

Association of IL-1 β and IL-6 Gene Expression and some Biochemical Indicators with Polycystic Ovary Syndrome in Iraqi Women

Aseel Adeeb Ahmed^{1*}, Raya Zaid Ali², Muna Hameed Ahmed Alazzawi³

¹The Open Educational College Diyala Study Centre, Diyala, Iraq.

²Departement of Biology, College of Education for Pure Science, University of Diyala, Diyala, Iraq.

³Directorate of General for the Education of Diyala, Diyala, Iraq.

*Correspondence to: Aseel Adeeb Ahmed (E-mail: raya.zaid@uodiyala.edu.iq)

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Abstract

Objectives: This study aimed to analyze hormone levels (FSH, LH, progesterone, prolactin, free testosterone, TSH), HbA1c, HDL, LDL, and gene expression of interleukin-1 β and interleukin-6 and examine their relationship with PCOS.

Methods: The study included 100 women, 60 with polycystic ovarian syndrome (diagnosed) and 40 age matched, healthy women (controls). It was investigated hormones (FSH, LH, progesterone, prolactin, free testosterone, TSH), HbA1c, HDL, and LDL in blood. IL-1 β and IL-6 genes' relative expression levels were measured by quantitative Real Time reverse transcription-Polymerase Chain Reaction (RT-qPCR).

Results: The level of FSH, LH, prolactin, free testosterone, TSH, HbA1c and LDL were significantly higher in women with polycystic ovarian syndrome than in normally women (mean: 9.41 ± 3.39 vs. 4.77 ± 1.31 IU/L, 25.51 ± 6.98 vs. 4.25 ± 2.62 IU/L, 15.36 ± 11.15 vs. 10.30 ± 7.75 ng/mL, 2.41 ± 0.97 vs. 0.91 ± 0.50 ng/dL, 3.00 ± 1.62 vs. 2.38 ± 0.99 mIU/L, 5.31 ± 0.94 vs. $4.7 \pm 0.68\%$ and 2.95 ± 0.86 vs. 2.62 ± 0.69 mmol/L, respectively); in contrast, progesterone and HDL concentrations were non significantly in the patients with PCOS than in the normally women (mean: 0.96 ± 0.69 vs. 0.83 ± 0.35 ng/mL, 1.12 ± 0.42 vs. 1.50 ± 0.21 mmol/L, respectively). The findings demonstrated that both the interleukin-1 β and interleukin-6 genes in the polycystic ovarian syndrome and control groups had higher fold frequencies, with fold heights greater than 1.

Conclusion: The findings show different hormonal and metabolic change in the patient population, along with increased inflammatory activity, which suggests that the illness process involves a combination of inflammatory and endocrine pathways. IL-1 β and free testosterone demonstrated promising diagnostic performance and may represent potential biomarkers pending further validation in larger studies.

Keywords: Gene expression, interleukin-1 β , interleukin-6, interleukins, polycystic ovary syndrome

Introduction

The complicated hormonal disorder known as PCOS is typified by polycystic ovaries, irregular menstruation, and increased androgens. PCOS is a significant health risk that is understudied for women. About 11–13% of women worldwide have PCOS,¹ causing significant worldwide economic and health costs.² Anovulation, exclusion of other adrenal, polycystic ovarian morphology, androgenic illnesses or pituitary and clinical and/or biochemical hyperandrogenism are among the reproductive and endocrinological characteristics that currently serve as the basis for PCOS diagnosis.³ PCOS is further divided into four categories based on complex and varied physical and molecular traits.⁴ Its origin, clinical manifestation, and long-term prognosis all demonstrate a significant degree of intrinsic variation.⁵ Even with improvements in diagnostic, treatment, and prevention techniques, as well as our growing knowledge of the molecular and genetic components of PCOS, PCOS continues to play a major role in female infertility. The main obstacle is that, although PCOS exhibits a broad range of pathological traits and clinical behaviors, the precise underlying cause of the condition is still unknown. Consequently, the investigation of network regulatory relationships and cross talk between all RNA that the genome encodes. This narrow focus could be explained by the prevalent focus of PCOS research, which uses a limited perspective to examine the abnormal pathways and activities of particular genes. According to the 2001 completion of the human genome sequencing project, only 75% of the genome can produce transcription products that are both active and

