronic pelvic pain between May 2012 and July 2013 in Istanbul University Cerrahpasa School of Medicine Hospital Department of Obstetrics and Gynecology were evaluated for this case–control study. Inclusion criteria were as follows: (1) Absence of any previous ovarian surgery, (2) 18–45 years of age with regular menstrual cycles and (3) Absence of any endocrine and autoimmune diseases. Exclusion criteria were as follows: (1) Evidence of post-menopausal FSH levels, (2) Presence of pregnancy, (3) Any suspicion of malignant ovarian disease, (4) History of oral contraceptive (OC) use or any other hormone therapy (HT) in past 3 months and (5) Presence of any non-endometriotic ovarian cyst/mass. About three patients were

excluded due to diagnosis of hemorrhagic corpus luteum cyst, two were excluded from the study due to diagnosis of serous cystadenoma and one patient was excluded due to presence of mucinous cystandenoma. 88 remaining patients were recruited for the study. Patients were allocated into two groups according to surgical findings [17,18]: The endometriosis group consisted of subjects with histologically proven endometriosis (*n*:53), and the control group was formed of women without any macroscopic endometriotic lesion, as checked during a thorough examination of the abdominopelvic cavity (*n*:35). For patients with endometriosis, the extent of disease was evaluated using the American Society of Reproductive Medicine (ASRM) revised classification [19]. Patients were classified as having minimal, mild, moderate and severe disease, and were distributed as 10, 18, 15 and 10 patients, respectively. In addition to this, two new groups were formed by combining patients with minimal and mild disease and with moderate and severe disease. Therefore, 28 patients were classified as having minimal-to-mild disease (Stages 1 and 2), while 25 patients were determined to be having moderate-to-severe disease (Stages 3 and 4). General information such as age, gravidity, parity, body mass index (BMI), existence and duration of infertility and existence of dysmenorrhea were collected and evaluated. An informed consent was obtained from all women and approval from the Human Ethics Committee of Istanbul University was obtained as well.

Biological features of inflammation such as high-sensitivity C-reactive protein (mg/l) and white blood cell count (n/ml) were also collected for each patient. Samples were collected in the operating room from all participants. Briefly, following the insertion of the peripheral venous catheter (PVC), 5–10 ml of venous blood samples were collected, using the PVC to draw blood. All samples were kept at room temperature for at least 30 min to allow the blood to clot and were then centrifuged at 5000 *g* for 10 min and serum supernatants were collected. Aliquots of those samples were stored at −80 °C until needed for analysis Serum YKL-40 concentrations were determined by a commercial enzyme-linked immmunosorbent assay (Quidel, Santa Clara, CA). The intra-assay and inter-assay variations were 3.6% and 5.3%, respectively. The sensitivity of the assay was 20 ng/ml.

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 18.0. Data were presented as mean ± SD. Mean values between the groups were compared by Student's *t* test. Pearson's correlation coefficient was used to determine the relationship between the variables. All reported confidence interval (CI) values are calculated at the 95% level. All reported *p* values are two-tailed and a $p < 0.05$ was considered statistically significant.

Results

About 53 women with endometriosis and 35 healthy women were included in this study. Clinical and demographic features of both endometriosis and non-endometriosis groups are shown in Table 1. No significant difference between the groups in terms of age, BMI, gravidity, parity, duration and the diagnosis of infertility was found. Presence of dysmenorrhea as a symptom was significantly higher in endometriotic group (*p*:0.003). Being inflammatory markers, serum CRP and WBC levels were not significantly different between the groups. However serum YKL-40 levels were statistically higher in the endometriotic group compared to the control group (*p*:0.001). In addition, serum CA 125 levels were significantly higher in the endometriosis group (*p*:0.001). Among the patients in the endometriosis group, 60% of endometriomas were located unilaterally, while rest of 39.7% of the patients had bilateral endometriomas. Douglas pouches were found to be completely obliterated in nine patients (16.9%). About 17 patients (32%) had partially obliteration while 25 (50.9%) patients had no signs of any obliteration in Douglas pouch. Presence of peritoneal endometriosis was documented in 19 (35.8%) patients, while rest of 34 (64.2%) patients with endometriosis did not have peritoneal disease. Diagnosis of deep infiltrating endometriosis was established during surgery in 13 patients (24.5%).

YKL-40 levels were significantly higher in Stage 3–4 group compared to Stage 1–2 group (*p*:0.001). Even though CA 125 levels were significantly higher in endometriosis patients, no significant difference was found between Stage 1–2 and Stage 3–4 patients in terms of CA 125 (*p*:0.301). In addition, no significant difference was found between the groups in terms of all parameters evaluated; age, BMI, CRP and WBC(*p* values 0.511; 0.109; 0.178; 0.056, respectively) (Table 2). Serum YKL-40 values according to stages of the disease and those of the control group are also depicted in Figure 1, showing a gradual increase in association with the advancing stage. In the correlation analysis, serum YKL-40 levels were found to be positively

Table 1. Demographic and clinical features of endometriosis and non-endometriosis groups.

