

Levels of Cytokines Profile in Polycystic Ovary Syndrome

Shafaq H. Hussein Al-Musawy, Ihsan E. Al-Saimary, Maysoon Sherif Flaifil

Department of Microbiology and Gynaecology, College of Medicine, University of Basrah, Basrah, Iraq

Abstract

Background: Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorder of female at reproductive age and its prevalence in general population as 20%–33%. **Objective:** The aim of this study was to measure the level of interleukin (IL) 18, IL 6, and tumor necrosis factor (TNF) in polycystic ovary women and compare their levels with apparently healthy control group. **Materials and Methods:** A case-control study was carried out in Basra. In this study, blood samples from 73 women with PCOS and 73 healthy control women were collected from outpatients and private gynecological clinics and primary health care centers from different area of Basra during August 2016–March 2017 for estimation of their serum levels of IL6, IL18, and TNF alpha by using enzyme-linked immunosorbent assay technique. **Results:** It is found that serum levels of IL6, IL18, and TNF alpha were elevated in PCOS women, and we conclude that serum levels of IL6, IL18, and TNF alpha are highly statistically significance in PCOS women than in healthy control group. **Conclusion:** Levels of IL6, IL18, and TNF alpha were highly statistically significant in PCOS comparing to normal women, and these high levels were related to PCOS independent on the presence of obesity or hyperandrogenism.

Keywords: Interleukin 18, interleukin 6, polycystic ovary syndrome, tumor necrosis factor alpha

INTRODUCTION

Polycystic ovarian syndrome is a heterogeneous collection of signs and symptoms that form a spectrum of a disorder with a mild presentation in some women and a severe disturbance of reproductive, endocrine, and metabolic function in others. The pathophysiology of polycystic ovary syndrome (PCOS) appears to be multifactorial and polygenic. Key features include menstrual cycle disturbance, hyperandrogenism, and obesity.^[1]

Polycystic ovaries are commonly detected by ultrasound or other forms of pelvic imaging, with estimates of the prevalence in the general population as 20%–33%.^[2]

PCOS has significant and numerous clinical issues, including reproductive, endocrine, and metabolic disorders such as hyperandrogenism and obesity, especially abdominal obesity, is an independent factor aggravating PCOS endocrine abnormalities, as subcutaneous abdominal adipose tissues and the liver tissues contribute to extragonadal aromatization.^[3]

There is evidence that PCOS is also a proinflammatory disorder, characterized by the presence of chronic low-grade inflammation and there is increased level of several

inflammatory cytokines that associated with insulin resistance (IR). Obesity and diabetes mellitus have also been found to be associated with the syndrome.^[4] Plasma interleukin (IL)-18 is found to be elevated in obesity and in women with PCOS and in patients with type 2 diabetes.^[5,6] It was found that common polymorphisms in the genes encoding tumor necrosis factor (TNF), type 2 TNF receptor, IL-6, and the IL-6 signaling molecule gp130 are associated with hyperandrogenism and PCOS or influence hyperandrogenic phenotypic traits.^[7,8]

Aim of this study

The aim of the study is to measure the level of IL 18, IL 6, and TNF alpha in polycystic ovary women and compare their levels between PCOS women with apparently healthy control group.

MATERIALS AND METHODS

A total number of 73 women with PCOS were involved in this case-control study. Patients were collected from outpatient

Address for correspondence: Shafaq H. Hussein Al-Musawy,
College of Medicine, University of Basra, Basra, Iraq.
E-mail: shafaqalmusawy@gmail.com

Access this article online

Quick Response Code:



Website:
www.medjbabylon.org

DOI:
10.4103/MJBL.MJBL_32_18

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How to cite this article: Al-Musawy SH, Al-Saimary IE, Flaifil MS. Levels of cytokines profile in polycystic ovary syndrome. *Med J Babylon* 2018;15:124-8.

and private gynecological clinics from different area of Basra regardless the marital status, whom age ranged from 20 to 40 years. The diagnosis of PCOS was based on Androgen Excess and PCOS society at 2006 criteria:^[9] (1) Oligoovulation and/or anovulation; (2) clinical and/or biochemical signs of hyperandrogenism (patients presented with hirsute, acne or alopecia, and/or increased circulating levels of testosterone); (3) polycystic ovaries (ovarian morphology was assessed using transvaginal ultrasound). (4) Exclusion criteria included all patients with hormonal therapy (or any medication known to interfere with follicular development or hormonal levels for last 4 months of sample aspiration), diabetic patients, and patients with oligomenorrhea, and amenorrhea due to other causes other than PCOS causes.