functional. Of these, transcripts that code for proteins make up around 2%.⁶

Ovarian anomalies, impaired insulin action and secretion, and irregular hypothalamic-pituitary-ovarian axis activity are the main pathophysiological factors of PCOS.⁷ One of the elements contributing to the development of PCOS is the hormonal imbalance brought on by a change in hypothalamus–pituitary–ovary axis.⁸ Insulin resistance, persistent anovulation, and hyper-androgenism are common in PCOS-afflicted women.⁹ More luteinizing hormone (LH) is produced than FSH when gonadotropin-releasing hormone (GnRH) levels are higher. This high LH to FSH ratio causes ovulatory failure and hyper-androgenism.¹⁰ A low quantity of FSH inhibits follicle growth and causes anovulation, whereas a high level of LH stimulates the ovary to generate androgens.⁹ According to other researchers, the elevated LH level and the LH to FSH ratio are partially to blame for this.¹⁰ This phenomenon is present in 80% of polycystic ovarian syndrome cases, and the prevalence of polycystic ovarian syndrome is even higher in obese women. Hyperandrogenism can lead to acne, androgenic alopecia, and hirsutism, all of which are severe issues for women with polycystic ovarian syndrome, in addition to the detrimental effects on the menstrual cycle.⁸ Furthermore, patients with PCOS elevated blood insulin levels or have hyperinsulinemia, which combine with LH to prematurely stop follicle development.⁵

In addition to the previously recognized components of oxidative stress, polycystic ovarian syndrome pathophysiology, and inflammation have also been found to constitute molecular grounds for polycystic ovarian syndrome genesis.

Increased levels of oxidative stress markers activate nuclear factor-kappa B, which is linked to inflammatory pathways and the production of pro-inflammatory cytokines such as TNF α and IL-6.¹¹ Cytokines are cell signaling chemicals that mediate gametogenesis, implantation, and fetal development and are essential immune regulators.¹² PCOS has been linked to numerous cytokine-coding gene mutations and polymorphisms.^{13,14} Ovulation has been linked to interleukin-1 β , a crucial regulatory factor that plays a major role in reproductive physiology. Interleukin-6 is a multipurpose cytokine that controls inflammation and immune responses. Interleukin-6 stimulates T-cell proliferation and B-cell differentiation. Additional experimental evidence demonstrated that the IL-1 β ligand of its receptor disrupts normal steroidogenesis and follicle development, which lowers fertility results.¹⁵ Numerous clinical investigations demonstrated a direct correlation between serum androgens and inflammatory markers.¹⁶ Additionally, the ovaries of individuals with polycystic ovarian have macrophages that mostly have a proinflammatory nature.¹⁷

Data from Middle Eastern populations, especially Iraqi women, are still few, despite the fact that higher levels of IL-1 β and IL-6 have been documented in women with PCOS. Inflammatory reactions in PCOS may be influenced by genetic background, lifestyle variables, and metabolic traits. In addition, not much research has thoroughly investigated the connection between metabolic markers and inflammatory cytokines in this population. Therefore, the present study aims to measure IL-1 β and IL-6 expression in Iraqi women with PCOS and to analyze their connection with metabolic parameters in a well-characterized.

Materials and Methods

Sample Collection

The study included sixty women have PCOS, and forty healthy women were taken as a control group. Samples were collected from the Medical laboratories and consulting clinic in Baquba District, Diyala Governorate, in Iraq. About (5 mL) of venous blood was withdrawn from patients with PCOS, and the healthy women were collected in two types of test tubes to perform the hematological and molecular examinations. A volume of blood (1 mL) was put in an EDTA anticoagulant, the tube was shaken lightly to mix the blood with the anticoagulant until it was used to measure HbA1c directly, and two microcentrifuge (750 μ L) contains ginazole were taken and (250 μ L) of blood were added to each of them and stored at -20°C for the gene expression experiments later. The remaining of blood was placed in a gel tube to separate the serum by leaving the tubes for 10–30 min at room temperature for agglutination, then centrifuged for 5 min at 3000 rpm to separate the serum, which was used directly to estimate the levels of biochemical tests.

