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Interleukin-6 Concentration in Peritoneal Fluid and Serum of Patients with and without Endometriosis

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ABSTRACT

Background: Endometriosis is a prevalent gynecological condition often diagnosed invasively through laparoscopy. The role of interleukin-6 (IL-6) in its pathogenesis suggests its potential as a non-surgical diagnostic marker.

Objective: This study aimed to compare the levels of IL-6 in the peritoneal fluid and serum of patients with and without endometriosis, proposing IL-6 measurement as a non-invasive diagnostic approach.

Patients and methods: This study was conducted at Ain Shams University Maternity Hospital from August 2008 to April 2010. This case-control study involved 80 patients undergoing laparoscopy for benign gynecological reasons. Group 1 included 40 patients with endometriosis, while group 2 consisted of 40 patients without pelvic pathology. IL-6 levels were measured using ELISA in both serum and peritoneal fluid collected during laparoscopy.

Results: IL-6 concentrations were significantly higher in both serum (103.290 vs. 16.798) and peritoneal fluid (153.506 vs. 16.474) of endometriosis patients compared to controls ($P < 0.05$). A positive correlation ($r=0.902$) was found between serum and peritoneal IL-6 levels in the cases group, with significant differences in IL-6 levels across different stages of endometriosis. The optimal cutoff value for serum IL-6 was determined to be 25.4 pg/ml with a sensitivity and specificity reflective of its diagnostic potential. **Conclusions:** Serum and peritoneal fluid IL-6 levels were significantly elevated in endometriosis patients, correlating with disease severity. Serum IL-6, with a cutoff value of 25.4 pg/ml, could serve as a non-surgical diagnostic marker for endometriosis. Further validation in larger cohorts is recommended.

Keywords: Endometriosis, Interleukin-6, Serum, Peritoneal fluid, Diagnostic biomarker, Laparoscopy.

INTRODUCTION

Endometriosis is a prevalent disorder that impacts the reproductive system. It is characterized by the extra-uterine location of endometrial glands and stroma. Between 6 and 10 percent of women of reproductive age have this condition. Clinical manifestations that are frequently documented encompass dysmenorrhea, pelvic pain, dyspareunia, and infertility. Nonetheless, these symptoms may differ considerably [1].

Clinical diagnosis of endometriosis is challenging due to the absence of specific symptoms. While non-invasive imaging modalities like ultrasonography and magnetic resonance imaging (MRI) can aid in the detection of specific forms of endometriosis, their diagnostic accuracy varies considerably between research studies. At this time, endometriosis is definitively diagnosed via laparoscopy followed by histological confirmation; this invasive procedure is required despite being regarded as the gold standard. Thus, the advancement of a minimally invasive diagnostic technique has the potential to enhance the management of endometriosis[2].

Endometriosis is an estrogen-dependent chronic inflammatory condition, according to a multitude of studies. Furthermore, extensive academic research has emphasized the critical importance of various cytokines in controlling the proliferation, activation, adhesion, and eventual implantation of endometrial cells. For example, the involvement of IL-1b in the proliferation and adhesion of endometrial cells has been established. It has been established that elevated levels of IL-1b and TNF-a can stimulate peritoneal mesothelial cells to produce IL-6; this may exacerbate the local inflammatory response observed in endometriosis [3].

Endometriosis patients have been found to have elevated concentrations of interleukin (IL)-6, a multifunctional cytokine implicated in numerous proliferative and immunological mechanisms, in their peritoneal fluid (PF)[4]. The aim of this study was to compare the levels of IL 6 in PF and serum between patients who underwent laparoscopy with and without endometriosis, as a non-surgical diagnostic tool for the condition.

PATIENTS AND METHODS

Patients: Eighty patients who were admitted to the Laparoscopic Unit of Ain Shams University Maternity Hospital between August 2008 and April 2010 for laparoscopy with benign gynecological indications participated in this case-control study. The patients were divided into two cohorts: Group 1 comprised forty individuals who were presented with chronic pelvic pain [(dysmenorrhea and/or dyspareunia) (endometriosis)] during laparoscopy (Cases), or infertility. Forty patients comprising group 2 (Controls) had no pelvic pathology as determined by diagnostic laparoscopy for polycystic ovaries or tubal ligation.