The control group consists of 73 fertile women collected from primary health care centers in Basra and who have regular menstrual cycle with no sign of hyperandrogenism and their age between 20 and 40 years and subjected to ultrasound examination and have normal hormonal level.

Body mass index (BMI) for both patients and control group was calculated as follows: Weight (kilograms)/height² (meters). The simplicity and ease of measurement have entrenched the widespread use of the BMI as a marker of adiposity, not only for epidemiological purposes but also in clinical practice.^[10] BMI is interpreted by using standard weight status categories that are the same for all ages and for both men and women. For adults, WHO defines overweight and obesity as follows:^[11] overweight: Is a BMI ≥ 25 and obesity: Is a BMI ≥ 30 .

BMI provides the most useful population-level measure of overweight and obesity as it is the same for both sexes and for all ages of adults. However, it should be considered a rough guide because it may not correspond to the same degree of fatness in different individuals.^[11] Blood sugar was measured to exclude diabetes mellitus.

Blood sample was collected (from each patients) in clot activator tube; serum was separated after centrifugation and divided into three Eppendorf tubes to avoid multiple freezing and thawing and kept frozen until time of analysis. Serum level of IL6, IL18, and TNF alpha was collected for both patients and control group by using enzyme-linked immunosorbent assay by using human ELIZA kits from R and D system USA company.

RESULTS

Demographic characteristics

This is a case-control study involved 73 patients with PCOS obtained from outpatient and private clinics in period between August 2016 and March 2017. These were compared with age matched 73 apparently healthy controls. The basic subject characteristics are shown in Table 1. There was no significant difference in mean age, BMI, or age group distribution between cases and controls. Around half of the patients (54.8%) have an evidence of hyperandrogenism and only three patients (4.1%) have normal menstrual cycle.

Distribution of individuals on different classes of obesity is shown in Figure 1. There was no significant difference between patients and controls regarding grades of obesity ($P > 0.05$).

The levels of cytokines are shown in Table 2. There was highly significant difference in levels of IL-6 and IL-18 between PCOS patients and controls. In addition, TNF α of PCOS patients was significantly higher than the controls (The difference in the number of sample in the table due to negative result so these samples excluded from the study).

Table 1: Demographic characteristics for polycystic ovary syndrome women and apparently healthy control women

	Cases (n=73)	Controls (n=73)	P
Age (years), mean $\pm$ SD	27.7 $\pm$ 5.8	29.4 $\pm$ 5.7	0.082
BMI (kg/m ²), mean $\pm$ SD	27.0 $\pm$ 3.4	26.0 $\pm$ 3.3	0.060
Age groups (years), n (%)			
18-20	5 (6.8)	6 (8.2)	0.274
21-25	24 (32.9)	13 (17.8)	
26-30	17 (23.3)	20 (27.4)	
31-35	18 (24.7)	19 (26.0)	
≥ 36	9 (12.3)	15 (20.5)	
History of infertility, n (%)	50 (68.5)	0	0.0
Oligomenorrhea, n (%)	70 (95.9)	0	0.0
Hyperandrogenism, n (%)	40 (54.8)	0	0.0

SD: Standard deviation, BMI: Body mass index

Table 2: Cytokines levels in cases and controls

Cytokines	Subjects	n	Mean $\pm$ SD	Range	P
IL-6 (ng/ml)	Cases	68	277.5 $\pm$ 292.4	0.0-736.4	<0.01
	Controls	71	5.8 $\pm$ 10.5	0.0-45.3	
IL-18	Cases	71	643.4 $\pm$ 171.8	361.0-997.6	<0.01
	Controls	71	328.0 $\pm$ 147.9	124.0-950.0	
TNF- α	Cases	64	31.9 $\pm$ 67.1	0.0-339.4	0.022
	Controls	60	10.7 $\pm$ 23.8	1.6-161.0	

