

# Association between Hormonal Imbalance and Interleulin-10 Level with Polycystic Ovarian Syndrome of Iraqi Women

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## Abstract

**Objectives:** To determine the level of interleukin-10 as inflammatory marker in PCOS patients and compared with healthy control and explore the correlation between IL-10 level and other biochemical markers in PCOS patients.

**Methods:** Whole blood samples of 80 PCOS obese patients and 80 healthy people were collected in duration from Dec., 2021 to April, 2022, at the gynecological and obstetric teaching hospital, Kerbala health directorate, Iraq. The Rotterdam criteria-2003 was accepted to PCOS females with age range between (18–40 years), while the apparently control group with age ranged between (18–40 years). BMI and WHR and hormonal status (LH, FSH, LH/FSH ratio, Prolactin, Free Testosterone) were determined. Elabscience/USA ELISA kit uses the Sandwich-ELISA principle was used to determined IL-10 level in serum.

**Results:** The obese PCOS women had a seriously decrease in IL-10 (pg/ml) level when compare with apparently healthy control group with mean  $\pm$  SD for patients ( $2.192 \pm 0.47$ ) and for control group ( $4.532 \pm 0.75$ )  $P$  value  $< 0.0001$ , and there are significant negative correlations between IL-10 and (BMI  $P < 0.0001$ ,  $r = -0.66$ ), (WHR  $P < 0.0001$ ,  $r = -0.66$ ), (LH  $P < 0.0001$ ,  $r = -0.63$ ), (LH: FSH ratio  $P < 0.0001$ ,  $r = -0.72$ ), (Prolactin  $P = 0.008$ ,  $r = -0.29$ ).

**Conclusion:** Our findings indicate that the IL-10 level decrease in PCOS obese patients and has significant association with pathogenesis and progression of disease.

**Keywords:** Interleukin-10, polycystic ovary syndrome, Iraq

## Introduction

Low-grade inflammation and inflammatory indicators have been linked to PCOS. Understanding the pathophysiology of PCOS and its therapy by inhibition or regulation of associated pathways can be improved by looking into the inflammatory mediators involved for its development. Because ovulation is a semi-inflammatory process, visceral adipose tissue can induce an inflammatory response and sustain the inflammation in adipocytes by influencing the release of inflammatory cytokines. Out of this regulated inflammation, PCOS can arise.<sup>1</sup> Interleukin-10 (IL-10), which was first discovered in 1991, is an immunosuppressant and an anti-inflammatory cytokine that is essential for the body's defense processes.<sup>2,3</sup> The IL-10 protein is a homodimer, and each of its components is 178 amino acids long.<sup>4</sup> IL-10 is a T-helper cell family member (TH2) that inhibits the function of TH1 cells.<sup>5</sup> Activated immune cells release interleukin-10, an anti-inflammatory cytokine. Monocytes are capable of producing IL-10 when they are stimulated.<sup>6</sup> as well as non-immune cells like epithelial or neuronal cells.<sup>7</sup> The trans-membrane receptor complex made up of IL-10R1 and IL-10R2 is how IL-10 exerts its effects and regulates the actions of lymphocytes, macrophages, and a variety of other cells.<sup>8</sup> It regulates the differentiation and proliferation of immune cells, such as macrophages, T cells, and B cells; reduces monocyte activation; and restricts the release of pro-inflammatory cytokines, such as TNF, IL-1, IL-6, IL-12, and IL-2.<sup>9</sup> To keep the ovary functioning properly, the levels of inflammatory markers must be in equilibrium. Alterations in

steroidogenesis, delayed follicular maturation, and ovarian issues might result from an imbalance between pro-inflammatory and anti-inflammatory cytokines caused by increased production of TNF- $\alpha$  and IL-6 and decreased production of IL-10. It is thought that since it lowers TH1 cell activity, progesterone is produced and the corpus luteum matures, maintaining pregnancy. Obesity and the metabolic syndrome are linked to decreased IL-10 levels. The fact that PCOS patients had lower plasma levels of IL-10 suggests that clomiphene citrate increases IL-10 and helps PCOS women become pregnant and ovulate more frequently.<sup>10</sup>

Therefore, this study aimed to determine the level of interleukin-10 as inflammatory marker in PCOS patients and compared with healthy control and explores the correlation between IL-10 level and other biochemical markers in PCOS patients of Iraqi women in Kerbala province.