	Endometriosis (*n*:53)	Control (*n*:35)	*p*
Age (years)	32.1 ± 8.1	30.8 ± 7.9	0.457
BMI (kg/m^2)	24.0 ± 4.5	24.6 ± 4.5	0.464
Gravidity (*n*)	0.9 ± 1.5	1.3 ± 1.7	0.239
Parity (*n*)	0.5 ± 0.7	0.9 ± 1.0	0.110
Diagnosis of infertility (*n*/*N*, %)	16/53, 30.1	8/35, 22.5	0.351
Duration of infertility (months)	69.5 ± 59.3	119.4 ± 120.1	0.266
Dysmenorrhea	34/53, 641	11/35, 31.4	0.003*
Ovarian endometriosis			
Unilateral	32/53; 60.3%	N/A	
Bilateral	21/53; 39.7%	N/A	
Obliterated Douglas pouch			
None	27/53; 50.9%	N/A	
Partially	17/53; 32.0%	N/A	
Complete	9/53; 16.9%	N/A	
Peritoneal endometriosis			
Yes	19/53; 35.8%	N/A	
No	34/53; 64.2%	N/A	
Deep infiltrating endometriosis			
Yes	13/50; 24.5%	N/A	
No	40/50; 75.5%	N/A	
CRP (mg/l)	3.4 ± 4.3	2.3 ± 2.1	0.040*
WBC (*n*/μl)	7336 ± 1615	6755 ± 1642	0.093
YKL-40 (ng/ml)	92.8 ± 27.8	61.8 ± 14.0	0.001*
CA 125 (U/ml)	81.1 ± 66.3	15.7 ± 7.0	0.001*

BMI, body mass index; CRP, C-reactive protein; WBC, white blood cell; N/A, not available.
*p Value <0.05.

Table 2. Comparison of age, BMI and inflammatory markers between Stage 1–2 and Stage 3–4 endometriosis patients.

	Stage 1–2 endometriosis (*n*:28)	Stage 3–4 endometriosis (*n*:25)	*p*
Age (years)	31.3 ± 7.8	32.8 ± 8.3	0.511
BMI (kg/m^2)	22.9 ± 3.7	25.2 ± 5.1	0.109
CRP (mg/l)	3.0 ± 3.2	4.7 ± 5.3	0.178
WBC (*n*/μl)	7735 ± 1600	6890 ± 1545	0.056
YKL-40 (ng/ml)	77.6 ± 20.4	109.8 ± 25.2	0.001*
CA 125 (U/ml)	72.3 ± 59.6	92.5 ± 73.9	0.301

BMI, body mass index; CRP, C-reactive protein; WBC, white blood cell.
*p Value <0.05.

