



Non-Invasive Diagnosis of Endometriosis based on the Evaluation of Serum TNF- α , IL-10 and TNF- α \ IL-10 Ratio

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Abstract : Endometriosis is a chronic gynecological disease manifested by the occurrence of ectopic foci of endometrial tissue in the pelvic cavity and/or ovary. Etiology of the disease is still not fully understood but there is a growing bulk of evidence that immunological abnormalities play a role in this disease. The aim of the present study was to evaluate the serum TNF- α , IL-10 and TNF- α \ IL-10 ratio as non-invasive diagnosis of endometriosis. This case-control study was conducted on 30 women with endometriosis and 30 normal women as control. Blood was collected from patients and controls, enzyme-linked immunosorbent assay was carried out for estimation the serum level of (TNF- α and IL-10) in patients and controls groups. The present findings showed that the median serum levels of TNF- α and IL-10 were significantly elevated in females patient as compared with healthy females ($P < 0.01$). On the other hand, the median ratio of TNF- α \ IL-10 was significantly higher in patients when compared to controls. The current study showed that both T-helper 1 (Th1) and T-helper 2 (Th2) cytokines (TNF- α and IL-10) underline the role of the immune processes in pathogenesis of endometriosis and can be used as a non-surgical diagnostic markers for disease.

Key words : *Endometriosis, TNF- α , IL-10, Th1, Th2, Cytokines.*

Introduction

Endometriosis is a common disorder defined as the growth of endometrial tissue outside the uterine cavity that causing diverse conditions, including infertility, pelvic pain, dysmenorrhea and constipation¹. 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 injury². 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 health³.

Multiple reports have tried to reveal the role of the immunological system in endometriosis and many abnormalities have been discovered in this association^{4,5}.

Immune system alterations are thought to be involved in the development of endometriosis, especially a dysfunction in immune-related cells and macrophages within the peritoneum secreting a number of products,

mainly cytokines and growth factors⁶. At this level, there is an immune-inflammatory reaction that activates immune cells, together with endometriotic implants, producing high amounts of cytokines, growth factors and angiogenic products⁷. The aim of this study was to evaluate the serum TNF- α , IL-10 and TNF- α \ IL-10 ratio as non-invasive diagnosis of endometriosis.

Materials and Methods

Thirty female patients with endometriosis their age range (20 – 45) years and 30 females as control their ages were matched with the patients were enrolled in this study. They were from attendants to Kamal Al-Samari hospital and Baghdad medical city teaching hospital from June 2014 to January 2015. The diagnosis was made by the consultant medical staff, which was based on clinical and ultrasonic examinations. They were newly diagnosed and all of the cases had received no treatment with no complain of chronic or systemic diseases. Serum samples were separated from the whole blood, aliquated and stored at -20°C until used. The level of TNF- α and IL-10 were determined by using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit and performed as recommended in leaflet with kit (TNF- α and IL-10 Boster/ USA).

Statistical analyses were done using SPSS v13. The outcome quantitative variable (TNF- α and IL-10) were 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

Table 1 showed that the mean age of endometriosis patients was 27.7 \pm 0.79 years, whereas for healthy controls was 29.6 \pm 1.40 years with no significant differences (*p*>0.05).

Table 1: Age distribution of the patients and controls group.

| | Patients n=30 | Control n=30 | P-value |
|---------------|------------------|-----------------|---------------------|
| Age | | | |
| Range | (20-45) | (20-41) | |
| Mean \pm SE | 27.7 \pm 0.79 | 29.6 \pm 1.40 | 0.747 ^{NS} |

SE= Standard error; NS=Non significant (*p*>0.05).

This study revealed a slight significant elevation in median serum levels of TNF- α in endometriosis patients (45.22 pg/ml) in comparison to that of healthy control (16.51pg/ml), (*p*<0.05), table (2). Moreover, the median serum levels of IL-10 are highly significant (*p*<0.05) among patients (6.52pg/ml) as compared to controls (1.71pg/ml), as shown in table (3). Determination the ratio between Th1 and Th2 ratio based on the cytokine levels in this study showed that median serum ratio of TNF- α \ IL-10 revealed significant difference between patients and control (*p*<0.01), (39.3 vs. 11.1) as observed in table (4).