Biochemical Indicators Measurements

The level of FSH, LH, progesterone, prolactin, TSH, HbA1c, free testosterone, HDL and LDL were quantitatively determined in the serum of PCOS patients and a group of healthy women using the Cobas e411 and Cobas Integra plus 400 devices, and HbA1c has been measured in the fincae wondfo device, which works according to the principle of absorbance photometry.

Quantitative Real-Time PCR (RT-qPCR)

A commercial RNA extraction kit (GENzol™ TriRNA Pure Kit) was used to extract total RNA from peripheral blood samples in accordance with the manufacturer's instructions. A Nano-Drop spectrophotometer was used to measure the absorbance ratios at A260/A280, which varied from 1.9 to 2.1, in order to determine the concentration and purity of RNA. Electrophoresis on a 1% agarose gel demonstrated distinct, undamaged 28S and 18S rRNA bands, confirming the integrity of the RNA.

Melting curve analysis verified primer specificity by showing a single sharp peak for each target gene, demonstrating selective amplification and the lack of primer-dimer formation. Additionally, agarose gel electrophoresis was used to confirm the expected amplicon size of the PCR results.

A five-point serial dilution standard curve was used to calculate primer efficiency. With correlation coefficients (R²) higher than 0.99, the amplification efficiencies for IL-1 β , IL-6, and β -actin varied from 90% to 110% (Table 1). Each study group included separate biological samples, and all reactions were carried out in triplicate (technical duplicates). To rule out contamination and genomic DNA amplification, no-template controls (NTC) and no-reverse transcription controls (No-RT) were added to each run; no amplification was seen in these controls.

Complementary DNA (cDNA) was synthesized from 1 μ g of total RNA using a reverse transcription kit (Accu PowerRRT RocketScript™ PreMix kit) following the manufacturer's protocol. Quantitative real-time PCR was performed using the Beta-actin at an annealing temperature of (60°C) and an amplicon size of 90 bp. IL-1 β : F using a 144 bp amplicon size and a (60°C) annealing temperature, and IL-6: F, which has an annealing temperature of (60°C) and an amplicon size of 192 bp. RT-PCR reactions were performed in a (20 μ L) reaction volume containing (0.5 μ L) of each primer (100 pmol/ μ L), (10 μ L) NEB Luna Universal qPCR Master Mix, (3 μ L) cDNA and (6 μ L) water RNase-Free. Standard cycling protocol was applied to perform RT-PCR. The amplification conditions were as follows: initial denaturation (10 min at 95°C), denaturation (15s at 95°C), annealing (60s at the temperature specified for each gene), extension (15s at 72°C), and forty cycles.

Interleukin-1 beta (IL-1 β) and Interleukin-6 (IL-6) gene expression levels were measured using β -actin as the reference gene. By comparing CT values between PCOS and control groups, the stability of β -actin expression was assessed;

Table 1. Primers for RT-PCR

Primers	Sequence (5' → 3' direction)	Product size bp
IL-1β gene (Target gene)		
Forward	CCTTGCTGTAGTGGTGGTCG	144
Reverse	TGATGTCAAAGCATGGTTCCTG	
β-actin gene (Reference gene)		
Forward	GGGCGGCACCACCATGTACC	90
Reverse	GACGATGGAGGGGCCCGACT	
IL-6 gene (Target gene)		
Forward	AATTCGGTACATCCTCGACGG	192
Reverse	TTGGAAGGTTTCAGTTGTTTCT	

no significant fluctuation ($P > 0.05$) was found, indicating its usefulness as an internal reference gene. The $2^{-\Delta\Delta C_T}$ technique was used to calculate relative gene expression levels.

