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Serum level evaluation of interleukin-18 in obese women with polycystic ovary syndrome

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Abstract

This study is designed to measure the level of interleukin (IL) 18 in polycystic ovary women and its association with obesity. In this study, blood samples from 50 women with PCOS and 30 healthy control women were collected from AL-Yarmouk Teaching, Baghdad Teaching hospitals During January 2018 - March 2018 for estimation of their serum level of IL18 by using enzyme-linked immunosorbent assay (ELISA) technique and evaluation serum levels of luteinizing hormone (LH), Follicle-stimulating hormone (FSH), Testosterone, prolactin (PRL) and Estradiol (E2) by using Electrochemiluminescence immunoassay (ECLIA). The results showed that there is a highly significant increase ($P < 0.001$) in serum level of IL18 in PCOS women than in healthy control group. As well as, the results of IL-18 value according to Body mass index (BMI) showed significant difference ($P<0.05$) between BMI and IL-18 level in all the PCOS patient subgroups (normal weight, overweight and obesity). Also, there was a significant increase in LH, FSH, PRL and T, and significant decrease in E2 was detected in PCOS patients.

Keywords: IL-18, polycystic ovary syndrome, Obesity

تقييم المستوى للبين ابيضاض ١٨في البدينات المصابات بمتلازمة تعدد الاكياس المبيضية

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الخلاصة

صممت هذه الدراسة لقياس مستوى البين ابيضاض ١٨ في النساء المصابة بمتلازمة تعدد الاكياس المبيضية ومقارنة مستواه مع النساء الطبيعيات ظاهريا وكذلك علاقته بالسمنة. في هذه الدراسة ، تم جمع عينات دم من 50 امرأة مصابة بمتلازمة تعدد الاكياس المبيضية و 30 امرأة طبيعية من مستشفى اليرموك التعليمي ومستشفى بغداد التعليمي خلال الفترة من كانون الثاني 2018 حتى اذار 2018 لقياس المستوى المصلي للانترلوكين ١٨ باستعمال تقنية enzyme-linked immunosorbent assay (ELISA) وقياس المستوى المصلي لهرمون اللوتيني (LH) ، الهرمون المنبه للجريب (FSH) ، التستوستيرون (T) ، البرولاكتين (PRL) و الاسترادول باستخدام تقنية Electrochemiluminescence immunoassay (ECLIA). بينت النتائج أن هناك زيادة معنوية عالية ($P <0.001$) في مستوى مصل الانترلوكين ١٨ في النساء المصابات بمتلازمة تعدد الاكياس المبيضية مقارنة مع النساء الطبيعيات. وكذلك قياس قيمة الانترلوكين ١٨ وفقًا لمؤشر كتلة الجسم (BMI). بينت النتائج وجود اختلاف معنوي ($P <0.05$) في جميع مجاميع المرضى لـمتلازمة تعدد الاكياس المبيضية الفرعية (وزن طبيعي ، زيادة الوزن والسمنة) بالمقارنة مع النساء الطبيعيات . وكما بينت النتائج هناك زيادة في LH، FSH، PRL و T، وانخفاض في E2 لدى النساء المصابات بـمتلازمة تعدد الاكياس المبيضية مقارنة مع النساء الطبيعيات.

Introduction

Polycystic ovary syndrome (PCOS), also known as Stein-Leventhal Syndrome, is one of the most common endocrine disorders in women, which is clinical manifestations of menstrual abnormalities, hair growth, obesity, high blood insulin, and insulin resistance [1]. The PCOS is very complicated and unclear, and many environmental and lifestyle factors greatly contribute to the pathogenesis of it [2-6].

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Inflammatory cytokines may be important factors in the pathogenesis of PCOS. There is convincing evidence describing the influence of low-grade inflammation and cytokines in PCOS. [7-11]. 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 are associated with insulin resistance (IR). Obesity and diabetes mellitus have also been found to be associated with the syndrome [12].