Inclusion criteria: Patients within childbearing period (less than 45 years old), patients with IUD as contraception, smoker or alcohol drinker and current infection. **Exclusion criteria:** Patients above 45 years of age, minimal amount of PF present during laparoscopy and bloody peritoneal fluid (PF).

METHODS

Patients were subjected to the following: Full history taking. A full clinical examination including general and abdominal examination and local pelvic examination. Ultrasound examination. HSG.

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Laparoscopic visualization of all patients: Displaying a range of lesions from bluish grey "powder burns" or "gunshot" appearances to subtle signs such as red implants and white scarring, alongside deep nodules in the rectovaginal septum and sub ovarian adhesions.

Peritoneal fluid sampling during laparoscopy for IL-6 level using ELISA. Also, Blood samples were taken to determine IL-6 level and estimated using ELISA.

Test procedures:

Reagents, samples, and standards were prepared, with 50 µl of standard and test sample added to wells, not requiring initial dilution. Following the addition and incubation of 50 µl of a green biotin antibody at room temperature for 1.5 hours, 100 µl of HFP-Streptavidin was introduced after a washing step and 30 minutes of incubation. Following the washing process, 50 µl of TMB Substrate reagent was introduced, followed by 20 minutes of incubation (halted with 25 µl Stop solution). Using a reference of 630 nm, the absorbance was promptly quantified at 450 nm.

Ethical considerations: The study was done after being accepted by The Research Ethics Committee, Ain Shams University (Approval code: MS-13-8-2008). Prior to enrollment, written informed consents were obtained from all patients. Their agreement to participate in the trial and for the publication of data was clearly delineated in the consent form, guaranteeing the preservation of their confidentiality and privacy. This research was conducted in adherence to The Code of Ethics of the World Medical Association (Declaration of Helsinki) including human subjects.

Statistical analysis

Statistical analysis was done by SPSS v26 (IBM Inc., Armonk, NY, USA). Quantitative variables were presented as mean and standard deviation (SD) and compared between the two groups utilizing unpaired Student's t- test. Qualitative variables were presented as frequency and percentage (%) and were analysed utilizing the Chi-square test or Fisher's exact test when appropriate. Pearson correlation was done to estimate the degree of correlation between two quantitative variables. Receiver Operating Characteristic curve (ROC-curve) analysis: The overall diagnostic performance of each test was assessed by ROC curve analysis, a curve that extends from the lower left corner to the upper left corner then to the upper right corner is considered a perfect test. The area under the curve (AUC) evaluates the overall test performance (where the area under the curve >50% denotes acceptable performance and area about 100% is the best performance for the test). A significance level of 0.05 or less was utilized to define a P value.

RESULTS

Mean serum and peritoneal IL-6 concentration: An analysis of the concentrations of IL-6 in serum and the PF between the two groups revealed that the cases' group had significantly higher levels of IL-6 in both serum (103.290 vs. 16.798) and the PF (153.506 vs. 16.474) than the control group. Both comparisons produced a P- < 0.05 (Table (**1**).

Table (1): Mean serum and peritoneal IL-6 concentration

		Group 1 (Cases)	Group 2 (Control)	P – value
Serum IL-6 concentration	Number	40	40	< **0.05***
	Mean	103.290	16.798	
	SD	24.2803	1.7251	
Peritoneal IL-6 concentration	Number	40	40	< **0.05***
	Mean	153.506	16.474	
	SD	± 14.8129	± 1.1483	

*Significant P-value. IL: Interleukin

Correlation of serum and peritoneal IL-6 with the infertility duration among cases:

In the cases' group, there was no statistically significant difference observed among the duration of infertility and the concentrations of IL-6 in the PF and serum (Table 2).

Table 2: Correlation between serum IL-6 and peritoneal IL-6 with the infertility duration among the cases group

Cases	Duration of infertility	
	r	P-value
Serum IL-6	-0.103	> 0.05
Peritoneal IL-6	-0.071	> 0.05

*Significant P-value. r: correlation coefficient

Correlation between serum and peritoneal IL-6 among cases & control group: PF IL-6 and serum IL-6 levels in the cases' group exhibited a statistically significant positive correlation (r=0.902). Conversely, in the control group, PF IL-6 and serum IL-6 levels demonstrated a negative correlation (r=0.352) (Table 3).