Table 2: Serum levels of TNF- α between patients and controls.

| Serum TNF- α | patients | Control | P-value |
|---------------------|---------------|--------------|-----------------|
| Range | (8.19-211.81) | (8.32-98.02) | |
| Median | 45.22 | 16.51 | <i>p</i> <0.01* |
| Total | 30 | 30 | |

*Significant differences

Table 3: Serum levels of IL-10 between patients and controls.

| Serum IL-10 | patients | Control | P-value |
|-------------|-------------|--------------|-----------------|
| Range | (0.75-8.43) | (0.92-13.78) | |
| Median | 6.52 | 1.71 | <i>p</i> <0.05* |
| Total | 30 | 30 | |

Table 4: Median serum TNF- α /IL-10 ratio in patients and controls.

| | Patients | Control s | P-value |
|----------------------------------|-----------|-----------|---------|
| serum TNF- α /IL-10 ratio | | | |
| Range | (0.6-132) | (0.7-44) | |
| Median | 39.3 | 11.1 | p<0.01* |
| Total | 30 | 30 | |

Discussion

The clinical data to date strongly support the pathophysiological implication of T-helper 1 (pro-inflammatory) and T-helper 2 (anti-inflammatory) cytokines in endometriosis^{8,9}. Cytokines play a major part in the pathogenesis of endometriosis and that selective targeting of these biomarkers may provide treatments that not only enhance our understanding of this disease but also provide substantial and prolonged clinical benefit. So this encouraged us to evaluate the serum TNF- α , IL-10 and TNF- α /IL-10 ratio as non-invasive diagnosis of endometriosis.

The present work is found increase in serum levels of TNF- α and IL-10 in women with endometriosis when compared to controls, which is in accordance with the observations of the previous researchers^{8,10}. Malutan and colleagues found that significantly higher serum level of TNF- α in female with endometriosis compared to healthy controls⁸. Other recent study has shown that the peritoneal TNF- α can be a reliable screening marker for the prediction of endometriosis in adolescents girls¹¹. Increased levels of cytokines in the serum and peritoneal fluid of women with endometriosis may reflect increased T-helper 1 synthesis of cytokines by peritoneal macrophages, lymphocytes, ectopic endometrial implants, or mesothelial cells of the peritoneum, all of which can produce cytokines^{12,13}. In addition Galo and colleagues reported that the serum level of TNF- α in endometriosis group was significantly elevated than that in without endometriosis group, and suggested that TNF- α serum levels are good diagnostic markers of endometriosis in the spectrum of noninvasive methods¹⁰. Moreover, gene and protein expression of IL-8 in the stromal cells of endometriotic tissues are up-regulated by TNF- α , and TNF- α stimulates the proliferation of the endometriotic stromal cells. This stimulatory effect of TNF- α was abolished by adding anti-TNF- α antibody or anti-IL-8 antibody. Therefore, TNF- α may act on stromal cells by mediating the proliferative effects of IL-8¹⁴. Furthermore, several studies examined the association of TNF- α gene polymorphisms and endometriosis, and it seems that some polymorphisms are involved in the pathogenesis of endometriosis¹⁵. On the other hand, blocking TNF- α appears to inhibit the development of the disease in animal models¹⁶.

In consistent with present finding other recent study conducted by Suen and colleagues showed that serum level of IL-10 was higher in endometriosis patients compared to both healthy subjects, and suggest that IL-10 may suppress immunity against endometrial implants, contributing to development of endometriosis¹⁷. Ho *et al.*, showed that the level of IL-10 in peritoneal fluid and noticed increased level of IL-10 in patients with endometriosis compared to normal women¹⁸. Punnonen and colleagues observed that IL-10 levels were elevated in the peritoneal fluid of endometriosis patients than that in control. These results support the concept that macrophage activity is increased and therefore disturbing immune function in endometriosis¹⁹. The other important result in this study was the determine the ratio between Th1 and Th2 ratio based on the cytokine levels of TNF- α / IL-10 in this study revealed significant elevation of TNF- α / IL-10 ratio among patients as compared to controls, confirming the findings of previous study²⁰, which found significant differences in the ratio of TNF- α / IL-10 in the serum of the group of patients with endometriosis. In conclusion the current study showed that both T-Th1 and Th2 cytokines (TNF- α and IL-10) underline the role of the immune processes in pathogenesis of endometriosis and can be used as a non-surgical diagnostic markers for disease.

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