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## Study of the Immunological Status of Iraqi Women suffering from Endometriosis and Polycystic Ovary Syndrome.

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### ABSTRACT

Endometriosis is chronic disease describe by the presence of endometrial- like tissue, outside the uterus. Polycystic Ovary Syndrome (PCOS) is one of the most endocrine disorders which effected in women with reproductive ages. The immune system play very important role in these diseases, so to test this role, certain cytokines (IL-6, IL-12 and TNF- $\alpha$ ), immunoglobulins (IgM, IgG) and complements (C3, C4), also estrogen and progesterone hormones were investigated in all participants. The study included 72 Iraqi women with age range between (16-43) year; 22 woman with endometriosis, 30 womam with PCOS and 30 apparently healthy controls. Serum was separated and cytokines were detected by using Enzyme Linked Immunosorbent Assay (ELISA); immunoglobulins and complements were detected by using Genus devise, while estrogen and progesterone hormones were measured by using ELFA technique (Enzyme Linked Fluorescent Assay) by Minividas Device. The results showed that the mean serum level of (IL-6 and TNF- $\alpha$ ) were high in endometriosis and PCOS patients compared with healthy control, but the increase statistically non significant ( $P>0.05$ ) between patients (Endometriosis and PCOS), but highly significant ( $P<0.01$ ) differences between patients and healthy control, while IL-12 were nearly similar in patients compared with control, and there was no significant differences between all groups. IgG mean serum level was low in endometriosis patients compared with PCOS patients and healthy control, there were no significant differences ( $P>0.05$ ) between patients and healthy control, also IgM serum level was low in endometriosis patients compared with PCOS patients and healthy control , there were significant differences ( $P<0.01$ ) between all investigated groups. Endometriosis patients showed low mean serum level of C3 compared with PCOS patients and healthy. There was no significant differences between patients groups ( $P>0.05$ ) and no significant differences between all investigated groups. Endometriosis group show low mean serum level of complement C4 compared with PCOS group and healthy subjects. There was no significant differences ( $P>0.05$ ) between patients groups and no significant differences between all investigated groups. The mean serum level of E2 was elevated in patients group comparing to control, also PRG level in patients group was higher compared with control, there was significant differences between all groups. From these findings we can conclude that marked high serum level of IL-6 and TNF- $\alpha$  in endometriosis and PCOS patients clarify their important participation in the pathogenesis of both of diseases, while low levels of immunoglobulines and complements indicate to use them up by immune system and the role of humoral immunity in patients.

**Key words:** Endometriosis, PCOS, IL-6, IL-12, TNF- $\alpha$ , IgG, IgM, C3, C4, E2 and PRG.

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## INTRODUCTION

A chronic gynecological disease which characterized by the presence of ectopic foci endometrial tissue in the pelvic cavity and or ovary [1]. It is effect in 10% of women with reproductive ages, about 2-3 % of adolescent who suffering from dysmenorhea and chronic pelvic pain have laparoscopic proof of endometriosis [2]. Some women with endometriosis have symptoms such as; menstrual cramps, chronic pelvic pain, or painful intercourse and other symptoms, also most of endometriosis patients suffering infertility [3]. Some reports show that the risk of increase endometriosis is six times higher in first degree relative of women with sever endometriosis comparing to other women [4]. The cause of endometriosis is unknown until know, many theories were told how endometriosis happened, but none of them are effectively explain all of factors that led to develop this disease [5]. Retrograde menstruation is the oldest theories to explain the occurrence of endometriosis and it is developed in the 1920 by Dr. John Sampson, when menstrual fluid (endometrial cells/ debris) flows back up to the fallopian tubes and in to the pelvic cavity during menstruation [6]. These cells deposited in different positions growth and development in to endometriosis causing adhesion, necrosis, inflammation, and internal bleeding. Then the symptoms and the physical distortion of pelvic anatomy that is seen in women with endometriosis begin to appear [7][8][9]. According to the American Society for Reproduction and Medicine (ASRM) endometriosis is classified in to four stages which are minimal (stage 1), mild (stage 2), moderate (stage 3), severe (stage 4) as in figure:(3) , every stage is depends on the presence and severity of adhesions , size, location, presence and size of ovarian endometriomas and depth of growth [10][3]. The laparoscopy with histological verification is still the golden method for diagnosis this disease, but this procedure is still have risk such as morbidity and also mortality [11]. transvaginal ultrasonography, history, physical and imagines assessments and (CA-125) all these methods used to diagnosis endometriosis [12][13]. Women with endometriosis at first treated with hormonal therapy, and that related to endometriosis consider estrogen depended inflammatory disease, so this therapy give to control the production of estrogen by suppression the monthly period, and when the therapy is not useful and the disease become more sever so the surgery is necessary by a procedure called laparotomy [5].

