Serum and Urine Levels of Cytokeratin-19 in Endometriosis

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Abstract

Background: Endometriosis is a debilitating disease with features of chronic inflammation. Although the pathogenesis of endometriosis is mysterious, immune abnormalities play a role in this disease. Aim of the study: To estimate the serum and urine levels of cytokeratin-19 (CK19) as non-invasive tool for diagnosis of endometriosis. Materials and Methods: A total of 79 subjects were enrolled in this case control study (44 patients with endometriosis and 35 apparently healthy women as control). Serum and urine samples were collected from all subjects and ELISA was used to detect of CK19 in serum and urine samples. Results: The present study showed a significant elevation (p<0.001) in median serum and urine levels of CK19 in patients as compared with control group. Concerning the comparison of CK19 levels according to the stages of endometriosis there was no important differences among patients group. Conclusion: CK 19 may be a valuable urinary and serum biomarker for noninvasive diagnosis of endometriosis.

Keywords: Endometriosis, Cytokeratin19, ELISA.

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Introduction

Endometriosis is a benign disease defined by presence endometrial glands and stroma outside of the uterus [1]. Endometriosis associated with both pelvic pain and infertility. The ectopic endometrial tissue usually is located in the pelvis but can appear anywhere in the body [2]. The best way in diagnosis of endometriosis is laparoscopic inspection with histological emphasis. In spite of it is an invasive procedure with possible dangers, which may include major vessel or bowel injury [3]. However, emergence of a non-invasive diagnostic test for endometriosis would have a groundbreaking impact on the patients’ quality of life, on the efficacy of available treatment as well as on the cost of endometriosis. So a simple blood test for prediction and diagnosis of endometriosis would overcome these problems and have a major impact on women’s health [4]. Several reports have tried to reveal the role of the immunological markers in endometriosis and many abnormalities have been discovered in this association [5-9]. Cytokeratins are keratin proteins found in the intracytoplasmic cyto of epithelial tissue. They are an important component of intermediate filaments, which help cells resist mechanical stress [10]. Cytokeratins 19 is present in virtually all epithelia as well as for instance in basal cells of mucous membranes [11]. Cytokeratin-19 (CFRA21-1) has a molecular weight of 40 kDa. It is found overload in the peritoneum and in the peritoneal fluid in lower concentrations. Ectopic endometrial tissue demonstrates a high immunoreactivity for CK19 [13]. It has been accepted that the expression of insuprabasal cells can be regarded as a marker of a precancerous process [14]. Various squamous cell cancers, such as lung cancer, intrahepatic cholangiocarcinoma, and urinary bladder cancer, are characterized by the release of CK19 [15]. Apparently, as a result of tumor necrosis and the accompanying disintegration of cytoskeleton elements, elevated CK19 fragment levels are observed in serum [16]. CK19 was detected in the urine and serum of patients with endometriosis as well. However, these observations were highly inconsistent, which is the reason why this marker has not been considered thus far [17]. The aim of this study is to estimate the serum and urine levels of CK19 as non-invasive tool for diagnosis of endometriosis.

Materials and Methods

This study was included 44 patients with endometriosis their age ranged (18-48) years, were collected from Aum Albanin center for infertility at Al-Emamain AL-Kadhemain Medical City and from Higher Institute of infertility diagnosis and assisted reproductive technologies at Al-Nahrain University from November 2015 to April 2016. The diagnosis of each case was done by a gynecologist confirmed by laparotomy or laparoscopy investigations confirmed by histopathology report. They were classified according to the American Fertility Society (now the American Society for Reproductive Medicine; ASRM) into different stages (minimal, mild,
moderate and severe endometriosis), in addition present study included 35 apparently healthy volunteers whose ages were matched with patients as control group. All of these healthy women were a symptomatic with regular menstrual cycle and fertile. Ethical approval and informed consent were obtained from each participant in this study according to the declaration of Helsinki-ethical agreement; it was obtained from the Institutional Review Board of College of Medicine /Al- Nahran University. Five ml of venous blood were drawn from patients and controls. Blood sample was collected in glasses gel tubs for serum separation. The serum sample was divided in to200 μL in each Eppendorf tube, and kept at -20 C° till used. Moreover, ten ml of urine were collected from patients and controls. Urine sample was collected in the urine cup and centrifuged at 3000 c rpm for 15 s., urine sample was divided into 1ml in each Eppendorf tube and kept at -20 C° till used. Detection of CK19 levels in the serum and urine were determined by using commercially available Human CK19 ELISA kit (My Biosource, USA). Statistical analyses were done using SPSS v13. The outcome quantitative CK19 was non-normally distributed. Such variable is described by median. The difference in median of quantitative non-normally distributed variable groups was calculated by Mann-Whitney-test. Analyses where the P -value was <0.05 were considered to be statistically significant.