Interleukin-18 (IL-18) is a proinflammatory cytokine that promote the production of tumor necrosis factor alpha (TNF-a), which in turn stimulate the synthesis of IL-6, and IL-6 adjust the synthesis of C-reactive protein (CRP) within the liver [13]. Plasma interleukin (IL)-18 is found to be elevated in obesity and in women with PCOS and in patients with type 2 diabetes [14] [15]. The aim of the study is to measure the level of IL 18 in polycystic ovary women and compare their level with apparently healthy control group, and its association with obesity**.**

Methods:

During January 2018 to March 2018, a total of 50 patients their age between 19 and 45 year with PCOS were selected from AL-Yarmouk teaching and Baghdad Teaching / Baghdad, Iraq. PCOS diagnosis depended on the 2003 Rotterdam ESHRE/ASRM criteria that included; oligoovulation and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries. All alternative etiologies (androgen-secreting tumors, congenital adrenal hyperplasia, Cushing's syndrome) were excluded [16]. Simultaneously, a total of 30 Apparently healthy woman without PCOS were randomly selected from Both hospitals. Controls had regular menstrual cycle with no sign of hyperandrogenism and their age between 19-45 years and subjected to ultrasound examination and have normal hormonal level. The exclusion criteria of controls were those with irregular menstrual periods, malignant tumors, autoimmune diseases and ovarian related diseases.

Blood sampling

A blood sample was collected from each woman of both PCOS and healthy control by using 5ml of disposal syringe in gel-containing tubes, left to clot at room temperature (20-25 ºC) for 10 minutes, then centrifuged at 5000 rpm for 5 minutes to obtain serum. Serum was separated after centrifugation and divided into two Eppendorf tubes to avoid multiple freezing and thawing and kept frozen for further.

Laboratory methods

Serum level of IL-18 was determined by Using Enzyme-linked immunosorbent assay (ELISA). As well as the reproductive hormones (LH, FSH, testosterone, prolactin and E2) associate with PCOS were determined on menstrual cycle day 3 using Electrochemiluminescence immunoassay technique "ECLIA. In both cases, the instructions of manufacturer were followed body mass index (BMI) was calculated according to equation: Body mass index= body weight(kg)/ squared body height (m2).

Statistical analysis

The data were expressed as a mean ± standard error, and independent t-test and ANOVA table were used to express the probability (two-tailed) at the level 0.05 and 0.001 by using the computer program IBM SPSS version 25.0.

Result

Demographic characteristics

The results showed there no significant difference in the mean of age between PCOS group and controls (P > 0.05). While there was a highly significant difference in the mean of BMI between PCOS group and controls (*P<0.001*), as shown in Table-1

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

Parameters	PCOS patients (n = 50)	Controls (n = 30)	Probability
Age (years)	27.9±0.89	26.3±1.39	P > 0.05
BMI (kg/m^2)	29.48±0.76	24.12±0.88	P < 0.001
History of infertility, *n* (%)	40(80%)	0	0
Oligomenorrhea, *n* (%)	47(94%)	0	0
hyperandrogenism, *n* (%)	35(70%)	0	0

Results described as means ±SE

Hormonal assay

In comparison Between control and PCOS patients, the results showed PCOS patients have significantly increased levels of LH, FSH, Testosterone, Prolactin, whereas significantly decreased level of E2, as shown in Table-2.

Table 2-Levels of reproductive hormones in blood samples PCOS women and healthy controls

Parameters	PCOS patients (n = 50)	Controls (n = 30)	Probability
LH (mIU/ml)	8.32±0.45	6.18±0.44	P < 0.05
FSH (mIU/ml)	5.80±0.35	5.11±0.26	P < 0.05
T (ng/ml)	0.54±0.13	0.05±0.01	P < 0.001
PRL (ng/ml)	18.25±2.81	14.23±1.35	P < 0.05
E2 (pg/mL)	81.53±8.79	93.49±6.69	P < 0.05

Results described as means ±SE

Serum level of IL-18

The results in Table-3 showed there was high significant increases in the level of IL 18 (P < 0.001) in PCOS patients compared to control.

Table 3-Interleukin-18 level of PCOS patients group compared to controls

Groups	No.	IL-18 level (pg/ml) (Mean±SE)	Probability
PCOS	50	609.73±26.58	P < 0.001
Control	30	477.67±10.25	

IL-18 value according to BMI in studying group

Table-4 showed the relationship between BMI and IL-18 level in PCOS patient's and control groups. There was a significant difference (P<0.05) between BMI and IL-18 level in all the PCOS patient subgroups (normal weight, overweight and obesity) in comparison with control group.

