ejpmr, 2016,3(3), 76-81

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

INFLAMMATORY MEDIATORS AS BIOMARKERS FOR ENDOMETRIOSIS

Fouad NT[1*], ElShennawy D[1], Soliman MY[2], Hemeda H[2], Khattab D[1], Shafik AS[2]

[1]Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
[2]Obstetric and Gynaecology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

***Correspondence for Author: Dr. Fouad N.T.**
Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt..

Article Received on 16/01/2016 **Article Revised on 10/02/2016** **Article Accepted on 02/03/2016**

ABSTRACT

The diagnosis of endometriosis, nowadays depends mainly on surgical laparoscopy, with all its known potential risks, due to the lack of a reliable diagnostic noninvasive biomarker. Serum and peritoneal TNF-α, IL-6, IL-8, hs-CRP, and plasma ccf nDNA levels were assessed in 120 women scheduled for diagnostic laparoscopy to investigate their roles as reliable non invasive biomarkers in the diagnosis and staging of endometriosis in Egyptian patients. The patients were divided into group I (endometriosis group = 80 patients), which is further subdivided into: group IA (stage I endometriosis =38 patients) and Group IB (stage II endometriosis = 42 patients), and Group II (non-endometriosis group= 40). Comparative statistics revealed significant difference between group I and group II regarding serum TNF-α, serum hs-CRP, serum and peritoneal IL-6 and IL-8, and plasma ccf DNA levels. Conclusion: Serum TNF-α, serum IL-6, serum IL-8, and plasma ccf DNA are possible highly sensitive reliable non-invasive biomarkers for screening of endometriosis and their routine use would indeed decrease the number of unnecessary laparoscopies. Moreover, serum IL-6 and IL-8 can be used to discriminate between various stages of endometriosis.

KEYWORDS: Endometriosis, TNF-alpha, IL-6, IL-8, ccfDNA.

INTRODUCTION

Endometriosis is a chronic pelvic inflammatory disease, characterized by the presence and the growth of ectopic endometrial glands and stroma at multiple locations outside the uterine cavity, and is usually manifested as pelvic pain or infertility.[1,2] It is reported that endometriosis affects about 10% of women in the reproductive age.[3] Currently, there are no reliable sensitive and specific diagnostic tests for the clinical diagnosis of endometriosis, and the diagnosis of endometriosis nowadays depends mainly on laparoscopy, which is highly invasive and has many potential risks.[4,5] Non-invasive tools for early diagnosis of endometriosis are thus needed to reduce the number of unnecessary laparoscopies without adversely affecting the clinical outcomes of endometriosis patients.[6]

The exact etiology and pathogenesis of endometriosis are still unclear, but it is evident that the growth of the ectopic endometrial tissue depends on many factors including neo-angiogenesis, adhesions, and dysregulation of apoptosis.[7,8] Dysfunction of the immune system cells with release of high amounts of pro-inflammatory cytokines and pro-angiogenic factors (both systemic and local), is thought to be involved in the pathogenesis of endometriosis.[5] Several studies reported that elevated serum levels of TNF-α and IL-8, which are potent angiogenic, pro-inflammatory, and growth promoting factors, were found in patients with endometriosis.[9] Moreover, elevated levels of IL-6, known to activate T cells and promote B cell differentiation, were found in the sera of endometriosis patients. Though, several studies reported that serum IL-6 levels correlate with the degree of severity of the disease, others reported a significant higher levels of serum IL-6 in early stages of endometriosis.[5,10]

Elevated levels of circulating cell-free DNA (ccf DNA) were reported in cancers and in various inflammatory diseases, serving as a reliable apoptotic and necrotic marker**.** As endometriosis is a chronic inflammatory condition, the possibility that ccf DNA could serve as a marker for the detection of endometriosis has been raised.[6]

The aim of the current study is to investigate the role of the pro-inflammatory cytokines: TNF-α, interleukin 6, interleukin 8, and markers of low grade and chronic inflammation: high sensitivity C- reactive protein (hs-CRP) and plasma ccf nDNA, as reliable non invasive biomarkers in the diagnosis and staging of endometriosis in Egyptian patients.

