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ORIGINAL RESEARCH **PERIOPERATIVE MEDICINE**

Association between interleukin-39 (IL-39) with hormonal and metabolic changes in women with polycystic ovarian syndrome

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ABSTRACT

Background & Objective: The most prevalent endocrine defect affecting women in their reproductive years is called polycystic ovarian syndrome (PCOS). It is strongly correlated to metabolic syndrome, insulin resistance, and an increased risk of diabetes and cardiovascular disease in the future. The heterodimer of interleukin-39 (IL-39) is 54 kDa. The IL-12 family subunits p19 and Ebi3 have been found to create a novel complex known as IL-39 (p19/Ebi3), according to the researchers. This study aimed to assess additional hormonal and metabolic abnormalities as well as the blood level of IL-39 in patients with PCOS.

Methodology: The study employed 180 samples of Iraqi women in the 20–40 age range. Ninety newly diagnosed PCOS patients and ninety healthy, fertile women made up the age-matched case-control research group. The groups were gathered between December 2023 and March 2024 from private laboratories and the Al-Sadr Teaching Hospital, Al-Hakeem Hospital, and Al-Zahraa Hospital in the Najaf Governorate. An enzyme-linked immunosorbent assay (ELISA) was used to measure each subject's IL-39 level and calculate their hirsutism scores. The relevant statistical methods were applied to analyze the data.

Results: The BMI, LH, LH/FSH ratio, TT, FAI, FIN, FSG, and HOMA-I R values of the PCOS patient women were considerably greater than those of the healthy women group. The difference in the blood level of IL-39 between the PCOS women and the control group was statistically significant ($P < 0.0001$). Furthermore, a noteworthy inverse relationship was noted between IL-39 and an IL-39 cut-off value (ng/mL), yielding 82.2% sensitivity and 80.4% specificity; (AUC: $P < 0.0001$; 95% CI: 0.848–0.965).

Conclusion: A lower level of IL-39 may contribute to the etiology of PCOS patients. Further research is necessary to fully comprehend the pathophysiology and clinical significance of the IL-39 system in PCOS. According to the study's findings, compared to healthy, fertile control subjects, women with PCOS had lower serum IL-39 levels.

Abbreviations: TT - Total testosterone; FT - free testosterone; FAI TC - Free androgen index; total cholesterol; TG - Triglycerides; "LDL-C" - low-density lipoprotein; "HDL-C" - high-density lipoprotein and cholesterol; PTX-3 - Pentraxin-3; WHR - Waist-to-hip ratio; BMI - Body mass index; FSG - Fasting serum glucose; HOMA-IR HOMA%S - Insulin Resistance Homeostatic Model Assessment, or: Insulin sensitivity HOMA, LH - Luteinizing hormone; FSH - Follicle stimulating hormone; IL-39 - interleukin-39.

Key words: IL-39, insulin resistance, obesity, polycystic ovarian syndrome; LH, FSH and LH/FSH.

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1. INTRODUCTION

A prevalent endocrine disorder affecting up to 15% of women of reproductive age is polycystic ovarian syndrome. Notably, in recent decades, there has been a discernible increase in the prevalence of PCOS along with the rising incidence of obesity. The cause of PCOS is still unknown, in spite of the condition's high frequency and expensive nature.[1] PCOS is the most common endocrinopathy that affects women who are sexually active. It is a complicated and diverse disorder. It is characterized by an overabundance of testosterone, prolonged anovulation, and a shift in the cardiometabolic profile.