Polycystic Ovary Syndrome (PCOS) is a combination of hyperandrogenism and an ovulation with or without the presence of PCOS in the ultrasound and it is one of the most endocrine disorders that effected in women with reproductive ages [14][15][16]. It is estimation that PCOS affected 3-15% of all women, and the abnormality in the ovary is the primary cause of this disorder and in addition to other agents such as; genetic, obesity and environmental factors [17]. According to the Rotterdam criteria, at least two of the following are enough to diagnosis of PCOS: oligo and or anovulation, clinical and or biochemical signs of hyperandrogenism, and polycystic ovary at ultrasound with exclusion of other androgen excess or related disorder [18]. PCOS is associated with presence of insulin résistance, diabetes mellitus type 2, obesity, metabolic syndrome, dyslipidemia, hypertension, hyperplasia, cardiovascular disease and endometrial carcinoma [19]. PCOS also causing infertility in rang (15-25%), and that related to the anovulation and irregular cycle [20]. Hormonal contraceptive (HCs) are used to treat PCOS by inductions of ovulation and screening, this type of medication is use either oral contrastive, patches and vaginal rings such as; Spironolactone, Dian and Yaz these oral contraceptives with anti-androgenic effects used as first line treatment of hairsutism, acne and menstrual abnormalities caused by PCOS [21][22][23]. Many studies show the role of cellular and humoral immunity in the pathogenesis of endometriosis and PCOS, as cytokines are important mediators of immunity, so that to identify the relationship between immunological function and both of endometriosis and PCOS; level of cytokines (IL-6, IL-12 and TNF- $\alpha$ ) and immunoglobulins (IgG and IgM) were measured in the sera of three investigated groups.

## MATERIALS AND METHODS

This study includes 72 Iraqi women (with ages from 16 to 43 year), who were referred to the Consultant Clinic at the Department of Gynecology and Obstetrics in both Al-Olwiia and Baghdad Teaching Hospitals during the period from the first of September 2016 to the end of February 2017. The diagnosis was made by the consultant medical staff at the clinic on the basis of International criteria. The current study includes three investigated groups; 22 patients with endometriosis, 30 patients with PCOS and 30 apparently healthy control who nearly in the same age with patients (20-43) years.

The blood samples were collected (8) ml from the venous blood of the patients of both groups and control group then they were transferred to plain tubes and left nearly an hour to clot at room temperature. Then, they were separated by centrifuging at speed (3000) round per minute for 10 minutes to collect the serum by using micropipette. After that the separated serum will be divided in to 6 aliquots (150) ml in Eppendorf tubes and until used it in immunological assay. The level of cytokines (IL-6, IL-12 and TNF- $\alpha$ ) were detected kept at -20 °C by using Enzyme Linked Immunosorbent Assay (ELISA) technique kits; immunoglobulins (IgG and IgM), and complements (C3 and C4) were detected by using Genus devise (antigen- antibody complex and turbidity) method, while estrogen and progesterone hormones were measured by using ELFA technique (Enzyme Linked Fluorescent Assay) by Minividas Device.

Data were entered and analyzed by using SPSS (Statistical Packages for Social Sciences- version 24), data were presented in simple measures of frequency, percentage, tables and graphs) were used. Data were presented in terms of mean  $\pm$  Standard Deviation (S.D.) and Standard Error (S.E.). The significance of difference of different means (quantitative data) was tested using Students-T-test for difference between two independent means or ANOVA test for difference among more than two independent means. The difference was considered significant when the probability (P) value was equal or less than 0.05 [24].