Subjects and methods

One hundred twenty women, with regular menstrual cycles, scheduled for diagnostic laparoscopy were recruited from Ain Shams University Maternity Hospital,

Cairo. Their ages ranged from 23 to 43 years with a mean of 27.8 ± 4.16.

Full history taking, clinical examination, and laparoscopic visualization for the presence or absence of endometriosis in the proliferative phase of the cycle were performed for all patients.

Informed consent was obtained from all participant individuals. The study was conducted in accordance with the stipulations of the local ethical and scientific committees of Ain Shams University and the procedures respected the ethical standards in Helsinki declaration of 1964.

Patients were divided according to laparoscopic finding into two groups: group I including 80 endometriosis patients which was further subdivided according to stages of endometriosis, according to the revised four-stage scoring system of American Society of Reproductive Medicine [11], into: group IA with stage I or minimal endometriosis (n=38), and group IB with stage II or mild endometriosis (n=42). Meanwhile, group II (non-endometriosis group/control group) included 40 cases with no detected pelvic pathology. The indication for laparoscopy are summarized in figure (1).

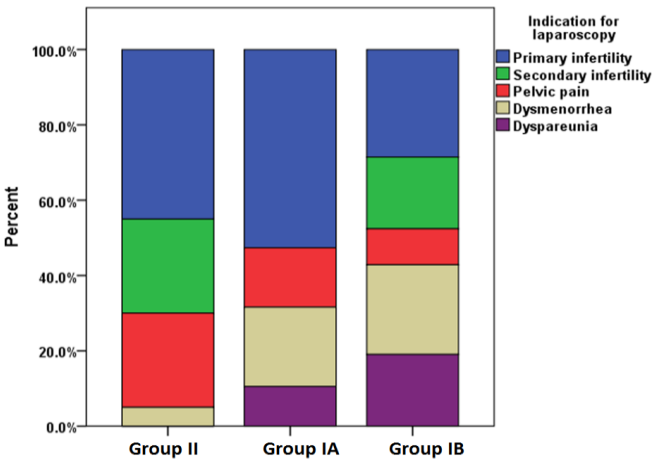

Figure (1): The indication for laparoscopy in each of group IA, IB and group II patients.

During the laparoscopy procedure, both peripheral venous blood (clotted and on EDTA), and peritoneal samples were withdrawn. Serum and plasma were separated from clotted venous samples and EDTA samples respectively and stored at -80°C till final analysis. Peritoneal samples were taken by flushing the pelvis and re-suction of 20 ml of normal saline, centrifuged and stored at -80°C till final analysis.

Concentration of TNF-α, IL-6, IL-8, and hs-CRP was measured by ELISA according to the manufacturers' instructions using AviBion Human TNF-α, Human IL-6, and Human IL-8 ELISA kits (Orgenium Laboratories, Finland), and Quantikine human high sensitivity C-reactive protein (hs-CRP) (R&D Systems, Minneapolis, MN).

Plasma cell free DNA assay

DNA was extracted from 500 µl plasma using the automated MagNA PureTM LC Instrument and MagNA Pure LC DNA Isolation Kit (Roche Applied Science, Switzerland). Real-time amplification reaction containing 5 µl of extracted DNA was set up to a volume of 25 µl DNA with 12.5 µl of TaqMan Universal PCR Master Mix, 0.3 µmol/l of each of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primer and 0.1 µmol/l of GAPDH probe. Gene sequence was amplified with forward 5′-CCCCACACACATGCACTTACG-3′ and reverse 5′-CCTAGTCCCAGGGCTTTGATT-3′ primers.

Real-time PCR was performed using ABI Prism 7000 Sequence Detector (Applied Biosystems, ABI, USA). As a single-step real-time PCR, FAM- and VIC-labeled probes (5′-MGB-GTGAACGTGGATGAAGTTGG) were used for determining selected DNA area of interest. The real-time quantitative PCR was performed using a 2 min incubation at 50°C, followed by an initial denaturation step at 95°C for 10 min and 40 cycles of 1 min at 60°C and 15 sec at 75°C. The positive reaction was detected by accumulation of a fluorescent signal.