Women with PCOS are more likely to have insulin resistance (IR), hyperinsulinemia, central obesity, nonalcoholic fatty liver disease (NAFLD), and type 2 diabetes mellitus (T2DM) as compared to women without PCOS who are matched for age and body mass index (BMI). Adipose tissue (AT) physiology impairment has been linked to PCOS. It seems that hyperandrogenemia is the primary source of the adipocyte hypertrophy linked to PCOS that is brought on by hyperinsulinemia. Changes in the activity of adipocytes impact the release of proinflammatory compounds called adipokines, which are obtained from adipose tissue and raise the possibility of low-grade inflammation.[2]

PCOS impacts women's health at every stage of life, beginning before conception and continuing into the postmenopausal years. These disorders include diabetes mellitus, obesity, dyslipidemia, hypertension, anxiety, and depression. The life cycle of women affected by PCOS is shown by the circle. Within the squares are the details and interactions of several relevant moderating factors, from the perinatal period to adolescence and maturity.[3]

Whether the symptoms are oligomenorrhea (65–87% had demonstrable polycystic ovaries on ultrasound), hirsutism (60–92%), acne (83%), or acne (45% in women with acne as a sole symptom), the characteristic ultrasound features of the polycystic ovary are easily the most common detectable sign associated with any of the typical symptoms.

The clinical and endocrinological aspects of the syndrome are known to be heterogeneous and inconsistent; thus, it makes sense to include an ultrasound examination as a fundamental component of the diagnostic process. This procedure is commonly performed in the fertile age range.[4] To identify polycystic ovarian changes using ultrasound, it was necessary for at least one ovary to have an increase in ovarian volume of more than 10 cm3, or for at least 12 follicles to form in ovaries with diameters between 2 and 9 mm. One of the fundamental features of PCOS ultrasound is the arrangement of follicles around the ovary's border, which resembles a "string of pearls.[5]

Antral and pre-antral follicle cell numbers are higher in PCOS patients, as multiple studies have shown. Additionally, individuals develop ovarian cysts because of an increase in abnormally formed follicles caused by a decrease in apoptotic activity in mature follicular cells.[6,7,8]

The heterodimer of IL-39 is 54 kDa. The IL-12 family subunits p19 and Ebi3 have been found to create a novel complex called IL-39 (p19/Ebi3),[9,10] the researchers discovered that Ebi3 and p19 join to form a complex in several cell subsets.[11,12]

An equilibrium in the levels of inflammatory markers is necessary to maintain optimal ovarian function. A mismatch in pro- and anti-inflammatory cytokines may lead to changes in steroidogenesis, delayed follicular maturation, and ovarian problems.[13]

The researchers verified that p19 and Ebi3 are co-expressed in the mouse RAW 264.7 macrophage cell line and dendritic cells (DC) from the bone marrow. The supernatant of cultivated RAW 264.7 macrophage cells contains the natural IL-39 (p19/Ebi3) complex. It was demonstrated that B lymphocytes activated by lipopolysaccharide (LPS) release a naturally occurring form of IL-39.[14] in Figure 1.

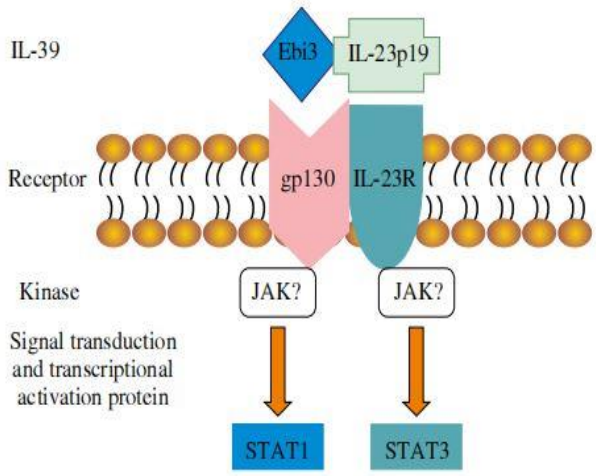

Figure 1: IL-39 and the signaling pathway. The Ebi3 and IL-23p19 subunits make up IL-39, a newly discovered member of the IL-12 family. It binds to the

gp130/IL-23R receptor and phosphorylates STAT1 and STAT3[14]

Interleukins are essential for both innate and adaptive responses in mammals. When cytokines that are normally produced by leukocytes and have a specific effect on leukocytes were initially described, the term "interleukin" was used to describe them. The sequencing of the human and mouse genomes has increased the number of known interleukins. However, the nomenclature of several of the more recently discovered interleukins are not clear and need to be confirmed. Although the majority of cloned non-mammalian interleukins have been identified in avian species, isolated IL-1 genes are now available for most vertebrate taxa.[15]

2. METHODOLOGY

Ninety women between the ages of twenty and forty who had received a PCOS diagnosis within the last year were included in this case-control research using the Rotterdam ESHRE/ASRM 2003 criteria. The study was carried out in the AL-Zahra teaching hospital for obstetrics and gynecology and the fertility center at AL-Sader medical city in Najaf, Iraq, between December 2023 and March 2024.

