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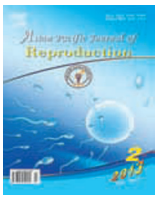

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Clinical signs, symptoms and serum level of interleukin-6 and tumor necrosis factor in women with or without endometriosis

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ABSTRACT

Objective: To evaluate clinical signs and symptoms and serum levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), matrix metalloproteinase-2 (MMP-2), and vascular endothelial growth factor (VEGF) as non-invasive methods to diagnose endometriosis. **Methods:** Eighty women scheduled to laparoscopy underwent blood sampling for measurement of IL-6, TNF-α, MMP-2, and VEGF. The diagnosis of endometriosis was established by laparoscopy using The American Fertility Society visual diagnosis. The presence or absence of endometriosis was correlated with clinical signs and symptoms and with serum levels of those substances. **Results:** The sensitivity and specificity to detect endometriosis of infertility (OR 134.3) were 78 % and 98 %, dysmenorrhoea (OR 11.7) were 63 % and 88 %, and chronic pelvic pain (OR 13.0) were 28 % and 100 %. The presence of rectovaginal nodules had a sensitivity 25 % and specificity 100 %. (OR 11.3, 95 %). The sensitivity and specificity of biologic markers IL-6 (OR 2.5) were 68 % and 53 %, and TNF-α (OR 28.1) were 68% and 60 %. **Conclusions:** History of infertility, dysmenorrhea, chronic pelvic pain, dyspareunia, cervical tenderness and rectovaginal nodule are clinical signs and symptoms suggesting endometriosis. IL-6 and TNF-α appears to be best serum markers for endometriosis.

1. Introduction

Endometriosis is frequently found in women of reproductive age and characterized by the presence of endometrial tissue (stroma and glands) outside of the uterine cavity. It is associated with chronic inflammatory process with defects in immune system. Peritoneal fluid (PF) in women with endometriosis contains increased number of macrophages that secrets inflammatory products including growth factors, cytokines, and various tumor markers[1]. Tumor markers such as interleukin-6 (IL-6), IL-8 and matrix metalloproteinase (MMP) have been associated with implantation process of endometrial tissue. Angiogenesis enables these endometrial cells to proliferate and it is mediated by an increase in vascular epithelial growth factor (VEGF). The development of endometriosis seems to be associated with IL-6 and tumor necrosis factor-alpha (TNF-α). Several studies have found elevated serum IL-6, MMP-2, TNF-α, and VEGF in women with endometriosis[2-4].

The purpose of our study was to evaluate clinical signs and symptoms and serum levels of interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), matrix metalloproteinase-2 (MMP-2), and vascular endothelial growth factor (VEGF) as non-invasive methods to diagnose endometriosis.

2. Materials and methods

Our study consisted of 80 consecutive women of reproductive age (18-42 years), 40 women underwent laparoscopy for chief complaints related to endometriosis and 40 others underwent laparoscopy for chief complaints unrelated to endometriosis at the Reproductive Health Division, Department of Obstetrics and Gynecology, Faculty of Medicine, University of Indonesia from January to April 2012. The study was approved by the Institutional Review Board of Department of Obstetrics and Gynecology, Faculty

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of Medicine, University of Indonesia. Women treated with hormonal medication in the last 6 months before laparoscopy were excluded from the study. The diagnosis of endometriosis was made by laparoscopic procedure as gold standard using The American Fertility Society visual diagnosis without any histology confirmation. The photos and videos of laparoscopic surgeries were seen by another laparoscopic experts as second opinion to confirm the diagnosis.

Blood sampling was taken before laparoscopic procedure. Method of measurement is ELISA with 680 bio-rad reader. Intra-assay variability for the biological markers is 4.4% for IL-6, 5% for TNF-α, 2% for MMP-2, and 4% for VEGF.

Statistical analysis: We used Shapiro Wilks test to evaluate the distribution of the data. Comparisons were analyzed using Student's *t* test or Mann-Whitney U test when appropriate. Proportions were compared with Chi Square test or Fisher exact test, *P* value of less than 0.05 was considered significant.

3. Results

Of total 80 women, 40 women were found to have endometriosis (study group) and 40 others had no endometriosis (control group). The profile of these women was demonstrated in Table 1. Mean age of endometriosis subjects was 32.8±4.7 years, and mean control age was 36.2±4.0 years (*P*: 0.001, 95% CI 1.4-5.3). Endometriosis subjects had higher education than control subjects, with difference mean of 6.3 years. Almost all women (96.2%) were married. 96.25% of the women had regular menstrual cycles. Of those experiencing endometriosis, 82.5% were in the work force (Table 1).