**RESULTS**

Out of 52 patients in this study, 22(42.31%) were patients with endometriosis and 30(57.69%) were patients with PCOS. Regarding patients group, the mean age was (31.14) years of endometriosis patients, and the mean age was (25.90) years of PCOS patients. Young individuals appeared to be more susceptible to infected both of endometriosis and PCOS as it show in (Table: 1).

**Table (1): Distribution and percentage of endometriosis and PCOS patients according to age groups.**

Age (years)	Endometriosis No. (%)	PCOS No. (%)
< 20	2 (9.1)	6 (20.0)
20-24	4 (18.2)	11(36.7)
25-29	4 (18.2)	-
30-34	2 (9.1)	9 (30.0)
35-39	4 (18.2)	3 (10.0)
> 40	6 (27.3)	1 (3.3)

**Serum Level of Interleukin-6, Interleukin-12 and Tumor Necrosis Factor- alpha:**

Endometriosis and PCOS showed high mean serum level of IL-6 (103.27 $\pm$ 39.17, 105.95 $\pm$ 44.83 pg/ml, respectively), as compared with healthy control (36.25  $\pm$ 12.47 pg /ml), there was no significant (P>0.05) differences between patients groups (Endo and PCOS P=0.824), while there was a highly significant (P<0.01) differences between patients and healthy control (P=0.0001) as in (Table 2), while endometriosis and PCOS patients show nearly similar mean serum level of IL-12 (15.49 $\pm$ 3.32, 15.05 $\pm$ 3.34 pg/ml respectively) compared with the control (14.39 $\pm$ 2.18 pg/ml), and there were no significant differences between patients (P>0.05) (P=0.640) , and there was no significant differences between all investigated groups P=0.506 as in (Table: 2). Also endometriosis and PCOS patients were show high mean serum level of TNF- $\alpha$  (84.48 $\pm$ 40.28, 116.19 $\pm$ 71.37 pg/ ml respectively) compared with healthy control (30.91 $\pm$ 4.66pg/ml). There were no significant differences between patients group (P>0.05) (Endo and PCOS, P=0.067) and high significant differences between patients groups and healthy subjects P= 0.0001 (Table: 2).

**Serum Level of Immunoglobulines IgG and IgM:**

Endometriosis patients show low serum level of immunoglobulin (IgG) (8.86 $\pm$ 5.04 g/L) compared with PCOS patients and healthy control (10.49 $\pm$ 5.67, 10.72  $\pm$ 3.51 g/L respectively). There were no significant differences between patients groups (P>0.05) (P=0.287) and no significant differences between patients and healthy subjects (Endo and control P= 0.176; PCOS and control P=0.873), also there were no significant differences

between all investigated groups  $P= 0.396$  as list in (Table: 3). Also endometriosis patients show low serum level of (IgM) ( $0.774\pm 0.577$  g/L) compared with PCOS patients and healthy control ( $1.371\pm 0.916$  ,  $1.708\pm 0.940$  g/L respectively). There were significant differences between patients groups ( $P= 0.010$ ) and significant differences between Endo group and control  $P= 0.0001$ , but no significant differences between PCOS group and control  $P= 0.214$ , while there were significant differences between all investigated groups  $P= 0.002$  as list in (Table: 3).

**Table (2): Mean serum level of IL-6, IL-12 and TNF- $\alpha$  (pg/ml).**

Samples	NO.	Mean $\pm$ S.D. IL-6 (pg/ml)	Mean $\pm$ S.D. IL-12 (pg/ml)	Mean $\pm$ S.D. TNF- $\alpha$ (pg/ml)
Endometriosis	22	103.27 $\pm$ 39.17	15.49 $\pm$ 3.32	84.48 $\pm$ 40.28
PCOS	30	105.95 $\pm$ 44.83	15.05 $\pm$ 3.34	116.19 $\pm$ 71.37
Healthy control	30	36.52 $\pm$ 12.47	14.39 $\pm$ 2.18	30.91 $\pm$ 4.66
Level of Sig.		0.0001*	0.506	0.0001*
*Significant difference between two independent means using Student-t-test or ANOVA test for difference among more than two independent means at 0.05 level				