Statistical Analysis

Statistical analysis was performed using SPSS V 15.0. Comparisons of variables were conducted between groups using the student-t test. In addition, correlations between variables within groups were performed using the Pearson correlation coefficient. $p < 0.05$ and < 0.01 were set as statistically significant and highly significant respectively.

An operator characteristic (ROC) curve was constructed to establish clinically cut off values for studied parameters with calculation of sensitivity, specificity, positive predictive value, negative predictive value.

RESULTS

Comparative statistics between group I (endometriosis patients) and group II (non endometriosis patients) revealed highly significant difference regarding serum TNF-α, serum IL-6, serum IL-8, peritoneal IL-6, peritoneal IL-8, and plasma ccf DNA, and significant difference regarding serum hs-CRP. On the other hand, there was no statistically significant difference between both groups as regards peritoneal TNF-α and CRP (table 1).

Table (1): Comparative statistics between group I (endometriosis patients) and group II (non endometriosis patients) as regards lab. Findings.

Parameters	Group I	Group II	t	p-value	Significance
	Mean ±SD	Mean ± SD			
Serum TNF-α (pg/ml)	285.2 ± 107.0	102.8 ± 36.7	10.462	<0.001	HS
Serum hs-CRP (ng/ml)	2.10 ± 1.86	1.27 ± 0.6	2.747	0.021	S
Serum IL-6 (pg/ml)	59.6 ± 9.9	13.7 ± 5.5	19.47	<0.001	HS
Serum IL-8 (pg/ml)	29.3 ± 2.2	5.7 ± 1.2	18.65	<0.001	HS
Plasma ccf. DNA (genome equivalent/ml)	25196.4 ± 20443.2	4903.0± 2172.4	6.248	<0.001	HS
Peritoneal TNF-α (pg/ml)	158.5 ± 39.6	146.3 ± 43.7	1.544	0.125	NS
Peritoneal hs-CRP (ng/ml)	0.86 ± 0.38	0.74 ± 0.4	1.602	0.111	NS
Peritoneal IL-6 (pg/ml)	28.5 ± 8.9	10.2 ± 5.1	22.9	<0.001	HS
Peritoneal IL-8 (pg/ml)	11.5 ± 3.4	5.1 ± 0.4	13.49	<0.001	HS

HS: highly significant, S: significant, NS: non significant

Moreover, a statistically significant difference was found between each of group I A (stage I endometriosis) and group I B (stage II endometriosis) when compared to group II, regarding serum TNF-α, hs-CRP, serum IL-6, serum IL-8, peritoneal IL-6, peritoneal IL-8, and plasma ccf DNA, while no statistically significant difference was found between them as regards peritoneal TNF-α and peritoneal hs-CRP. Moreover, there was no significant difference between Group I A and I B regarding serum and peritoneal levels of TNF-α, and hs-CRP, while there was a significant difference between the two groups regarding serum and peritoneal lev els of IL-6, IL-8 and plasma ccf DNA (table2).

Table (2): Comparative statistics between each of group IA, group IB, and group II as regards lab findings
HS: highly significant, S: significant, NS: non significant