The most frequently found symptom in women with endometriosis was infertility (77.8%), followed by dysmenorrhea (62.5%), dyspareunia (35%), and chronic pelvic pain (27.50%). The most common clinical signs were cervical tenderness (37.5%), rectovaginal nodule (25%), and adnexal tenderness (15%). In bivariate analysis, a significant correlation was found betwen infertility, dysmenorrhea, chronic pelvic pain, dyspareunia, cervical tenderness, and rectovaginal nodule, serum concentration of IL-6 and TNF-α and the presence of endometriosis. (Tables 2 and 3).

Table 1
Patient characteristics and their correlations with endometriosis.

Variable	Category	Endometriosis by laparoscopy diagnostic		Total	OR (95%CI)	*P*
		Positive	Negative			
Age (year)	Mean±SD	32.83 ± 4.73	36.16 ± 4.02	-	1.19 (1.07-1.33)	0.01
Education (year)	Mean±SD	15.70 ± 9.74	9.43 ± 6.61	-	15.08 (4.71-48.21)	< 0.001
Occupation	Active	33	5	38	33 (9.53-114.29)	< 0.001
	Inactive	7	35	42	Ref	
Married	Yes	37	40	77	0.49 (0.042-5.60)	0.56
	No	3	0	3	Ref	
Menstruation	Regular	38	39	77	2.05 (0.18-23.59)	
Profile	Irregular	2	1	3	Ref	0.56
Non hormonal contraception	Yes	5	32	37	0.04 (0.01-0.12)	
	No	35	8	43	Ref	< 0.001

Table 2
Signs and symptoms and their correlations with endometriosis.

Variable		Category	Endometriosis by laparoscopy diagnostic		OR (CI)	*P*
			Positive	Negative		
Symptoms	Infertility	Yes	31	1	134.33 (16.49-5606.41)	< 0.001
		No	9	39	Ref	
	Dysmenorrhea	Yes	25	5	11.67 (3.40-45.08)	< 0.001
		No	15	35	Ref	
	Chronic pelvic pain	Yes	11	0	13.00 (1.64-578.18)	< 0.001
		No	29	40	Ref	
	Dyspareunia	Yes	14	0	18.78 (2.47-817.80)	< 0.001
		No	26	40	Ref	
Signs	Portio tenderness	Yes	15	0	21.00 (2.78-909.95)	< 0.001
		No	25	40	Ref	
	Recto vaginal nodule	Yes	10	0	11.32 (1.40-508.62)	0.001
		No	30	40	Ref	
	Adnexal tenderness	Yes	6	1	6.88 (0.76-324.46)	0.108
		No	34	39	Ref	

We also performed Area Under the Curve (AUC) with Receiver Operating Characteristic (ROC). From clinical symptoms of endometriosis, the highest AUC value was in the symptoms of infertility (0.88), followed by dysmenorrhea (0.75); chronic pelvic pain (0.68); and dyspareunia (0.64). The highest AUC value for clinical signs was cervical tenderness (0.69), followed by rectovaginal nodule (0.63), and adnexal tenderness (0.56). From biological markers, the highest AUC value was in TNF-α (0.71), followed by IL-6 (0.64), MMP-2 (0.58), and VEGF (0.48).

Table 3
Serum markers and their correlations with endometriosis.

Variable	OR (95%CI)	*P*
IL-6 (pg/mL)	2.51 (1.08;5.82)	0.029
TNF- α (pg/mL)	28.13 (3.77;209.96)	0.001
MMP-2 (ng/mL)	1.01 (0.99;1.03)	0.307
VEGF (pg/mL)	0.82 (0.47;1.44)	0.729

Table 4
Location of endometriosis lesions in laparoscopic visualization

Location	Total	%
Endometriosis cyst	16	40.0
Tuba fallopii	2	5.0
Sacrouterine	31	78.0
Parametrium	6	15.0
Rektovaginal septum	10	25.0
Vesicouterine fold	1	2.5
Rectosigmoid	2	5.0
Posterior fornix	4	10.0
Ovarian Fossa	6	15.0

4. Discussion

The mean age of endometriosis in our patients with endometriosis was 32.8 years which was older than that in the controls. In our culture, women usually marry between 20 and 30 years of age and seek medical assistance if no conception occurs within a year. Most of them will undergo laparoscopic examination. Women with high level of education are usually established financially and have the means to undergo medical treatment. These are career women who might have a stressful life. Stress has been associated with uterine spasm that might lead to retrograde menstruation predisposing the occurrence of endometriosis.1 Indeed; patients with endometriosis were younger and had higher level of education than control patients.