**Table (3): Mean serum level of IgG and IgM (g/L)**

Samples	NO.	Mean $\pm$ S.D. IgG (g/L)	Mean $\pm$ S.D. IgM (g/L)
Endometriosis	22	8.86 $\pm$ 5.04	0.774 $\pm$ 0.577
PCOS	30	10.49 $\pm$ 5.67	1.371 $\pm$ 0.916
Healthy control	30	10.72 $\pm$ 3.51	1.708 $\pm$ 0.940
Level of Sig.		0.396	0.002*
*Significant difference between two independent means using Student-t-test or ANOVA test for difference among more than two independent means at 0.05 level			

**Serum Level of Complements (C3, C4):**

Endometriosis patients showed low mean serum level of C3 ( $0.771\pm 0.514$  g/L) compared with PCOS patients and healthy control ( $0.880\pm 0.512$ ,  $0.842\pm 0.462$  g/L respectively). There were no significant differences between patients groups ( $P>0.05$ ) (Endo and PCOS  $P=0.606$ ) and no significant differences between all investigated groups ( $P=0.767$ ), while endometriosis group show low mean serum level of complement C4 ( $0.235\pm 0.190$ g/L) compared with PCOS group and healthy subjects ( $0.441\pm 1.111$ ,  $0.409\pm 0.617$  g/L respectively). There were no significant differences ( $P>0.05$ ) between patients groups (Endo and PCOS  $P= 0.395$ ) and no significant differences between all investigated groups ( $P= 0.634$ ) as in table (4):

**Table (4): Mean serum level of C3 and C4 (g/L)**

Samples	NO.	Mean ± S.D. C3 (g/L)	Mean ± S.D. C4 (g/L)
Endometriosis	22	0.771±0.514	0.235±0.190
PCOS	30	0.842±0.462	0.441±1.111
Healthy control	30	0.880±0.512	0.409±0.617
Level of Sig.		0.767	0.634
*Significant difference between two independent means using Student-t-test or ANOVA test for difference among more than two independent means at 0.05 level			

**Serum Level of Estrogen (E2) and Progesterone (PRG) hormones**

Table: (5) showing the results of hormonal tests (estrogen and progesterone). The mean of estrogen (E2) to endometriosis group were (58.87pg/ml), while in PCOS patients E2 mean were (59.42pg/ml) and both of patients groups have elevated serum level of E2 comparing to control with no significant differences between all groups P=0.907. According to our results that mean serum level of E2 was elevated in patients group comparing to control, while there were nearly similar level of E2 between patients group.

Progesterone (PRG) also made to all investigated groups and the results were the mean of PRG in Endo group (1.50pg/ml), while in PCOS group the mean of PRG (1.64pg/ml) and according to results PRG level in patients group was higher compared with control, there was significant differences between all groups P=0.017 as list in table (5).

**Table (5): Mean serum level of E2 and PRG (pg/ml)**

Samples	NO.	Mean ± S.D. E2 (pg/ml)	Mean ± S.D. PRG (pg/ml)
Endometriosis	22	58.87±18.86	1.50±0.49
PCOS	30	59.42±19.36	1.64±1.50
Healthy control	30	56.70±27.56	0.79±0.42
Level of Sig.		0.907	0.017*
*Significant difference between two independent means using Student-t-test or ANOVA test for difference among more than two independent means at 0.05 level			

## DISCUSSION

Endometriosis is clinically identify by the presence of endometrial tissue outside the uterus, while Polycystic Ovary Syndrome (PCOS) is one of most endocrine disorder that associated with increased activity of rennin- androgen system and hyperandrogenism in women. Both of endometriosis and PCOS are affecting in women with reproductive age. The mean age of endometriosis patients in this study were (31.14) in rang (16-43) year and that agree with Wood *et al* [25] who reported that the typical ages of endometriosis are in late 20s and early 30s in age and also reported that teenagers (13-19) also have endometriosis, but this type of age group have the disease symptoms but unfortunately may not have diagnosis for this disease until 20s or 30s, in our study there were two patients in this group age. Also PCOS patients were in young age, the mean age of PCOS patients in this study were (25.09) and in rang from (17-40) , It is found that all over the world 105 million women have PCOS with age from (15-49) year [19] and that agree with our results .