SD: Standard deviation, TNF- α : Tumor necrosis factor alpha, IL: Interleukin

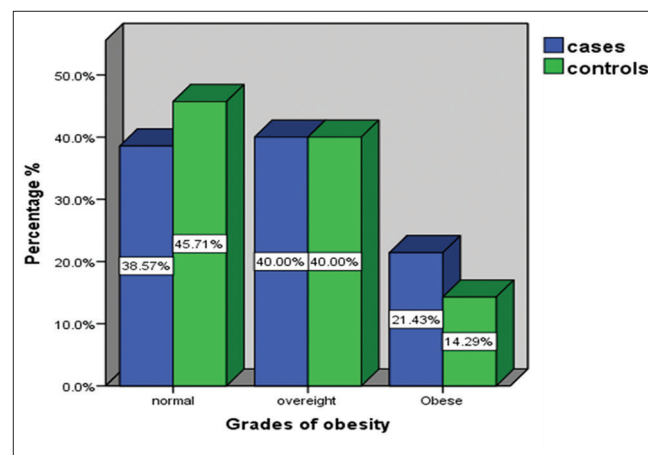


Figure 1: Distribution of individuals on different grades of obesity

Table 3 shows the effect of obesity on cytokines levels in patients with PCOS. There is no significant difference in the level of IL between obese and nonobese PCOS women.

The effect of hyperandrogenism on cytokines levels in PCOS women is shown in Figure 2. There is no difference in IL levels in women with PCOS in the presence or absence of hyperandrogenism.

The effect of infertility on cytokines levels in PCOS women is shown in Figure 3. There is no significant difference in the levels of cytokines in PCOS women in the presence or absence of infertility regardless primary or secondary infertility ($P > 0.05$).

The effect of luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio on cytokines level in PCOS women is shown in Figure 4. Only nine patients with pcos found to have LH/FSH ratio higher than 3. No statistically significant difference was found in the levels of cytokines between PCOS women with LH/FSH ratio higher than three and PCOS women with LH/FSH ratio lower than three.

DISCUSSION

PCOS is one of the most common endocrine disorders in humans. In fact, emphasis on its importance rises from increased prevalence of cardiovascular diseases and higher cardiovascular morbidity even in young and thin women with PCOS; this may be partially due to low-grade inflammation such as is seen in individuals with an excess of visceral/ectopic fat, a feature of most women with PCOS which plays an important role in cardiovascular disorders, in addition to atherosclerosis.^[12]

Evidence of low-grade chronic inflammation in PCOS is indicated by the presence of several elevated markers such as inflammatory cytokines (i.e., IL-6 and IL-18).^[13]

Immune system is affected by estrogen/progesterone ratio.^[14] Patients with PCOS present low progesterone level as a result of oligoovulation or anovulation; therefore, the immune system could be overstimulated by excess estrogen leading to production of autoantibodies in these patients.^[15]

Several mechanisms related to estrogen effects on the immune system, estrogens stimulate the production of IL-4 in Th2 lymphocytes, IL-1 in monocytes, IL-6 in T-lymphocytes and interferon- γ in Th1 cells. The stimulatory effect of estrogens on the immune system could be inhibited by progesterone.^[16]

Table 3: Effect of obesity on cytokines levels in polycystic ovary syndrome women

	Normal (BMI ≤ 25 kg/m ²)	Overweight and obese (BMI > 25 kg/m ²)	P
IL-6 (ng/ml)	254.0 $\pm$ 277.9	292.1 $\pm$ 306.2	0.612
IL-18 (ng/ml)	626.9 $\pm$ 191.4	651.7 $\pm$ 162.0	0.569
TNF- α	36.1 $\pm$ 76.7	31.0 $\pm$ 63.8	0.778

BMI: Body mass index, TNF- α : Tumor necrosis factor alpha, IL: Interleukin

In this study, 73 women with PCOS were involved, the average age was 27.7 ± 5.8 years which is in agreement with Mehde^[17] (27.85 ± 4.23 years) but lower than that obtained by ELMekkawi^[18] (31 ± 5 years) and higher than in studies of both Alteia *et al.* and Agacayak *et al.*^[3,19] (25.8 ± 4.4 years) (mean 26.2 ± 4.0 years).