Approximately 20-40 percent of infertile women suffered from endometriosis[5]. In this study, more than three-fourths of women with endometriosis experienced infertility. Bivariate analysis showed that the odd ratio for infertility was 134.3. Sensitivity and specificity of infertility variable were 78 % and 98 % respectively. They are many theories suggesting the relationship between infertility and endometriosis. These include increased number of peritoneal macrophages that might phagocytize spermatozoa, increased concentration of peritoneal cytokines that might impair ovulation, and the presence of autoantibody antialanin-111, which plays a role in the implantation failure[6].

Other risk factors include dysmenorrhea and chronic pelvic pain. Chronic pelvic pain might be due to irritation or direct infiltration of the nerve on the pelvic floor by endometriosis. It usually occurs when the endometriosis lesion is located on the lateral pelvic wall[7] and in the presence of adhesions[7,8]. This condition was responsible for the low sensitivity of chronic pelvic pain, which was only 28 percent. Sensitivity of dysmenorrhea was only 63 %. Perhaps this is due to the fact that dysmenorrhea is only encountered in women with endometriosis situated on the pelvic wall. In our study, endometriosis on the lateral pelvic wall was found in 15 % of the subjects only. Chronic pelvic pain had 100% specificity with an odd ratio of 13.0.

Sensitivity for dyspareunia was very low (35%). It seems that dyspareunia is encountered only when the implants were located on the vaginal wall, posterior fornix, and rectovaginal septum[8]. We found endometriosis on those locations in 25% of cases. In addition, dyspareunia could be multifactorial including psychological[8].

On pelvic examination, the objective signs of women with endometriosis vary according to the location and size of lesions. Occasionally, one might see blue or red endometriotic implants on the posterior vaginal wall. These lesions are tender and easily bleed on touching[1]. Cervical tenderness had a low sensitivity (38 %), but high specificity (up to 100 %). In the current study, the prevalence of endometriosis lesion on the posterior fornix was 10 %, and on the rectovaginal septum was 25 %. Rectovaginal nodule develops due to chronic endometriosis causing fibrotic nodule containing endometrial tissue, mixed with fat and fibromuscular tissue[9]. We found that the sensitivity was low but it had high specificity (100 %). It seems that in our study all patients with palpable rectovaginal nodule had endometriosis. The sensitivity of adnexal tenderness was only 15%.

Both epithelial and stromal cells of eutopic and ectopic endometrium produce IL-6. It plays a role in the stromal-epithelial interactions in both eutopic and ectopic endometrium of endometriosis patients[10-12]. The odd ratio for IL-6 in our study was 2.51. Bedaiwy et al reported that serum IL-6 could be used for differentiating women with and without endometriosis with high-level sensitivity and specificity[13]. Its level is not affected by the menstrual cycle.

Tumor Necrosis Factor-alpha (TNF- α) is secreted by active immune cells and has strong inflammatory, cytotoxic and angiogenic nature. It has been advocated in the development of endometriosis and infertility[1,14,15] and it affects the local and hormonal regulation in the growth

of ectopic endometrium. TNF-α increases prostaglandin production by endometrial epithelium and triggers adhesions of stromal cells with mesothelial cells, resulting in the formation of endometrial cell implant in the peritoneum[16]. In agreement with the findings of Chae *et al*, we found increased serum TNF-α level in women with endometriosis[17]. Harada et al found a positive correlation between TNF-α level and the size and number of endometrial lesion[3].

We could not find relationship between the presence of endometriosis and other serum markers such matrix-metalloproteinase-2 (MMP-2) and VEGF. Serum MMP-2 level increases during menstruation and decreases at proliferative and secretory phases of the cycle[18]. In our study, laparoscopic examination was performed outside the menstrual period. Similar to MMP-2, VEGF level varies with menstrual cycle.

In conclusion, history of infertility, dysmenorrhea, chronic pelvic pain, dyspareunia is clinical symptoms suggesting endometriosis. Cervical tenderness and palpation of a rectovaginal nodule are equally important. In view of serum markers for endometriosis, IL-6 and TNF-α appears to be best markers.

Conflict of interest statement

We declare that we have no conflict of interest.

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