## Materials and Methods

This study is a case-control study involves 80 PCOS patients. and 80 non-PCOS women as a control in childbearing age at the Reproductive Fertility consultant of gynecological and obstetric teaching hospital, Kerbala health directorate Iraq and Iraq's University of Kerbala College of Medicine during the duration from Dec., 2021 to April, 2022. An exhaustive interview gathering personal and family history, blood pressure, demographic information and laboratory examination was carried out. The Rotterdam criteria-2003 was presumed to 80 PCOS females with ages ranged between (18–40) years.

Patients with any 2 of the next 3 items can be recognized in diagnosis: oligomenorrhea or amenorrhea, increase androgen levels, ovarian volume >10 mL on U/S, and follicles  $\geq 12$  with diameter 2–9 mm.<sup>11</sup> Controller group has 80 ladies which ages reached between (18–40 years). They have regular menstruation, with normal ovaries as they were detected by the gynecologist. Body mass index were calculated from the following equation: BMI = Weight (kg)/Height (m<sup>2</sup>). Normal BMI level is (20–24.9) kg/m<sup>2</sup> and (25–29.9) kg/m<sup>2</sup> for overweight. When BMI  $\geq 30$  kg/m<sup>2</sup>, the woman is considered as obese.<sup>12</sup> The WHR diagnostic standard for obesity is 0.85 for women.<sup>13</sup> The volume withdrawn from each patient was 3.0 mL was used for serum separation and used for hormonal assays. The hormonal levels of each of LH, FSH and Prolactin were measured by the chemiluminescent automated immunoassay system (Cobas e411, Roche diagnostic, Germany). Free testosterone level was measured by Competitive Enzyme Immunoassay using Monobind/USA ELISA kit and Elabscience/USA ELISA kit uses the Sandwich-ELISA principle was used to determined IL-10 level in serum. The protocol for study was certified by the ethical research commission of College of Medicine, University of Kerbala and Kerbala Health Directorate. Approval also taken from administration of gynecological and obstetric teaching hospital and from each patient after explaining the nature and purpose of study. All statistical analyses were performed with the graph pad Prism 9.0.0 was released on October 28, 2020. Data were analyzed by *T*-test in statistical analysis the highly significant value is ( $P < 0.01$ ) and the significant value is ( $P < 0.05$ ). The data are presented as mean  $\pm$  SD (standard deviation). The correlation coefficient Spearman *r* test was calculated to examine association among parameters.

## Results

Based on inclusion and exclusion criteria, 160 women were involved in the last data analysis, the women involved within the study finally were with an age ranged between (18–40) years and the mean  $\pm$  SD of them were  $26.1 \pm 5.3$  years. The results of this study were displayed in Table 1. They incorporate the mean  $\pm$  SD of the patients with and without hirsutism and those with primary or secondary infertility and (regular or irregular) menstruation pattern. It is clear that the two groups are almost well matched, thus obtained results could be estimable. The results of this study were displayed in Table 2 using statistical unpaired *T*-test; age, BMI and WHR as well as using statistical Mann Whitney test. Significant elevations in LH concentrations ( $P < 0.0001$ ), LH/FSH ratio ( $P < 0.0001$ ), free testosterone levels ( $P < 0.0001$ ) and prolactin level ( $P < 0.0001$ ) were prevailed in the PCOS patients group when contrasted with the control group. However, while significantly decrease in FSH level ( $P = 0.01$ ) and IL-10 level ( $P < 0.0001$ ) during a comparable evaluation between PCOS patients and control group.