Ninety-nine volunteer women without PCOS who appeared healthy were compared to an age-matched control group of women with PCOS. The study was approved by the Najaf Health Directorate of the Hospital Administration for Obstetrics and Gynecology and the Ethics Committee of the University of Kufa's Faculty of Science. Every individual signed an informed consent form. Patients with any form of chronic illness, including those with impaired ovarian reserve, hypertension, dyslipidemia, cardiovascular diseases, thyroid problems, Cushing's syndrome, androgen-secreting tumors, were not allowed to participate in this study, deficits in enzymes, particularly 21-hydroxylase, and high blood pressure. An anthropometric measurement known as body mass index (BMI) is calculated. On cycle day 2, between 8 and 9 a.m., 5 mL samples of venous blood were taken following a 12-hour fast. After that, the serum was divided and stored at -20°C until analysis was completed.

Using colorimetric techniques and commercial test kits, serum glucose (FSG) and lipid profile (total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) levels) were assessed during the fasting examination. The amount of serum free testosterone was determined using ELISA kits (Monobind, USA). Sex hormone binding globulin (SHBG), IL-39, and fasting insulin (FINS) were measured using ELISA kits (ELabscience/USA). Total testosterone (TT), follicle stimulating hormone (FSH), and luteinizing hormone (LH) were measured using the immune-fluorescence method (Minividas, Biomerieux, France). Insulin resistance was calculated using the homeostatic model assessment (HOMA-IR), which was derived using a typical computation as follows. Weir and Jan (2019) state that the formula for calculating HOMA-IR is fasting insulin (IU/L) + fasting glucose (mmol/L) /22.5, with a cutoff value of > 2.5.In addition, the free androgens index (FAI) was calculated using the traditional technique FAI = total testosterone (TT)/SHBG × 100.[16]

2.1. Statistical analysis

For statistical analysis, SPSS software (version 25.0, SPSS Inc., Chicago, IL, USA) was utilized. The standard deviation and mean of each result were recorded. An unpaired student t-test was performed to determine the statistical significance of the study's groups, and t-tests were employed to compare two independent samples. In order to compare the parameters among women with PCOS, the relationship between the variables was investigated using Pearson's correlation analysis. The statistical significance was denoted by a P-value of 0.005.

2.2. Ethical approval

The project received ethical approval from Kufa University's Kufa College of Science (license number 4000 dated January 28, 2024). After each patient was told about the nature and objectives of the study, participants underwent a medical examination by a specialized physician to check for any indications or symptoms of PCOS.

3. RESULTS

Table 1 presents the baseline characteristics of the study groups. Ninety people with PCOS made up the 180 samples; ninety seemingly healthy women served as the control group. There is no discernible variation in the age variables between the groups under study. WHR and BMI were considerably greater in the patient group compared to the control group.

Table 2 compares the biochemical parameters of the research groups. The levels of prolactin ($P < 0.0001$), free testosterone ($P < 0.0001$), LH ($P < 0.0001$), and the LH/FSH ratio ($P < 0.0001$) were significantly higher in the PCOS patient group than in the healthy women. Comparable testing revealed a substantially lower level of FSH ($P = 0.01$) in women with PCOS compared to healthy women.