The similarities between these studies regarding the same age group administration because PCOS appeared at menarche and the females became symptomatic later but most women with

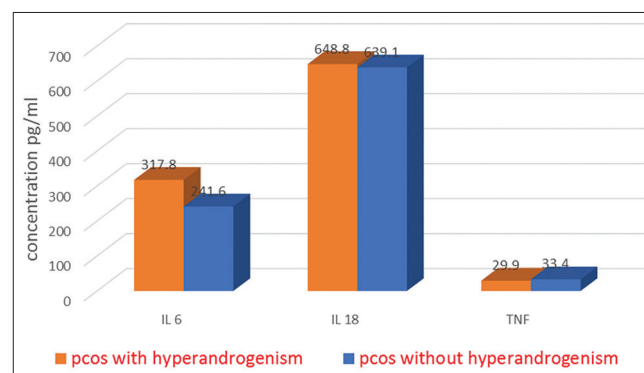


Figure 2: Effect of hyperandrogenism on interleukin 6, interleukin 18, and in polycystic ovary syndrome women

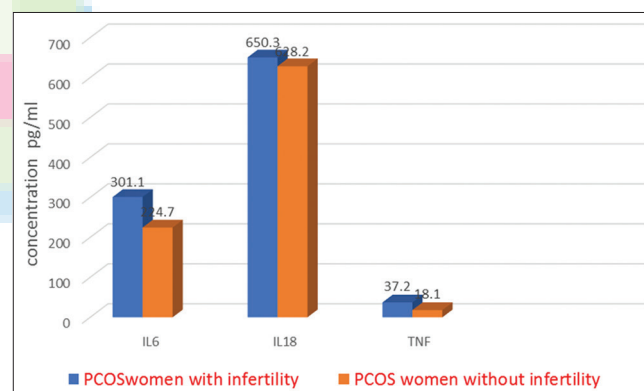


Figure 3: Effect of infertility on cytokines level in polycystic ovary syndrome women

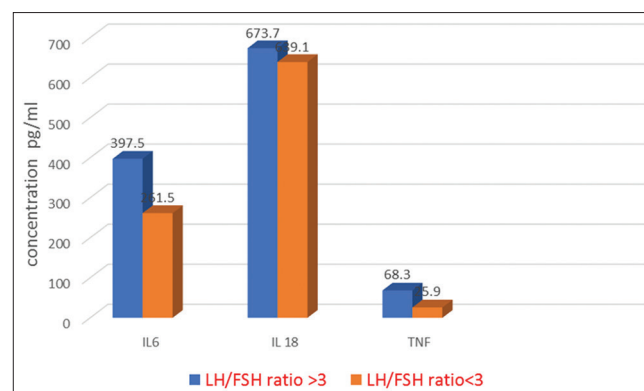


Figure 4: Effect of luteinizing hormone/follicle-stimulating hormone ratio on cytokines levels

polycystic ovarian syndrome are diagnosed when their age between 20 and 30 years.^[20] The mean BMI of PCOS women was within overweight range (27.0 ± 3.4) kg/m² which is in agreement with Alteia *et al.*^[19] and higher than the results obtained by several authors.^[21,3] On the other hand, the BMI of PCOS women in other studies was found to be within obese range (33.14 ± 1.88) (kg/m²).^[17,8] PCOS women had BMI of 31.8 ± 9.2 kg/m². Obesity is a common feature in PCOS ladies; the relation between adiposity with menstrual disturbance and hyperandrogenic status in PCOS is confirmed by data that detect an improvement in these parameters with weight loss.^[22] In this study, 95% of cases had menstrual disturbance, 54% of cases had hyperandrogenism, and 68% of cases had infertility.

Menstrual irregularity might be considered as a marker for IR in PCOS. Oligomenorrhea has been associated with hyperinsulinemia and with increased prevalence and future risk of type II diabetes mellitus.^[23] It is found that PCOS women had infertility rate 66%,^[24] but these studies are based on data collected from fertility clinics and hospitals, and there is no study which determined the natural history of the prevalence of infertility in PCOS, so further study is needed. It was found that the average level of IL 18 is to be significantly higher in PCOS ladies (643.4 ± 171.8 pg/ml) than in control group (328.0 ± 147.9 pg/ml) ($P < 0.01$), and it is the strongest predictor of PCOS among the studied parameters by using stepwise multiregression analysis but the difference in the level of IL18 between lean and obese ladies with PCOS was not significant ($P > 0.05$) and this result agrees with Yang *et al.*'s study and disagrees with other study done by Escobar-Morreale *et al.*^[13] which reported that both polycystic ovarian syndrome women and obesity induced an elevation in serum levels of IL-18; this disagreement may be due to the effect of different demographical factors and inclusion criteria.