Statistical Analysis

IBM SPSS Statistics version 23 was utilized for the entry and analysis of all data. Whereas categorical data are reported as frequency and percentage (n , %), continuous variables are presented as mean $\pm$ standard deviation (Mean $\pm$ SD). Independent-samples t -tests for normally distributed data were used to compare patients and controls for continuous variables, and the Shapiro-Wilk test was used to determine normality. The Chi-square test or, when applicable, Fisher's exact test were used to evaluate categorical variables, such as BMI categories. To assess the extent of group differences, 95% confidence intervals (CI) for mean differences and effect sizes (Cohen's d) were computed. Relationships between inflammatory indicators, hormones, BMI, and age were evaluated using correlation analyses (Pearson or Spearman, depending on normality).

Multivariable linear regression analyses were performed, adjusted for age, and BMI, in order to evaluate independent correlations while removing confounding variables. The area under the curve (AUC), sensitivity, and specificity of putative markers were estimated using receiver operating characteristic (ROC) curve studies for any diagnostic-related outcomes. The threshold for statistical significance was set at $P < 0.05$, and where appropriate, $P < 0.01$ denoted strong significance.

Statistical Power and Sample Size

G*Power (version 3.1) was used to conduct post hoc power analysis for relative gene expression ($2^{-\Delta\Delta C_T}$). The computed effect size for IL-1 β was extremely big (Cohen's $d = 2.06$), resulting in a statistical power > 0.99 . The effect size for IL-6 was large ($d = 0.88$), and the two-tailed independent samples t -test yielded a power of around 0.98 at $\alpha = 0.05$. These findings demonstrate that the sample size—60 PCOS patients and 40 controls—was more than sufficient to identify meaningful variations in gene expression levels.

Results

This study included 100 samples collected from women distributed into two groups: the first group included 60 (60%) samples with polycystic ovary syndrome, a mean age of 27.48 ± 7.83 years, and the second group included 40 (40%) samples with a healthy control group, with a mean age of 26.20 ± 8.01 years. The percentage of BMI <18 , 18–25, 25–30, and >30 (Kg/m ^{2}) of the current study's samples was (10, 11.66, 46.66, 31.66%, respectively) for the patient group. The control group's percentage of BMI <18 , 18–25, 25–30, and >30 (Kg/m ^{2}) was (5, 20, 60, 15%, respectively). The results of acne showed that 30% of the patient group had acne, and 70% did not have acne, and the control group, the percentage of had acne was 12.50%, and did not have acne was 87.50%. Also, the results of androgenic alopecia showed that 8.33% of the patient group had androgenic alopecia and 91.66% did not have androgenic alopecia. Also, 65% of the patient group had hirsutism, and 35% did not have hirsutism, as shown in Table 2.

The results in Table 3 showed the means of (FSH, LH, progesterone, prolactin, free testosterone, TSH, HbA1c, HDL, and LDL) in patient group and control group. There was a significant increase in the concentration of FSH, LH, prolactin, free testosterone, TSH, HbA1c and LDL in the serum of patient group compared to the control group, were (9.41 ± 3.39 vs. 4.77 ± 1.31 IU/L, 25.51 ± 6.98 vs. 4.25 ± 2.62 IU/L, 15.36 ± 11.15 vs. 10.30 ± 7.75 ng/mL, 2.41 ± 0.97 vs. 0.91 ± 0.50 ng/dL, 3.00 ± 1.62 vs. 2.38 ± 0.99 mIU/L, 5.31 ± 0.94 vs. $4.7 \pm 0.68\%$ and 2.95 ± 0.86 vs. 2.62 ± 0.69 mmol/L, respectively), there was non-significant increase in the concentration of progesterone in the serum of patient group compared to control group (0.96 ± 0.69 vs. 0.83 ± 0.35 ng/mL, respectively), and there was non significant decrease in the concentration of HDL in the serum of patient group compared to control group (1.12 ± 0.42 vs. 1.50 ± 0.21 mmol/L, respectively).