Table 1: Biochemical and demographic characteristics of the participating women's groups

Variables	PCOS Group	Healthy Group	P-value
Age (y)	30.21 ± 5.67	30.34 ± 7.16	0.325
BMI (kg/m²)	29.66 ± 1.19	23.62 ± 1.04	0.002
BMI :18.9-24.9	10 (12)	90 (100)	
BMI: 25-29.9	35 (38)	-	
BMI: ≥ 30	45 (50)	-	
WHR	1.08 ± 0.06	0.74 ± 0.05	0.001
With hirsutism	54 (60)	-	-
Without hirsutism	36 (40)	-	-
Primary infertility	60 (67)	-	-
Secondary infertility	30 (33)	-	-
Irregular cycle	68 (76)	-	-
Regular cycle	22 (24)	-	-

The information displayed as mean ± SD. TT - Total testosterone and free testosterone (FT) Free androgen index, or FAI TC stands for total cholesterol; TG - Triglycerides, "LDL-C" and "HDL-C" refer to low-density and high-density lipoprotein and cholesterol, respectively. Pentraxin-3, or PTX-3 SD stands for standard deviation; WHR - Waist-to-hip ratio; BMI - Body mass index; FSG - Fasting serum glucose; HOMA-IR HOMA%S - Insulin Resistance Homeostatic Model Assessment, or: Insulin sensitivity HOMA, LH - Luteinizing hormone; FSH - Follicle stimulating hormone; IL-39 - interleukin-39.

Table 2: biochemical traits of both the control and registered patient groups

Parameters	PCOS Group	Healthy Group	P-value
LH (mIU/L)	13.02 ± 3.16	4.95 ± 2.18	0.0001
FSH (IU/L)	6.37 ± 3.01	5.29 ± 2.43	0.05
LH/FSH	2.28 ± 0.69	0.804 ± 0.23	0.0001
TT (ng/mL)	2.49 ± 0.92	1.01 ± 0.37	0.025
FT (pg /mL)	13.14 ± 2.03	3.14 ± 3.05	0.0001
SHBG (pg/mL)	30.99 ± 10.2	74.31 ± 12.86	0.0001
FAI	10.48 ± 3.36	3.58 ± 2.02	0.0002

The data represented as mean ± SD, LH: luteinizing hormone, FSH: follicular stimulating hormone and FT: free testosterone, TT: total testosterone, SHBG: Sex binding globulin Hormone.

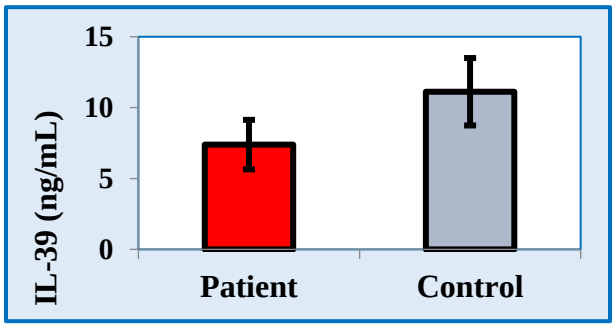

Figure 2: Comparison between IL-39 level in the study groups

Comparison between IL-39 level in the study groups revealed in Table 3 and Figure 2. The result demonstrates that IL-39 level significantly lower in PCOS group (P <0.0001) when compared with healthy control group.

The correlations between anthropometric and biochemical markers and interleukin - 39 in the PCOS patient group are shown in Table 4. The results showed that the IL-10 level showed non-significant positive correlations with age and FSH, and significant negative correlations with the following: LH (P < 0.0001, r=), Prolactin (P = 0.008, r=), BMI (P < 0.0001, r=), WHR (P < 0.0001, r=), LH (P < 0.0001, r=), and LH / FSH ratio (P < 0.0001, r=).