Group / Parameter	Group II	Group IA (n=38)			Group IB (n=42)			Group IA versus group IB	
	Mean ± SD	Mean ± SD	Group IA versus group II		Mean ± SD	Group IB versus group II			
			p	sig		p	sig	p	sig
Serum TNF-α (pg/ml)	102.8 ± 36.7	147.4 ± 65.7	0.023	S	242.8 ± 89.1	<0.01	HS	0.051	NS
Serum hs-CRP (ng/ml)	1.27 ± 0.6	2.08 ± 1.31	0.044	S	2.1 ± 2.1	0.017	S	0.960	NS
Serum IL-6 (pg/ml)	13.7 ± 5.5	51.3 ± 5.8	<0.01	HS	67.1 ± 6.2	<0.01	HS	<0.01	HS
Serum IL-8 (pg/ml)	5.7 ± 1.2	27.5 ± 1.4	<0.01	HS	31.0 ± 1.6	<0.01	HS	<0.01	HS
Plasma ccf. DNA (genome equivalent/ml)	4903.0 ± 172.4	5944.7± 2192.7	0.040	S	7108.4± 2975.5	0.013	S	0.047	S
Peritoneal TNF-α (pg/ml)	146.3 ± 43.7	151.3 ± 70.3	0.703	NS	177.4 ± 95.7	0.068	NS	0.263	NS
Peritoneal hs-CRP (ng/ml)	0.74 ± 0.4	0.78 ± 0.4	0.664	NS	0.36 ± -0.96	0.339	NS	0.559	NS
Peritoneal IL-6 (pg/ml)	10.2 ± 5.1	20.5 ± 5.4	<0.01	HS	35.8 ± 5.3	<0.01	HS	<0.01	HS
Peritoneal IL-8 (pg/ml)	5.1 ± 0.4	8.4 ± 1.3	<0.01	HS	14.3 ± 1.96	<0.01	HS	<0.01	HS

A significant correlation between serum TNF-α and each of serum hs-CRP, serum IL-6, serum IL-8 and plasma ccf DNA was found among group I (r=0.520, p<0.001, r=0.666, p<0.001, r=0.251, p=0.021, and r=0.631, p<0.001 respectively). (figures 2 and 3).

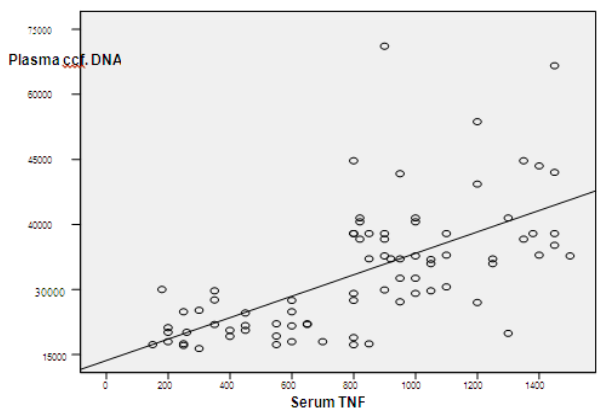

Fig. (2): Correlation between serum TNF-α and plasma ccf DNA among group I (endometriosis patients).

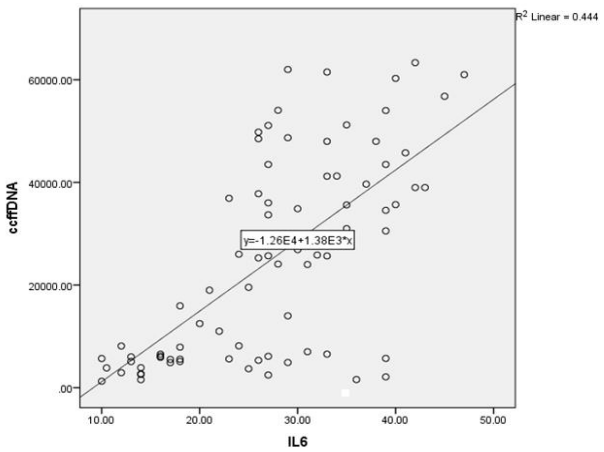

Fig. (3): Correlation between serum IL-6 and plasma ccf DNA among group I (endometriosis patients).

Receiver operating characteristics (ROC) curve was used to define the best cutoff value of the tested biomarkers with the highest sensitivity (table 3 and table 4).