There was no statistical difference between PCOS women with or without hyperandrogenism, and the difference in IL18 in PCOS ladies having infertility and those who do not have infertility is not significant. IL 18 has positive correlation with BMI in nonobese PCOS women, while Mehde^[17] and Yang *et al.*^[21] found that this correlation to involve both obese and nonobese women. This difference may be due to the difference in inclusion criteria and inability to exclude all factors that elevate serum IL 18 levels. It is found that IL 18 plays role in the pathogenesis of insulin resistant,^[13] so this may explain the higher level of IL 18 level present in PCOS ladies.

Increment of IL 18 has been postulated to have several deleterious effects. Higher serum IL-18 levels appear to be associated with atherosclerosis.^[25] Serum IL-18 levels are associated with cardiovascular death in patients with ischemic heart disease and with coronary events.^[26]

Increased risk of ischemic heart disease has been reported in PCOS women in comparison with healthy controls.^[27] PCOS is associated with endothelial dysfunction, decreased vascular compliance, and early carotid atherosclerotic changes. It is possible that these findings are due in part to increased

IL-18 levels.^[28] In the present study, it was found that IL6 is significantly higher in PCOS (277.5 ± 292.4 pg/ml) than control (5.8 ± 10.5 pg/ml), and this result was in agreement with other studies that done in Iraq,^[17,19,29] but this result differed with Agacayak *et al.*^[3] who found that there is no significant differences found in the level of IL6 between polycystic ovaries women and healthy normal women. This difference may be due to difference in BMI between this study and Agacayak *et al.*'s study.^[3]

In the present study, there is no statistical significant differences in the levels of IL-6 between obese (292.1 ± 306.2 pg/ml) and non-obese polycystic ovaries women (254.0 ± 277.9 pg/ml) ($P > 0.05$) and no difference in level of IL-6 between PCOS women in existing or lacking of hyperandrogenism. This result was in agreement with Fulghesu *et al.*^[30] who found that serum IL 6 was at higher levels in all polycystic ovaries women with IR independent of BMI. However, this disagreed with Escobar Morreale *et al.*^[8] who found significant difference in the level of IL6 between polycystic ovaries women with obesity and lean polycystic ovaries women and they detected that inflammatory marker is completely dependent on obesity, and low grade level of inflammation is related to excess body fat. These dissimilarities in the finding may be related to difference in the ethnicity and inclusion criteria. It is well recognized that IL 6 has played a significant role in the cardiovascular atherosclerosis, hyperlipidemia, and elevated blood pressure.

Furthermore, it is a strong activator of hepatic-reactive protein so the increased risk of ischemic attack in PCOS women is related to high level of IL6.^[31] The mean TNF alpha level in PCOS women in this study was 31.9 ± 67.1 pg/ml and in normal control group was 10.7 ± 23.8 pg/ml, so there is statistical significant differences between polycystic ovaries ladies and healthy normal women ($P < 0.05$) and this result was in agreement with several authors.^[3,17,32] In the present study, it was found that the difference in the level of TNF alpha between polycystic ovaries obese women and polycystic ovaries lean women is not significant ($P > 0.05$), and this finding was in agreement with Escobar-Morreale *et al.*^[8] who found that the difference in level of TNF alpha between obese polycystic ovaries ladies and lean polycystic ovaries women is not significant and also agreed with Agacayak *et al.*^[3]

TNF alpha is produced by a several cell types such as macrophages, lymphocytes, adipocytes, cardiac muscle, and fibroblasts, and it is produced in response to different stimulus and pathogen that lead to elevate its level,^[33] so the similarity in its level in both obese PCOS women and lean PCOS group may be related to difficulty in excluding all factors that stimulate TNF alpha production in both groups. The elevation of these inflammatory markers (IL6, IL18, and TNF) in polycystic women are signals for alteration of immune function in polycystic women, and this may help in identification of individuals with increased risk of cardiovascular disease and metabolic syndrome and also help in prevention of this disease. This may give a hint on the use of anti inflammatory agent to reduce these markers and prevent complications.

CONCLUSION

The level of IL6, IL18, and TNF alpha were highly statistically significant in PCOS comparing to normal women, and these high levels were related to PCOS independent on the presence of obesity or hyperandrogenism, and there is a positive correlation between IL6, IL18, and TNF alpha and a negative correlation between these markers and obesity and hyperandrogenism.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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