4. ISCUSSION

Elevated LH is one of the common symptoms of PCOS, while it is not necessary for a diagnosis. LH is one of the primary causes of hyperandrogenism in PCOS patients because it is known to enhance androgen synthesis in the ovaries in addition to inducing ovulation and luteinization. Primarily, luteinizing hormone promotes androgen production in ovarian theca cells containing LH receptors. Higher LH concentrations appear to be linked to severe PCOS cases. In the past, there has been a positive correlation shown between ovarian volume and follicle count. Additionally, PCOS individuals with higher levels of LH have been associated with more severe disturbances to their cycles and an increased risk of infertility. LH hypersecretion in PCOS women also reflects the severity of the condition.[16]

Table 3: IL-39 levels in the patient and healthy PCOS control groups

Parameter	Healthy Group	Patient Group	P-Value
IL-39 (ng/mL)	7.3915 ± 1.7473	11.2111 ± 2.3791	< 0.0001

The data represented as mean ± standard deviation, IL-39: Interleukine-39

On the other hand, follicular development is impaired by a relative FSH deficiency.[17] A higher frequency of the LH pulse inhibits the synthesis of FSH and estrogen, which stops the development of follicles and ovulation. In the end, this causes polycystic ovaries in PCOS patients.[18] The persistently rapid (GnRH) pulsatility that

favors pituitary LH over FSH synthesis and leads to increased LH concentrations and altered LH / FSH ratios that are diagnostic of PCOS is thought to be one neuroendocrine feature of the condition.

High levels of LH boost the generation of ovarian androgen, while low levels of FSH limit follicular growth.[19] The brain and pituitary gland produce FSH hormones, which are responsible for the variations in these hormone levels in the afflicted women.[20]

According to the Rotterdam agreement, circulating free testosterone, or FAI measures, should be used instead of serum total testosterone to diagnose hyperandrogenism in women with PCOS.[21] There are several possible reasons why PCOS may result in increased prolactin release. the impact of estrogens, which boost prolactin synthesis and secretion as well as lactotrophic pituitary cell proliferation. In PCOS, elevated estrogen levels may lead to an increase in prolactin concentrations.[22]

It is well known that prolactin causes islets to release more insulin. According to research, prolactin can have an impact on essential enzymes and transporters in the target organs that are engaged in glucose and lipid metabolism as well as the equilibrium of metabolism.[23] Therefore, a close association between prolactin and thromboembolic stroke, hypertension, insulin resistance, and coronary syndrome has been hypothesized.[24] Among the most common reasons of infertility in women are hyperprolactinemia and polycystic ovarian syndrome (PCOS).[25] increased serum LH levels are closely connected with increased blood testosterone levels.[26]

This study supports Chakrabarti, J. (2013) by showing a favorable association between age, WHR, BMI, and serum PCOS in both groups. In the PCOS population, there were higher mean BMI, LH, and LH: FSH ratios. Additionally, PCOS women's fasting insulin levels and androgen levels were noticeably higher.[27] Furthermore,

Table 4: Serum IL-39 level correlation with anthropometric and biochemical markers in a cohort of registered PCOS patients P-value with parameter r

Parameters	R	P-value
Age (y)	-0.291	0.035
BMI (kg/m^2)	-0.411	0.001
WHR	-0.376	0.001
LH (IU/L)	-0.204	0.073
FSH (IU/L)	-0.226	0.065
LH/FSH	0.194	0.109
SHBG (pg/mL)	0.298	0.031
TT (ng/mL)	-0.129	0.183
FT (pg/mL)	-0.317	0.001
FAI	-0.278	0.053
FSG (mg/dL)	-0.317	0.001
FIN (mIU/L)	-0.331	0.001
HOMA- IR	-0.292	0.034
HOMA- %β	0.270	0.059
TC (mg/dL)	-0.373	0.010
TG (mg/dL)	-0.016	0.920
HDL-C (mg/dL)	0.129	0.420
LDL-C (mg/dL)	-0.061	0.603
VLDL-C (mg/dL)	-0.026	0.772