Interleukin – 10 (IL-10) correlation with anthropometric and biochemical parameters in PCOS patients group was evaluated by Spearman *r* test are showed in Figure 1 and Table 3. The result showed that there are significant negative correlations between IL-10 and (BMI  $P < 0.0001$ ,  $r = -0.66$ ), (WHR  $P < 0.0001$ ,  $r = -0.66$ ), (LH  $P < 0.0001$ ,  $r = -0.63$ ), (LH: FSH ratio  $P < 0.0001$ ,  $r = -0.72$ ), (Prolactin  $P = 0.008$ ,  $r = -0.29$ ), and there are non-significant correlations with age and FSH revealed with IL-10 cytokine.

Table 1. Demographic parameters of the registered patients and the control

Demographic parameters	Control <i>N</i> = 80 Mean $\pm$ SD	Patients <i>N</i> = 80 Mean $\pm$ SD
Menstruation Pattern (regular)	80	17
Menstruation Pattern (Irregular)	–	63
With Hirsutism	–	67
Without Hirsutism	80	13
Primary infertility	–	52
Secondary infertility	–	28

Table 2. Biochemical parameters of the registered patients and the control

Biochemical parameters	Control <i>N</i> = 80 Mean $\pm$ SD	Patients <i>N</i> = 80 Mean $\pm$ SD	<i>P</i> -value
Age, year	$26.8 \pm 5.175$	$26.1 \pm 5.3$	0.15
BMI (kg/m <sup>2</sup> )	$23.3 \pm 1.156$	$32.5 \pm 6.357$	<0.0001
WHR	$0.777 \pm 0.0143$	$0.912 \pm 0.0563$	<0.0001
LH (m.iu/ml)	$106 \pm 0.555$	$11.89 \pm 3.188$	<0.0001
FSH (m.iu/ml)	$6.73 \pm 0.65$	$5.36 \pm 1.36$	0.01
LH/FSH ratio	$0.986 \pm 0.041$	$2.414 \pm 0.379$	<0.0001
Free testosterone (pg/ml)	$2.97 \pm 1.812$	$18.7 \pm 14.98$	<0.0001
Prolactin (ng/ml)	$12.19 \pm 2.92$	$16.14 \pm 4.05$	<0.0001
IL-10 (pg/ml)	$4.53 \pm 0.75$	$2.19 \pm 0.47$	<0.0001

## Discussion

The pathogenesis and progression of polycystic ovarian syndrome have been implicated in chronic low-grade inflammation as a major factor (PCOS).<sup>1</sup> IL-10 levels were lower in PCOS patients than in controls. Th1 cells and macrophages' expression of pro-inflammatory cytokines is down-regulated by the anti-inflammatory cytokine and suppressor IL-10. alters steroidogenesis, delays follicular development, and causes ovarian dysfunction due to an imbalance between pro- and anti-inflammatory cytokines.<sup>14</sup> The main characteristics of the metabolic abnormalities typical of PCOS include insulin resistance and hyperinsulinemia. Although there is mounting evidence that TNF $\alpha$ , IL-6, and IL-10 are critical players in mediating insulin resistance, low IL-10 levels have been linked to obesity and the metabolic syndrome.<sup>15</sup> Subeka Abraham Gnanadass' research demonstrated a connection between metabolic syndrome and obesity and low IL-10 levels. Patients with PCOS experienced a decrease in plasma IL-10.<sup>10</sup> Interleukin (IL)-10, which M. Karadeniz demonstrated in his work, is a significant anti-inflammatory cytokine that has been linked to obesity and type 2 diabetes and that controls the production of these pro-inflammatory cytokines. Patients with high BMI and insulin levels have been found to have low levels of IL-10.<sup>16</sup> In the past ten years, a lot of study has concentrated on the immunosuppressive and anti-inflammatory effects that are mediated by a variety of variables, including the anti-inflammatory cytokine interleukin (IL)-10. The pathogenesis