**Results**

The current study showed that the mean age of endometriosis patients was 30.54±8.39 years, whereas for healthy controls was 33.92±14.7 years with no significant differences (p>0.05), as shown in table (1). Regarding the staging of endometriosis, stage 1 (minimal endometriosis) was found in (17 %), stage 2 (mild endometriosis) consist of (19 %), stage 3 (moderate endometriosis) was found in (27%), while the rest (37 %) were represented stage 4 (severe endometriosis), as clearly observed in figure (1).

Table-1: distribution of patients and control groups according to the age.

<table>
<thead>
<tr>
<th></th>
<th>Patients n=44</th>
<th>Controls n=35</th>
<th>P value (T-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(18-48)</td>
<td>(18-48)</td>
<td></td>
</tr>
<tr>
<td>Mean±SD</td>
<td>30.54±8.39</td>
<td>33.92±14.7</td>
<td>0.192NS</td>
</tr>
</tbody>
</table>

NS=Non significant (p>0.05).

![Figure-1: Stages of endometriosis in patients group.](http://doi.org/10.36295/ASRO.2020.231440)
Table-2: The differences in median serum and urine levels(ng/ml) of CK19 between patients and healthy control.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients n=44 Median/ (Range)</th>
<th>Controls n=35 Median/ng/ml (Range)</th>
<th>P value (Mann Whitney U test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK19 in serum</td>
<td>3.8 (0.9-28)</td>
<td>0 (0-1.5)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CK19 in urine</td>
<td>4.1 (2.1-4.7)</td>
<td>0 (0-1.1)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Table-3: The differences in mean serum and urine of CK19 level (ng/ml) by stage of disease.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stage 1 n=4 Mean±SD</th>
<th>Stage 2 n=9 Mean±SD</th>
<th>Stage 3 n=13 Mean±SD</th>
<th>Stage 4 n=18 Mean±SD</th>
<th>P value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK19 in serum</td>
<td>3.14+1.16</td>
<td>3.56+0.98</td>
<td>3.3+1.46</td>
<td>3.41+1.0</td>
<td>0.910</td>
</tr>
<tr>
<td>CK19 in urine</td>
<td>3.54+0.96</td>
<td>4.06+0.43</td>
<td>4.34+0.41</td>
<td>4.1+0.39</td>
<td>0.105</td>
</tr>
</tbody>
</table>

Regarding ELISA technique which showed highest sensitivity and specificity for CK19 in urine (100% for both), whereas in serum the sensitivity and specificity were (93%) for both, table (4).

Table-4: Sensitivity and specificity percentage of ELISA regarding CK19.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cutoff value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK19 in serum</td>
<td>93.2%</td>
<td>93.8%</td>
<td>1.15</td>
</tr>
<tr>
<td>CK19 in urine</td>
<td>100%</td>
<td>100%</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Discussion
Endometriosis is one of the most frequent benign gynecological diseases that cause pelvic pain, dysmenorrhea, dyspareunia and infertility in about 10–20% of the female population of reproductive age (18). Factors responsible for a delayed diagnosis include the followings: asymptomatic course of disease, nonspecific symptoms, unremarkable findings in noninvasive examinations, and/or symptoms masked by contraceptive agents. Often, sociocultural factors play a role as well women tend to trivialize menstrual pain because they often face lack of understanding within their families (familial clustering) (19).

Current study showed highly significant increase in the levels of CK19 in serum and urine of patients with endometriosis as compared to controls group. Other study conducted by Tokushige et al. found the same result by using Western blot analyses (20). Also this study appeared significant differences between patients and controls regarding urine CK19 level. However, it still unknown about the pathophysiological significance of CK19 in endometriosis (21, 22). It still remains to be elucidated why it is sometimes detected in the urine when the corresponding serum levels are unremarkable. A possible explanation for this observation is that CK19 in serum acts as a substrate for enzymes such as, for instance, neutrophil elastase, and is therefore quickly degraded in inflammatory disease states such as endometriosis (23). In other prospective study demonstrate the usefulness of CK19 as a biomarker for the diagnosis of endometriosis through urine and serum by ELISA (24). Furthermore the current study demonstrates no significant differences in the median levels of CK19 in serum and urine among all stages of disease. These result agreements with Gupta et al. 2008 and Weiss, et al., 2009 (25, 26). In conclusion CK19 may be a valuable urinary and serum biomarker for noninvasive diagnosis of endometriosis.

Conflicts of Interest: The authors declare that they have no conflict of interest.

References