Table (3): Receiver-operating characteristic (ROC) curve analysis for diagnosis of endometriosis

Variable	Cutoff	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %
Serum IL-6 (pg/ml)	>24.03	100	100	100	100
Peritoneal IL-6 (pg/ml)	> 19.78	82.5	100	100	74.1
Serum IL-8 (pg/ml)	>7.1	90	92	96	82.2
Peritoneal IL-8 (pg/ml)	>5.85	100	100	100	100
Serum TNF-α (pg/ml)	165	95	86.2	77.6	97.2
Serum hs-CRP (ng/ml)	1.5	85	93.7	2	92,6
Plasma ccf. DNA (genome equivalent/ml)	5950	97.5	71.2	62.9	98.3

Table (4): Receiver-operating characteristic (ROC) curve analysis for discrimination between group IA and group IB

Variable	Cutoff point	Sensitivity %	Specificity %	Positive predictive value	Negative predictive value (NPV)
Serum IL-6 (pg/ml)	> 57.75	100	100	100	100
Peritoneal IL-6 (pg/ml)	> 27.77	100	100	100	100
Serum IL-8 (pg/ml)	> 28.91	100	100	100	100
Peritoneal IL-8 (pg/ml)	>10.49	100	100	100	100
Plasma ccf. DNA (genome equivalent/ml)	≥ 22900	69.6	97.1	97.0	70.2

DISCUSSION

Despite the great advances in the diagnostic tools, the diagnosis of endometriosis, which affects about 10-15% of women in the reproductive age, still depends mainly on laparoscopic detection of endometriosis lesions, with its known costs and risks, due to the lack of a reliable diagnostic noninvasive biomarker.[12,13] The development of a sensitive and reliable peripheral biomarker that can diagnose endometriosis at its various stages represents a great challenge, and would indeed profoundly improve the patients' quality of life, as well as the cost of diagnosis of endometriosis.[13] The most important point to consider while evaluating any biomarker of endometriosis is that it should have high sensitivity rather than a high specificity, so as to ensure the diagnosis of all women with endometriosis or other significant pelvic pathology, who might benefit from laparoscopic surgery.[3]

It is thought that endometriosis is associated with dysfunction of immune cells, with up-regulation of release of pro-inflammatory cytokines as TNF-α as well as other inflammatory mediators.[5,14] TNF-α is secreted mainly by activated macrophages and is reported to stimulate angiogenesis, and adhesion molecules and matrix metalloproteinases expression as well[8,15], a mechanism that would probably favor the growth of endometrial tissue. In agreement with the results of other previous studies[9,16,17], serum TNF-α levels in the current study were significantly elevated in patients with endometriosis compared with non endometriosis patients, as well as in both group IA and group IB patients when compared with non endometriosis patients respectively. These findings together with the observation that TNF-α enhances the proliferation of endometrial tissue as reported by Cho etal., 2007[16], enforce the hypothesis that TNF-α may be implicated in the pathogenesis of endometriosis. Moreover, our finding that peritoneal TNF-α levels did not differ significantly between endometriosis and non endometriosis patients, points to a systemic rather than a local inflammatory process. In that context and in accordance with previous studies[18], a significant difference in serum hs-CRP levels, but not in peritoneal hs-CRP levels, was found in the endometriosis group when compared with the non endometriosis group. Using ROC curve analysis, TNF-α had a cutoff of 165 pg/ml to discriminate serum endometriosis patients from non endometriosis patients that exhibits a high sensitivity (95%), while a cutoff of serum hs-CRP of 1.5 ng/ml didn't have such high sensitivity (85%), and thus would be less reliable in screening of endometriosis. Similarly, Foda and Abdel Aal[17], in their study reported that a threshold value of serum TNF-α >12.45 pg/ml was able to diagnose endometriosis with a sensitivity and specificity, of 89.23% and 86.87% respectively. As the reliability of any biological marker resides in its ability not to miss any possible case of endometriosis, we thus recommend to use our higher cutoff level as it has better sensitivity.