Cytokines are effective immune modulator glycoproteins with autocrine and paracrine effects that play a role in mitosis, angiogenesis and chemo taxis [26] [27]. Cytokines which are secreted by many types of cells including endometriotic tissue play important role in the pathogenesis of endometriosis and endometriosis-associated infertility [28]. Some of cytokines such as IL-6, IL-12, and TNF-  $\alpha$  play important role in different disease. Also these cytokines play a vital role in decrease immunologic observation, recognition and distraction of ectopic endometrial cells and prevent the implantation of ectopic endometrial tissue [29], and also these cytokines play a role in the pathogenesis of PCOS. According to our results there were elevated in the serum level of IL-6 in endometriosis patients compared with control and that agree with Bedaiwy and Falcone [30], who reported that there were elevated level of IL-6 in sera of endometriosis patients compared with control. Also there was elevated level of IL-6 in patients with PCOS and this result were compared with Peng *et al* [31] who found there was elevated in IL-6 level in PCOS women compared with control. High level of IL-6 has been found in most of diseases processes including endometriosis and PCOS. IL-6 play important role in the pathogenesis of endometriosis and PCOS as a regulator of inflammation and immunity, and might be work as a link between endocrine and immune system, according to several studies IL-6 is implicated in proliferation pathways, because it acts with other factors such as, heparin-binding epithelial growth factor and hepatocyte growth factor [32] [33] [34]. Different studies show high serum level of TNF- $\alpha$  in most of diseases. Our results also show elevated level of TNF- $\alpha$  in patients groups compared with the healthy subjects and that agree with Malutan *et al* [35] who was found elevated TNF - $\alpha$  in sera of patients with endometriosis compared with healthy control. Also our result show elevated serum level of TNF- $\alpha$  in PCOS investigated group compared with control and that agree with

Escobar- Morreale *et al* [36] that were found elevated mean serum level of TNF- $\alpha$  in PCOS woman compared with control. Even though TNF- $\alpha$  acts through autocrine- paracrine mechanism in adipose and muscle tissue, increase serum level of TNF- $\alpha$  were found in specific situations such as; insulin resistance, obesity and diabetes mellitus type 2, also increase production of TNF- $\alpha$  in PCOS patients might contribute in the pathogenesis of hyperandrogenism, in depend on obesity and insulin resistance [37] [38], while depended on several studies, increase level of TNF - $\alpha$  in the peritoneal fluid and serum of endometriosis patients were correlated with disease stages. TNF- $\alpha$  presence in the peritoneal fluid might play an important role in adherence of ectopic endometrial tissue to the peritoneum and that led to increase the implantation and development of the disease [39] [40]. According to our results there was nearly similar mean serum level of IL-12 in patients groups compared with control, The results of this study were compared with Malutan *et al.*, who found that was no significant differences in the serum level of IL-12 between the investigated groups, as all most of reports that found the level of IL-12 was no differ in all investigated groups. It was found that the decrease level of IL-12 may be associated with defective clearing of ectopic endometrial cells shed to the abdominal cavity through retrograde menstruation or formed passing through metaplasia [41]; some studies reported that endometriosis patients with low IL-12 concentrations are potentially more suspected to development to endometrial superficial cells and deep endometriotic lesion and the local increase of immunological activity in the peritoneal cavity is attenuates the adhesion potential of endometrial stromal cells [42][43]. Different studies reported that inflammatory mediator, IL-12 one of them were over product PCOS, obesity, diabetes and cardio vascular diseases [44], while Gallinelli *et al* [45] reported that IL-12 was found in low concentration in follicular fluid of women with PCOS compared with healthy subjects, but in our results there were similarity in IL-12 level in PCOS patients compared with control. Studies have been assessed total immunoglobulin level in patients with and without endometriosis, but have no role or no effected in the disease [46] [47], also El-Roeiy and colleagues found that there were a positive correlation between increasing disease stages and the level of IgG and IgM, while other study show that IgG level were decrease in follicular phase from the cycle,