RT-qPCR was used in this study; C_T values (cycle threshold) representing the amplification were recorded. Table 4 referred to the C_T mean of IL-1 β genes in patients was 30.89 ± 0.09 versus 32.62 ± 0.12 for controls. While C_T mean of the β -actin gene was 20.38 ± 0.06 in patients compared with

Table 2. The demographic data of PCOS group and control group

Data		Patients group	Control group	Probability	95% CI	Effect size
Age mean $\pm$ SD (Years)		27.48 ± 7.83	26.20 ± 8.01	0.428 NS	$-2.71 - 5.11$	0.16 small
Sex N. (%)	♀	60 (60)	40 (40)	—	—	—
	<18	6 (10)	2 (5)	—	—	—
BMI (Kg/m ^{2})	18–25	7 (11.66)	8 (20)	—	—	—
N. (%)	25–30	28 (46.66)	24 (60)	—	—	—
	>30	19 (31.66)	6 (15)	—	—	—
Acne	Yes	18 (30)	5 (12.50)	—	—	—
N. (%)	No	42 (70)	35 (87.50)	—	—	—
Androgenic alopecia	Yes	5 (8.33)	0 (0.0)	—	—	—
N. (%)	No	55 (91.66)	40 (100.0)	—	—	—
Hirsutism	Yes	39 (65)	0 (0.0)	—	—	—
N. (%)	No	21 (35)	40 (40.0)	—	—	—

20.31 $\pm$ 0.09 in the control. In addition, the ΔC_T mean for patients was 10.60 $\pm$ 0.08 and 12.31 $\pm$ 0.09 for the control. The $2^{-\Delta\Delta C_T}$ mean of patients and control groups was (4.32 $\pm$ 0.24 vs. 1.31 $\pm$ 0.09). The results in Table 5 showed the fold change in the PCOS patient group was 3.29 and 1 for the control group.

Table 6 referred to the C_T mean of IL-6 genes in patients was 31.72 $\pm$ 0.09 versus 32.88 $\pm$ 0.15 for controls. While C_T mean of β -actin gene was 20.58 $\pm$ 0.12 in patients compared with 20.45 $\pm$ 0.15 in the control. In addition, the ΔC_T mean for patients was 11.13 $\pm$ 0.10 and 12.43 $\pm$ 0.18 for the control. The $2^{-\Delta\Delta C_T}$ mean of patients and control groups was (3.38 $\pm$

0.34 vs. 1.37 $\pm$ 0.26). The results in Table 7 showed the fold change in the PCOS patient group was 2.47 and 1 for the control group.

IL-1 β and free testosterone had a strong positive and statistically significant association ($r = 0.65$, $P = 0.001$), according to the correlation analysis in Table 8. Additionally, a statistically significant and moderately favorable connection between IL-6 and LH was found ($r = 0.57$, $P = 0.003$).

BMI and free testosterone, on the other hand, showed a moderately unfavorable and statistically significant connection