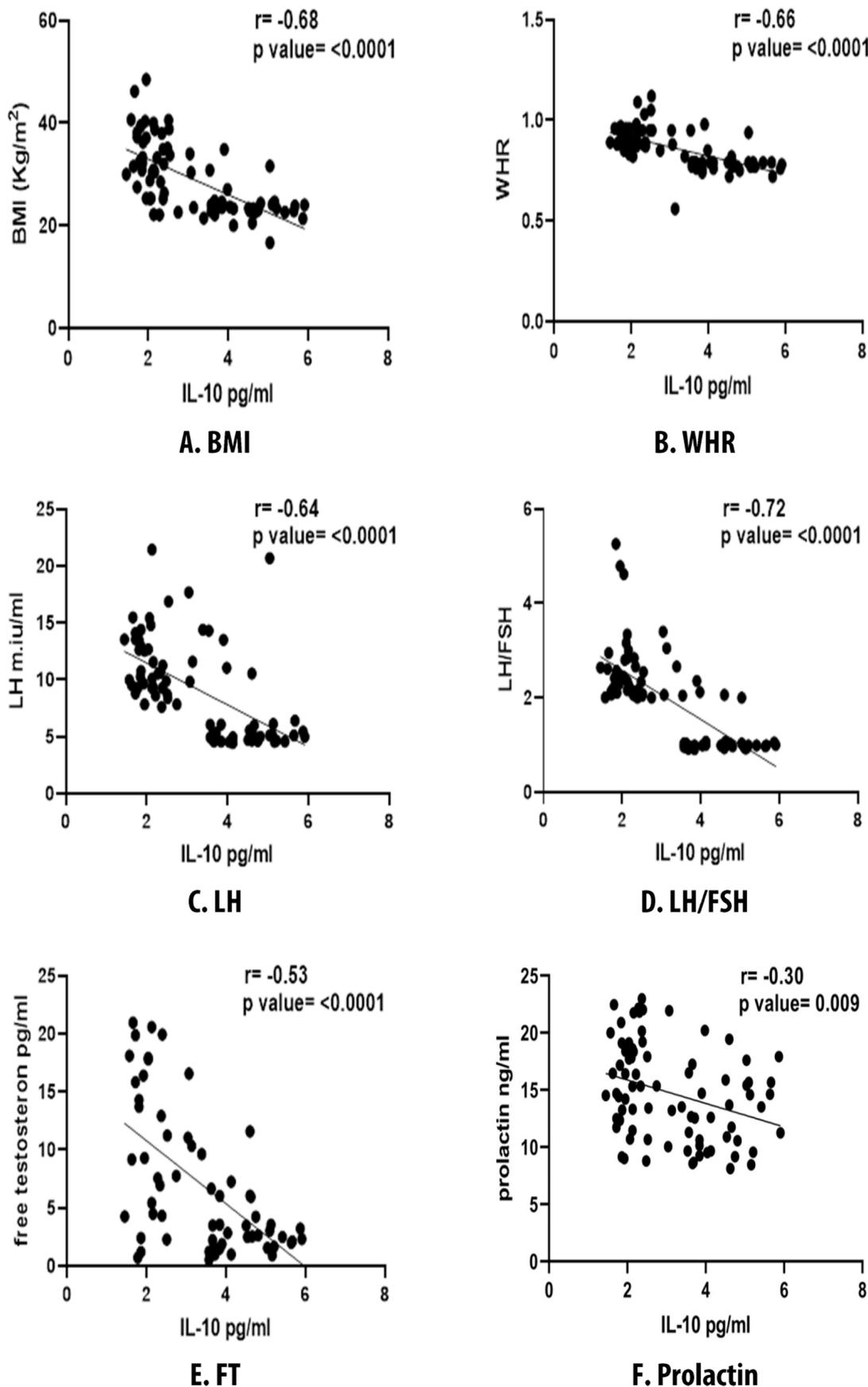


Fig. 1 Correlation between IL-10 cytokine level and A. BMI, B. WHR, C. LH, D. LH: FSH ratio, E. Free Testosterone hormone and F. Prolactin hormone level in PCOS patients group.