TNF-α is known to promote the release of other pro-inflammatory cytokines as IL-6 and IL-8. IL- 6, a pleitropic cyokine secreted by T lymphocytes as well as activated macrophages, stimulates B- lymphocytes, enhances T cell activation and upregulates expression of adhesion molecules, meanwhile, IL-8, secreted by activated macrophages, epithelial and endothelial cells, stimulates chemotaxsis of neutrophils and

angiogenesis[5,19], and thus both are expected to be elevated in endometriosis and may play a role in its pathogenesis as well. Although the results of previous studies, assessing the serum levels of IL-6 in endometriosis, are inconsistent, most of these studies[5,10, 17, 20,], reported a significant high serum level of IL-6 in endometriosis patients when compared to controls. In agreement with these previous studies.[17,20,21], serum IL-6 and IL-8 levels in the present study were significantly elevated in patients with endometriosis compared with non-endometriosis patients. Moreover, peritoneal IL-6 and IL-8 levels were significantly elevated in endometriosis patients compared to non endometriosis patients which suggests possible additional local peritoneal source of both cytokines. By using ROC curve analysis, we found that serum IL-6, IL-8 had cutoff of 24.03 and 7.1 pg/ml with sensitivity of 100%, and 90%, and specificity of 100% and 92.5% respectively. Because of their high sensitivity and specificity, serum IL-6, serum TNF-α, and serum IL-8 are probable highly reliable biomarkers for screening of endometriosis. In contrast to TNF-α, serum IL-6 and IL-8 showed significant difference between group IA and group IB patients and thus can be used to discriminate between patients of both groups and used for staging of endometriosis. Using ROC curve analysis, serum IL-6 and serum IL-8 cutoff of > 57.75 and > 28.91pg/ml respectively can discriminate between group IA and group IB patients with a sensitivity of 100%.

ccf-DNA was proved to be of diagnostic importance in a variety of chronic inflammatory conditions[22] In the current study, a highly significant difference regarding plasma ccf DNA between endometriosis and non endometriosis patients was found as well as a significant difference between each of group IA and group IB patients when compared to non endometriosis patients, supporting its role as inflammatory and necrotic biomarker in chronic inflammatory diseases as endometriosis. Using ROC analysis, a plasma ccf DNA cutoff of 5950 was able to discriminate cases from controls, with a sensitivity of 97.5% and specificity of 71.2%, while a plasma ccf DNA cutoff of 22900 was able to discriminate group IA from group IB cases, with a sensitivity of 69.6% and, specificity of 97.1%. This was in agreement with a study by Zachariah et al.[6], who found significantly increased concentrations of ccf DNA in plasma from patients with endometriosis compared with the age-matched controls. Though plasma ccf-DNA exhibits significant difference between group IA and group IB patients, its relatively low sensitivity made us recommend to restrict its use for screening of endometriosis only. It could be concluded that, serum TNF-α, serum IL-6, serum IL-8, and plasma ccf DNA are possible highly reliable non-invasive biomarkers for screening of endometriosis that exhibit high sensitivity and we recommend their use in routine screening of endometriosis patients to decrease the number of unnecessary laparoscopies. Moreover, serum IL-6 and IL-8 can be used to discriminate between various stages of endometriosis.