The information displayed as mean ± SD, Total testosterone (TT) and free testosterone (FT) Free androgen index, or FAI TC stands for total cholesterol; TG - Triglycerides, "LDL-C" and "HDL-C" refer to low-density and high-density lipoprotein and cholesterol, respectively. Pentraxin-3, or PTX-3 SD stands for standard deviation; WHR - Waist-to-hip ratio; BMI - Body mass index; FSG - Fasting serum glucose; HOMA-IR HOMA%S - Insulin Resistance Homeostatic Model Assessment, or: Insulin sensitivity HOMA, LH - Luteinizing hormone; FSH - Follicle stimulating hormone; IL-39 -

this study demonstrates the possible association between reproductive-age females' SHBG levels and PCOS risk. This meta-analysis comprised 39 papers that have been published to date in order to estimate the effect size for SHBG levels.[28] Low serum SHBG is associated with the complications and long-term prognosis of PCOS which plays an important role in its pathogenesis. [29]

Serum IL-39 levels were considerably lower in the PCOS individuals in this study (P = 0.000). The plasma IL-39 level and the serum LH level had a negative connection (P = 0.004). Reception operating characteristic analysis was used to establish what the PCOS cut-off value for IL-39 was. IL-39 (OR [95% CI] [0.848, 0.965], P < 0.001) was shown in the multiple

binary logistic regression analysis to have a significant correlation with PCOS.[30]

According to Zheng et al. (2016), a group analysis, high levels of IL39 were found in PCOS in both obese and lean patients, and these levels were significantly correlated with the T ratio (total testosterone ratio) and the HOMA-IR (homeostasis model assessment of insulin resistance) ratio. These findings are supported by the current study. Compared to controls with a comparable BMI, women with PCOS had considerably greater levels of IL-39. Compared to control participants, PCOS-affected women with higher levels of IR and total testosterone also had higher levels of IL-39. Interestingly, people with PCOS who were slim and/or overweight had higher levels of IL39. There was a great deal of variation between the trials, with the degree of IR being the primary factor.[31]

This study discovered that PCOS-affected women had significantly lower levels of IL-39 than did healthy control women. These outcomes agree with what Talaat et al. (2016) found. This finding may have implications for persistent low-grade inflammation, which is thought to play a major role in the etiology and progression of polycystic ovarian syndrome (PCOS). There was a significant correlation found between PCOS and the decrease in IL39 secretion.[32] Changes in steroidogenesis, delayed follicular maturation, and ovarian dysfunction are caused by an imbalance between pro- and anti-inflammatory cytokines. Insulin resistance and hyperinsulinemia are PCOS's two main metabolic disorders. [33]

The most prevalent cause of infertility in women is thought to be polycystic ovarian syndrome (PCOS), an endocrine condition characterized by hyperandrogenism, anovulation (infrequent ovulation), and polycystic ovary shape. Interleukin-10 and other anti-inflammatory chemicals were released in lower quantities when there were fewer T regulatory cells present.[34,35] This phenomenon relates to the hypothesis that circulating hormone abnormalities (e.g., elevated blood testosterone and LH concentrations in ovulatory women with polycystic ovaries) are associated with social group disparities. hindered growth.[36]

The current study found that elevated IL-39 levels were significantly predicted by hyperandrogenism and PCOS in PCOS women, who also had observably elevated IL-39 levels.

5. CONCLUSION

PCOS risk factors include obesity, hyperinsulinemia, hyperlipidemia, and HOMA-IR. Low serum SHBG is crucial to the pathophysiology of PCOS and is linked to the long-term prognosis and repercussions of the condition. This study addresses decreased related IL-39 and hormone levels, the connection between SHBG and PCOS, and associated therapeutic approaches. Further investigation is required to examine the connection between SHBG and PCOS, assess the effects of various medications on SHBG levels, and determine whether these interventions can be beneficial for PCOS.

6. Acknowledgments

The authors thank the patients and all the medical staff for their cooperation in fertility center in AL-Sader Medical city, and AL-Zahra Teaching Hospital for Obstetrics and Gynecology in Najaf-Iraq.

7. Data availability

The numerical data generated during this research is available with the authors.

8. Acknowledgement

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9. Conflict of interest

The study utilized the hospital resources only, and no external or industry funding was involved.

10. Authors' contribution

KMH: Concept, conduct of the study, translation

HAA: Statistical analysis, collection of the patient data, editing the draft manuscript

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