Table 3. Correlation of IL-10 with anthropometric and biochemical parameters in registered PCOS patients group

Parameters	R	P-value
Age (Y)	0.13	NS
BMI (kg/m <sup>2</sup> )	-0.68	<0.0001
WHR	-0.66	<0.0001
LH (m.iu/ml)	-0.63	<0.0001
FSH (m.iu/ml)	0.13	NS
LH : FSH	-0.72	<0.0001
Prolactin (ng/ml)	-0.29	0.008
Free Testosterone (pg/ml)	-0.53	<0.0001

of PCOS is firmly implicated as being driven by chronic low-grade inflammation. In several diseases, including PCOS, IL-10 has an anti-inflammatory and immune-suppressive effect. Recent studies showed that women with PCOS had considerably reduced serum levels of IL-10.<sup>17-19</sup> In his research, Angel Mercy Sylus also demonstrated that inflammation is frequently linked to PCOS, which affects these women's ovarian folliculogenesis, abnormal steroidogenesis in the ovary, and hyperinsulinemia. An anti-inflammatory cytokine called interleukin-10 (IL-10) controls how pro-inflammatory cytokines behave when there is inflammation. Reduced IL-10 levels have been observed in women with PCOS and IL-10 gene variation has been associated with PCOS.<sup>20</sup> This study showed negative correlation between IL-10 cytokine level and BMI, WHR, LH, LH/FSH ratio, prolactin hormone and Free Testosterone. While there is no correlation with age or FSH level. In his work, Po-Kai Yang demonstrated that obesity has been suggested to have a modulatory effect on PCOS patients' ovulatory functioning. Through increased IL-10 synthesis in visceral fats, obesity may interfere with normal folliculogenesis.<sup>21</sup> In PCOS compared to controls, IL-10 concentration was decreased. With respect to WHR, which measures visceral adiposity, there were significant relationships.<sup>14</sup> In PCOS patients, a substantial positive link between the ratio of T regulatory cells and LH levels was found, however other hormones like FSH will not impact the ratio of T regulatory cells. T regulatory cells release anti-inflammatory cytokines like

interleukin 10 (IL-10). In his research, Yiqing Yang discovered that PCOS patients had lower levels of the cytokine IL-10 associated to T regulatory cells.<sup>21</sup> According to Umit Cabus' study, serum IL-10 levels were greater in study participants than in controls, and the LH/FSH ratio was noticeably higher in PCOS-affected women.<sup>22</sup> When compared to controls, PCOS patients' mean plasma concentration of IL10 was significantly lower, and it had no effect on the levels of FSH, LH, or PRL.<sup>23</sup> Hyperinsulinemia stimulates the pituitary's reaction to gonadotropin-releasing hormone (GnRH), which increases the release of luteinizing hormone and androgen. This influences how well the hypothalamus-pituitary-ovarian gonadal axis works (HPO axis). Hyperandrogenism may prevent the growth of follicles, cause follicular atresia, and encourage insulin resistance as a result of feedback. The pathogenesis of PCOS is associated with elevated pro-inflammatory cytokines and decreased anti-inflammatory (IL-10) factors, and this inflammatory condition may harm insulin sensitivity and advance the onset of PCOS.<sup>24</sup>

## Conclusion

The observed results indicated that there is decrease in the mean of Interleukin 10 cytokine in PCOS patients. Various hormones which are free testosterone, Prolactin and LH in obese PCOS women are increase as compared with control, while there is significant decrease in FSH values was obtained. The result also showed that there are significant negative correlations between IL-10 and BMI, WHR, LH, LH: FSH ratio, Prolactin, and there are non-significant correlations with age and FSH revealed with IL-10 cytokine.

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## Conflict of Interest

The authors advertise that they have no conflict of interest. ■

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