Table 3: Correlation between serum and peritoneal IL-6 among cases & control group

		Peritoneal IL-6 (Cases)	Peritoneal IL-6 (Control)
Serum IL-6 (Cases)	r	0.902	
	P - value	**<0.05***	
Serum IL-6 (Control)	r		0.352
	P – value		>0.05

*Significant P-value. r: correlation coefficient

Correlation between serum and peritoneal IL-6 with each stage of endometriosis among the cases: A significant direct correlation (p<0.05) was identified between the levels of IL-6 in the serum and PF of the cases' group and the grade of endometriosis **(Table 4).**

Table 4: Correlation between serum and peritoneal IL-6 with each stage of endometriosis among the cases

Grade		Serum IL-6	Peritoneal IL-6
I	Number	26	26
	Mean	33.507	68.168
	SD	3.5688	6.1366
II	Number	7	7
	Mean	140.617	236.053
	SD	27.8927	35.5847
III	Number	4	4
	Mean	235.700	306.658
	SD	23.1305	51.3192
IV	Number	3	3
	Mean	444.433	496.300
	SD	40.5315	2.4249

Data are presented as mean ± SD or number (%).

Cut-off values for serum and peritoneal IL-6 level in relation to their sensitivity and specificity:

The curve in figure (1) indicated that the optimal cutoff value for serum IL-6 was 25.4 pg/ml, based on its sensitivity and specificity. The AUC was 0.739, representing a 73.9% overall predictive accuracy of serum IL-6, with the best cutoff value being 25.4 pg/ml. The curve demonstrated that the optimal cutoff value for PF IL-6 was 44.8 pg/ml, considering its sensitivity and specificity. The area under the curve (AUC) was 0.831, representing an 83.1% overall predictive accuracy for peritoneal IL-6, with the optimal cutoff value being 44.8 pg/ml.

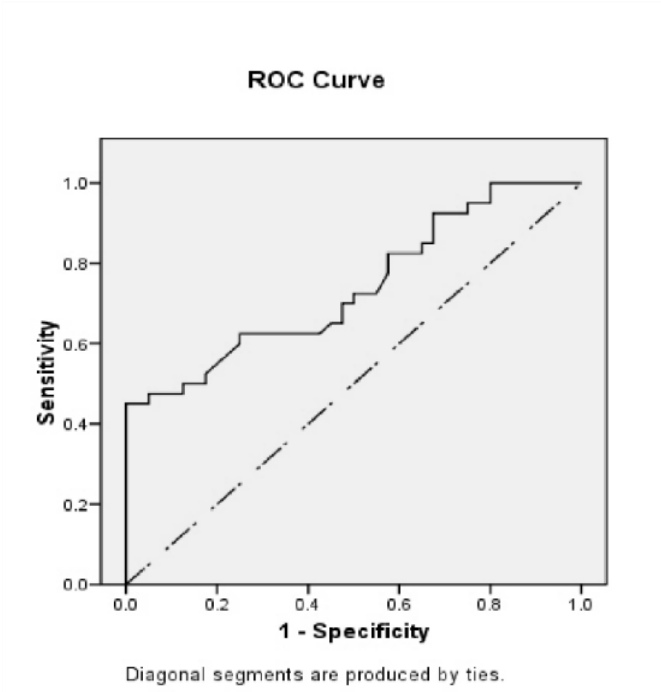

Figure (2): Receiver operator characteristic curve (ROC) for serum IL-6 level in diagnostic endometriosis.

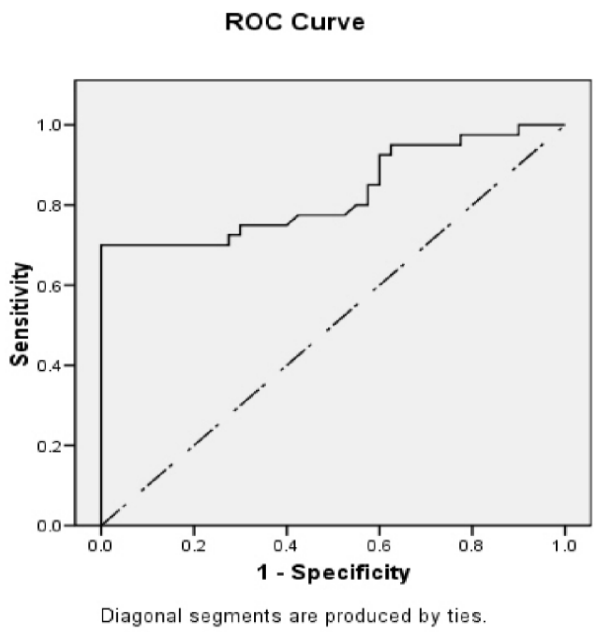

Figure (2): Receiver operator characteristic curve (ROC) for peritoneal fluid IL-6 level in diagnostic endometriosis.