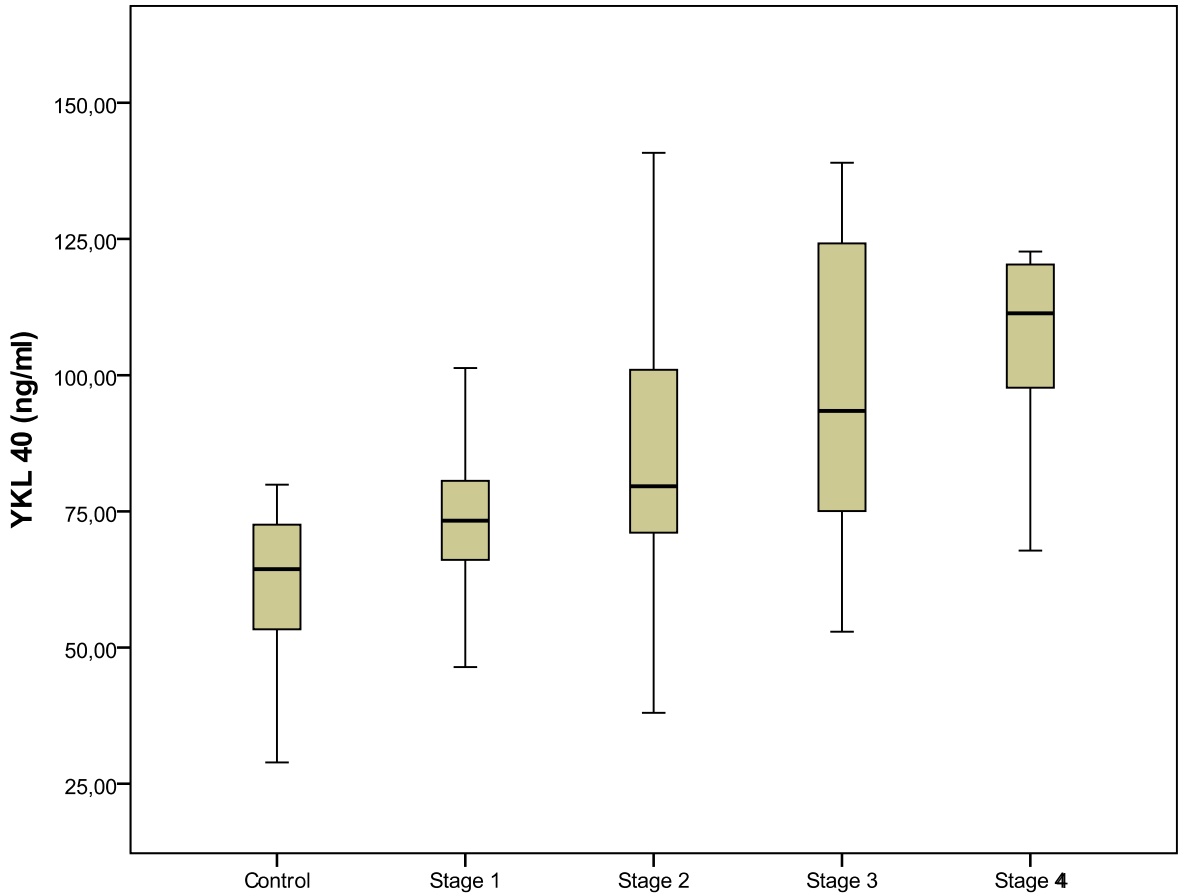

Figure 1. Serum YKL-40 values according to stages of endometriosis depicted together with the control group.

correlated with the stage of the disease and serum CA 125 values(r:0.649; p:0.001; r:0.317; p:0.010) (data not shown in the table).

Discussion

This is the first study to investigate serum YKL-40 levels in endometriosis patients so far. Results of the study revealed significantly higher serum YKL-40 levels in patients with endometriosis. Results also revealed that serum YKL-40 levels were significantly higher in patients with moderate–severe disease (Stage 3–4) compared to early–mild (Stage 1–2) group. In addition to this, increased serum YKL-40 levels in patients with endometriosis were found to be positively correlated with stage of endometriosis.

Various studies have investigated the relationship between endometriosis and chronic inflammatory process. Pelvic inflammation exclusively stands as one of the most characteristic pathological features of endometriosis. Some studies showed that innate immunity plays an important role in this phenomenon. Inflammatory cytokines including IL-1, IL-6, IL-8 and TNF are associated with endometriosis-associated pelvic inflammation [20–22]. Some of these cytokines may directly trigger infiltration by peritoneal leukocytes and may directly or indirectly support implantation and progression of endometrioid tissue implants [21]. Peritoneal cytokines may originate from endometrioid tissue itself; however, the major source appears to be peritoneal exudate cells, particularly macrophages [20,21,23].

YKL-40 is not a disease specific inflammatory biomarker and is shown to be secreted by activated macrophages and neutrophils in different tissues with inflammation; such as vascular smooth muscle cells (VSMCs), cancer cells and arthritic chondrocytes [4,6,24–27]. We hypothesized that serum YKL-40 levels might be associated with endometriosis due to the inflammatory process which is suggested to be involved in the pathophysiology of the disease. There is one study available from Kim et al. that evaluated the association between endometriosis and YKL-40. In that report YKL-40 was demonstrated immunohistologically in peritoneal endometrial lesions and significant association was found between serum levels of YKL-40 and the severity of disease [28]. Supporting this data, results of our study has demonstrated elevated YKL-40 levels in serum as well. In addition to this, serum YKL-40 levels were positively correlated with the advancing stage which might be suggested as a result of increasing extension of the disease. However, serum YKL-40 levels were not found to be correlated with size of endometriomas.

Renowned as an inflammatory marker, serum CRP levels in patients with endometriosis have been investigated. No significant difference in serum CRP levels between the patients with endometriosis and control patients was found. In addition to this, the severity of the disease and CRP levels are not found to be correlated as well. Results of the studies which evaluated CRP levels in peripheral blood of endometriosis patients are relatively controversial [29–32]. This might be due to differences in patient selection, study design and methodology used to detect CRP levels in peripheral blood. In a recent study, hs-CRP assay has been introduced as a diagnostic marker with relatively higher sensitivity and specifity values for diagnosing endometriosis and evaluating its severity.

CA 125 is widely used as a diagnostic marker for endometriosis. Interestingly, the results revealed no significant difference between serum CA 125 levels of Stage 1–2 and Stage 3–4

patients, while serum YKL-40 levels were significantly different between these groups.

In conclusion, serum YKL-40 levels are found to be significantly higher in patients with endometriosis compared to healthy controls. Moreover, severity of the disease is found to be correlated with serum YKL-40 levels. These findings contribute to the hypothesis which explains the involvement of inflammatory processes in pathophysiology of endometriosis. In addition, we may speculate that YKL-40 may be utilized as a marker for determining the severity of endometriosis, but this suggestion needs to be supported with the assistance of further studies with larger populations.

Declaration of interest

The authors declare no conflict of interest.

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