Table 4-Interleukin-18 concentration of PCOS patients group compared to controls according to BMI groups

Groups	PCOS	No.	Control	No.	Probability
Underweight (< 18.5 Kg/m^2)	-	0	406.33	1	-
Normal weight (18.5 – 24.9 Kg/m^2)	573.92±21.79	12	499.01±13.53	17	P < 0.05
Overweight (25 – 29.9 Kg/m^2)	586.69±30.93	16	474.11±21.99	9	P < 0.05
Obesity (≥30 Kg/m^2)	668.28±69.04	22	446.46±15.48	3	P < 0.001
Total	609.73±26.58	50	477.67±10.25	30	P < 0.001

Discussion

IL-18 was previously known as an IFN-γ inducing factor, has many functions that include the synthesis of IFN-γ by T and NK cells, stimulation of Th1-type immune response, increasing the proliferative response and cytokine production by the activated T cells. In the meantime, IL-18 leads to many activities against the pathogens through activating the effector cells that involved in the cellular interactions during the inflammation [17, 18].

In this study, 50 women with PCOS were involved, the average age was 27.9±0.89 years which is in agreement with Mehde [19] (27.13 ± 4.22 years) but lower than that obtained by ELMekkawi [20] (31 ± 5 years) and higher than in studies of both Alteia *et al*. (25.8 ± 4.4 years) [21] and Agacayak *et al*. (26.2 ± 4.0 years) [22]. 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 polycystic ovarian syndrome are diagnosed when their age between 20 and 30 years [23].

In the present study, higher level of BMI was encountered in PCOS patients. This result disagreed with Al-Musawy *et al*. [24] who reported that no significant difference in patient group BMI compared to control group. In addition, this result agreed with Nory and Abadi [25], Mohammed *et al*. [26] and Wang [27] who they reported that the women with PCOS have a high BMI than a healthy control woman. Obesity is a common feature in women with PCOS; 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 [28]. In this study, 94% of cases had menstrual disturbance, 70% of cases had hyperandrogenism, and 80% of cases had infertility. Menstrual irregularity might be considered as a marker for IR in PCOS. Oligomenorrhea has been related with hyperinsulinemia and with expanded predominance and future danger of type II diabetes mellitus [29].
The current study found that the average level of IL 18 is significantly higher in PCOS ladies (609.73±26.58 pg/ml) than in the control group (477.67±10.25pg/ml) ($P < 0.001$). This result agreed with Косей *et al*. [30] and Dawood *et al*. [31] reported the serum IL-18 increase in PCOS group. The same findings in Iraqi population reported by Al-Musawy *et al*. [24] found that IL-18 plays role in the pathogenesis of insulin resistant. Thus, this might be explained the increasing level of IL-18 in PCOS female patients [32,19]. Kretowski et al. [33] expected that the increased level of IL-18 in PCOS might be due to the genetic polymorphism in IL-18 encoding gene which might be related to PCOS, obesity, and insulin resistance.

The present study shows a significant difference ($P<0.05$) between BMI and IL-18 level in all PCOS patient's subgroups (normal, overweight and obesity) in comparison with control subgroups (573.92±21.79 *vs.* 499.01±13.53 Kg/m^2, 586.69±30.93 *vs.* 474.11±21.99 Kg/m^2 and 668.28±69.04 vs. 446.46±15.48 Kg/m^2, respectively). This results agreed with Escobar-Morreale *et al*. [34] and Dawood *et al*. [31] who reported that both polycystic ovarian syndrome women and obesity induced an elevation in serum levels of IL-18 , but disagrees with Yang *et al*. [32] and Al-Musawy *et al.* [24]
Increase IL-18 in PCOS patients was directly correlated with obesity through an important role in the development of insulin resistant and increasing the cardiovascular mortality by some mechanisms including low-grade inflammation and secretion of inflammatory cytokines (32,31]
Another study suggested that IL-18 considered as an adipogenic cytokine associated with the increasing of adiposity. Also, the adipocytes in the obese individuals produce highly levels of IL-18 in comparison with lean individuals. The increased level of circulating IL-18 was noticed in obese individuals and also in those with a high BMI value [35].

Conclusion

The current findings showed a significantly increased level of IL-18 in PCOS group. Also, there was a positive correlation between the serum level of IL-18 and the obesity. Whereas, patients with PCOS were more likely to have significantly increased levels of LH, FSH, T, PRL, and significantly decreased level of E2 in comparison with controls.

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