DISCUSSION

The primary method of diagnosis for endometriosis is laparoscopic examination, followed by histological confirmation. The absence of symptoms and the dearth of sensitive biomarkers may therefore impede the timely identification and treatment of this condition. Recently, a proliferation of academic inquiries has emerged, emphasizing the critical role that cytokines play in the development of endometriosis. Peritoneal fluid (PF) analysis revealed increased levels of IL-6, which were compared to serum levels in women without endometriosis and patients who had been diagnosed with the condition. Moreover, IL-6 concentrations increased with the progression of the disease. The aim of this study was to assess the non-invasive diagnostic potential of IL-6 in PF and serum samples from patients in order to identify endometriosis. Elevated concentrations of various cytokines have been detected in the PF of women diagnosed with endometriosis, indicating that these cytokines potentially contribute to the advancement and progression of endometriosis and endometriosis-associated infertility [5]. **Kalu *et al.*** [6] conducted an investigation and found that the endometriosis group demonstrated significant increased concentrations of PF MCP-1, IL-8, and IL-6 ($P < 0.012$, $= 0.003$, and $= 0.015$ correspondingly).

Similar to the findings of **Harada *et al.*** [7] and **Martínez *et al.*** [8] our results support the notion that IL-6 levels correlate significantly with the stage of endometriosis. Specifically, their researches revealed that patients with endometriosis had significantly higher concentrations of IL-6 in their peritoneal fluid than those without the condition. Furthermore, these studies observed that IL-6 levels increased in direct proportion to the score of active lesions. The findings of these investigations align with the research conducted by **Mahnke *et al.*** [9], which demonstrated that women with more advanced endometriosis (implant scores of 6 or higher) have increased levels of IL-6 in peritoneal fluid in comparison with normal controls or women with less severe endometriosis (implant scores of 5 or less).

Primary infertility impacted 72.5% (n=29) of the forty patients comprising the cases group, according to the findings of this study and secondary infertility affected 27.5% (n=11). The outcomes presented here align with the research conducted by **Roger *et al.*** [10], which revealed that endometriosis affects 20-40% of subfertile women and 5% of fertile women respectively.

Bedaiwy *et al.* [11] discovered that the sensitivity and specificity of IL-6 varied between 67% and 89% in their investigation. Furthermore, **Matrinez *et al.*** [8] observed that the AUC was 0.829, and the optimal serum IL-6 threshold, as determined by the ROC curve, was 25.75 pg/ml. This value corresponds to a specificity of 83.3% and a sensitivity of 75%. **Othman *et al.*** [12] reported that serum IL-6 has a specificity of 66% and a sensitivity of 71% in distinguishing endometriosis patients from controls, with a cutoff point of 1.9 pg/ml. According to **Mihalyi *et al.*** [13] the identification of minimal to mild endometriosis required a specificity of 61% and a sensitivity of 94%. The diagnostic accuracy for moderate to severe endometriosis was one hundred percent, with a specificity of 84%. Disparities in the outcomes of multiple investigations may be ascribed to variations in sample size, study designs, and population characteristics. The study was limited in scope and execution by the utilization of a single center and a small sample size.

CONCLUSION

In summary, the current findings indicated that individuals diagnosed with endometriosis exhibited considerably elevated concentrations of serum and PF IL-6 in comparison with those without the condition. The concentrations in patients with active endometriosis demonstrated an increasing correlation with the number and size of active lesions, which were impacted by the Revised American Fertility Society classification systems for endometriosis stages. A non-surgical diagnostic tool for endometriosis is the concentration of serum IL-6, which had 25.4 pg/ml cutoff value. A larger study, nevertheless, is necessary to validate these findings.

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Conflict of Interest: Nil.

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