but IgM level not change [48], One study appeared that anti- endometrial IgG antibodies found in 56% of endometriosis patients and only 5% in healthy control women [49], while other studies show that anti-endometrial IgG antibodies found in 33% in endometriosis patients and IgM antibodies found in 27% [50]. and depended on the studies who either found high, no change and low level of endometrial antibody, and according to our results we have low serum level of IgG and IgM and that might related as it was suggested by Mathur *et al.*[51] to the differences of antigenicity of endometrium, and that related to the complexity of human endometrial protein expressed at the different phases of menstrual cycle and also might associated with the severity of the disease and used these immunoglobulin's by immune complex . Also PCOS patients show little low IgG and low serum level of IgM and that not agree with Petrikova *et al* [52], who reported that anti- ovarian antibody (IgG, IgA and IgM) were found significantly higher for women with PCOS compared with control, In spite of lower levels of IgG and IgM in PCOS patients which that might related as endometriosis to use up these immunoglobulins by immune complex and that depend on the severity of the disease or complement might have no role in the pathogenesis of endometriosis and PCOS.

According to our results, endometriosis patients show low serum mean level of complements (C3, C4), and depended on different studies C3 and C4 increase in some reports [53][54] while in the recent reports complements concentration were decrease in women with endometriosis [55][48] and that related to be secondary to general burning up of the complement factors by immune complex as a result to the possibility of the autoimmune respond, while Abouseeda and Sabri (1991) [56] show that there were normal concentrations of C3 and C4 in the serum of endometriosis patients and control group so our results not agree with him, Serum level of C3 in PCOS patients was nearly similar compared with control and that not agree with Dehdashtihaghighat *et al.*, [57] and Snyder *et al.*, [58] who both of them found that there were high serum level of C3 in PCOS group compared with control, but no significant differences between investigated groups. It is found that C3 increase in adults with Cardiovascular Disease (CVD) and associated with tissue damage at the site of myocardial infection, also C3 increase in adults with risk of coronary heart disease and women with Systemic Lupus Erythematosus (SLE) [59] [60] [61], and because of patients with PCOS suffering from most of disease mention above in addition to obesity and insulin resistance C3 serum level elevated in PCOS patients. But, our results show high serum level of C4, and that might related to consumption of C3 by immune complex more than C4 and also associated with stages and severity of disease, by development the disease, immune complex used up the complements to decrease the progress of the disease.

According to our results that mean serum level of E2 was elevated in patients group comparing to control, while there were nearly similar level of E2 between patients group. Some studies show that high levels of testosterone and insulin in women with PCOS sometimes converted to estrogen. Orio *et al.*, [62] reported that the mean of E2 for PCOS patients were (58.6pg/ml), so that our results were agree with him, while Fulghesu *et al.*, [63] regarded that the mean of E2 in PCOS group was (27.3pg/ml), our results was in variance with him, also according to results PRG level in patients group was higher compared with control. Takahashi *et al.*, [64] measured estradiol levels in peritoneal and menstrual blood of patients with endometriosis, adenomiosis and controls. They found no differences in estradiol levels in peritoneal fluid. Though menstrual blood showed highest level of estrogen in patients with adenomiosis then followed endometriosis patients than controls, suggestion a uterine production of estrogen in these diseases, while progesterone is potent antagonist of estrogen- induced proliferation in the endometrium and may play an essential role in the pathogenesis of endometriosis. Related to several studies PRG in both endometriosis and PCOS are low comparing to the normal level in healthy people, because in the normal women who have normal cycle ovulation (release egg from the ovary) accord and the ovary produce progesterone hormone for consequently 12 to 15 days that makes women get her period normally every month, while women with PCOS ovulate infrequently or not have cycle at all that led to infrequently produce progesterone or not produce at all, so that led to irregular or absence the cycle [65].

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