REFERENCES

1. Boyle K and Torrealday S: Benign Gynecologic Conditions. Surgical Clinics of North America., 2008; 88(2): 245- 264.
2. Ahn S, Edwards A, Singh S, Young S, Lessey B, and Tayade C: IL-17A Contributes to the Pathogenesis of Endometriosis by Triggering Proinflammatory Cytokines and Angiogenic Growth Factors. J Immunol., 2015; 195(6): 2591- 2600.
3. Mihalyi A, Gevaert O, Kyama C, Simsa P, Pochet N, De Smet F, De Moor B, Meuleman C, Billen J, Blanckaert N, Vodolazkaia A, Fulop V, and D'Hooghe T: Non-invasive Diagnosis of Endometriosis Based on a Combined Analysis of Six Plasma Biomarkers. Human Reproduction., 2010; 25(3): 654-664.
4. Mounsey A, Wilgus A, and Slawson D: Diagnosis and Management of Endometriosis. American Family Physician., 2006; 74(4): 594- 600.
5. Malutan A, Drugan T, Costin N, Ciortea R, Bucuri C, Rada M, and Mihu D: Pro-inflammatory cytokines for evaluation of inflammatory status in endometriosis. Cent Eur J Immunol., 2015; 40(1): 96- 102.
6. Zachariah R, Schmid S, Radpour R, Buerki N, Fan A, Hahn S, Holzgreve W, and Zhong X: Circulating cell-free DNA as a potential biomarker for minimal and mild endometriosis. Reproductive BioMedicine Online., 2009; 18(3): 407-411.
7. Bertschi D, McKinnon B, Evers J, Bersinger N, and Mueller M: Enhanced inflammatory activity of endometriotic lesions from the rectovaginal septum. Mediators Inflamm., 2013; 2013: 450950
8. da Vilaça Belo A, Passos Andrade S, Peixoto Campos P, Ferreira M, s da Silva-Filho A, and Carneiro M: Identification of local angiogenic and inflammatory markers in the menstrual blood of women with endometriosis. Biomed Pharmacother., 2014; 68(7): 899- 904.
9. Xavier P, Belo L, Beires J, Rebelo I, Martinez-de-Oliveira J, Lunet N, and Barros H: Serum levels of VEGF and TNF-a and their association with C-reactive protein in patients with endometriosis. Arch Gynecol Obstet., 2006; 273: 227–231.
10. Sikora J, Mielczarek-Palacz A, Kondera-Anasz Z, and Strzelczyk J: Peripheral blood proinflammatory response in women during menstrual cycle and endometriosis. Cytokine., 2015; 76(2): 117.
11. Haas D, Shebl O, Shamiyeh A, and Oppelt P: The rASRM score and the Enzian classification for endometriosis: their strengths and weaknesses. Acta Obstet Gynecol Scand., 2013 Jan; 92(1): 3- 7.
12. Fuldeore M, Chwalisz K, Marx S, Wu N, Boulanger L, Ma L, and Lamothe K: Surgical procedures and their cost estimates among women with newly diagnosed endometriosis: a US database study. J Med Econ., 2013; 14: 115–123.
13. Borrelli G, Abrão M, and Mechsner S : Can chemokines be used as biomarkers for

endometriosis? A systematic review. Hum Reprod., 2014; 29(2): 253- 266.
14. Kobayashi H, Higashiura Y, Shigetomi H, and Kajihara H: Pathogenesis of endometriosis: the role of initial infection and subsequent sterile inflammation (Review). Mol Med Rep., 2014; 9(1): 9-15.
15. Eisenberg V, Zolti M, and Soriano D: Is there an association between autoimmunity and endometriosis? Autoimmunity Reviews., 2012; 11: 806– 814.
16. Cho S, Oh Y, Nam A, Kim H, Park J, Kim J, Park K, Cho D, and Lee B: Evaluation of serum and urinary angiogenic factors in patients with endometriosis. Am J Reprod Immunol., 2007; 58: 497– 504.
17. Foda A and Abdel Aal I: Role of some biomarkers in chronic pelvic pain for early detection of endometriosis in infertile women. Middle East Fertility Society Journal., 2012; 17: 187– 194.
18. Lermann J, Mueller A, Körber F, Oppelt P, Beckmann M, Dittrich R, and Renner S: Evaluation of high-sensitivity C-reactive protein in comparison with C-reactive protein as biochemical serum markers in women with endometriosis. Fertility and Sterility., 2010; 93(7): 2125- 2129.
19. Socolov R, Butureanu S, Angioni S, Sindilar A, Boiculese L, Cozma L, and Socolov D: The value of serological markers in the diagnosis and prognosis of endometriosis: a prospective case-control study. Eur J Obstet Gynecol Reprod Biol., 2011; 154: 215– 217.
20. Martinez S, Garrido N, Coperias JL, Pardo F, Desco J, Garcia-Velasco JA, Simon C, and Pellicer A: Serum interleukin-6 levels are elevated in women with minimal-mild endometriosis. Hum Reprod., 2007; 22: 836– 842.
21. Ohata Y, Harada T, Miyakoda H, Taniguchi F, Iwabe T, and Terakawa N: Serum interleukin-8 levels are elevated in patients with ovarian endometrioma. Fertil Steril., 2008; 90: 994– 999.
22. Zhong X and Holzgreve W: Circulating Cell-free DNA in Women's Medicine, Glob. Libr. Women's Med., 2009; 1756- 2228.