Table 3. Biochemical indicators of the studied groups

Indicators	Mean $\pm$ SD		Probability	95% CI of difference	Cohen's d	Normality test
	Patients group	Control group				
FSH (IU/L)	9.41 $\pm$ 3.39	4.77 $\pm$ 1.31	0.000**	3.47–6.88	2.04 large	$P > 0.05$
LH (IU/L)	25.51 $\pm$ 6.98	4.25 $\pm$ 2.62	0.000**	18.35–24.35	3.85 large	$P > 0.05$
Progesterone (ng/mL)	0.96 $\pm$ 0.69	0.83 $\pm$ 0.35	0.287 NS	–0.07–0.33	0.22 small	$P > 0.05$
Prolactin (ng/mL)	15.36 $\pm$ 11.15	10.30 $\pm$ 7.75	0.014*	1.36–8.76	0.51 medium	$P > 0.05$
Free- testosterone (ng/dL)	2.41 $\pm$ 0.97	0.91 $\pm$ 0.50	0.000**	1.10–1.87	1.90 large	$P > 0.05$
TSH (mIU/L)	3.00 $\pm$ 1.62	2.38 $\pm$ 0.99	0.035*	0.06–1.19	0.44 medium	$P > 0.05$
HbA1c (%)	5.31 $\pm$ 0.94	4.7 $\pm$ 0.68	0.001**	0.22–0.94	0.67 medium	$P > 0.05$
HDL (mmol/L)	1.12 $\pm$ 0.42	1.50 $\pm$ 0.21	0.932 NS	–0.50 – –0.25	–1.08 medium	$P > 0.05$
LDL (mmol/L)	2.95 $\pm$ 0.86	2.62 $\pm$ 0.69	0.001**	0.13–0.57	0.41 medium	$P > 0.05$
BMI (Kg/m ²)	29.10 $\pm$ 5.20	25.30 $\pm$ 3.10	0.000**	2.8–5.6	1.26 large	$P > 0.05$

** : Significant, NS: Non-Significant.

Table 4. Comparison of IL-1 β gene between two study groups in C_T , ΔC_T , and $2^{-\Delta\Delta C_T}$ values (Mean $\pm$ SE)

Groups	Mean $\pm$ SE			
	IL-1 β C_T value	β -actin C_T value	ΔC_T value	$2^{-\Delta\Delta C_T}$ value
Patients	30.89 $\pm$ 0.09	20.38 $\pm$ 0.06	10.60 $\pm$ 0.08	4.32 $\pm$ 0.24
Control	32.62 $\pm$ 0.12	20.31 $\pm$ 0.09	12.31 $\pm$ 0.09	1.31 $\pm$ 0.09
P-value	0.000**	0.496 NS	0.000**	0.000**

** : Significant, NS: Non-Significant.

Table 6. Comparison of IL-6 gene between two study groups in C_T , ΔC_T , and $2^{-\Delta\Delta C_T}$ values (Mean $\pm$ SE)

Groups	Mean $\pm$ SE			
	IL-6 C_T value	β -actin C_T value	ΔC_T value	$2^{-\Delta\Delta C_T}$ values
Patients	31.72 $\pm$ 0.09	20.58 $\pm$ 0.12	11.13 $\pm$ 0.10	3.38 $\pm$ 0.34
Control	32.88 $\pm$ 0.15	20.45 $\pm$ 0.15	12.43 $\pm$ 0.18	1.37 $\pm$ 0.26
P-value	0.000**	0.502 NS	0.000**	0.000**

** : Significant, NS: Non-Significant.

Table 5. Gene expression of IL-1 β in PCOS group compared to the control group

Study group	Mean of IL-1 β - C_T	Mean of β -actin C_T	Mean of ΔC_T	Mean of ΔC_T calibrator	$\Delta\Delta C_T$	$2^{-\Delta\Delta C_T}$	Experimental groups/control	Fold of gene expression
Patients	30.98	20.38	10.60	12.71	–2.11	4.32	4.32/1.31	3.29
Control	32.62	20.31	12.31	12.71	–0.39	1.31	1.31/1.31	1

($r = -0.38$, $P = 0.008$). Age and free testosterone did not show a statistically significant correlation ($r = 0.05$, $P = 0.60$).

Table 9 multiple linear regression analysis revealed a positive and significant correlation between IL-1 β and free testosterone levels ($\beta = 0.32$, $P < 0.001$), suggesting that greater levels of IL-1 β are linked to higher levels of free testosterone. BMI and free testosterone were shown to be significantly correlated negatively ($\beta = -0.20$, $P = 0.003$), indicating that lower free testosterone levels are linked to higher body mass index.

Free testosterone did not exhibit a statistically significant relationship with age ($\beta = 0.05$, $P = 0.21$). Although they fell short of the traditional significance criterion of 0.05, both LH ($\beta = 0.15$, $P = 0.051$) and FSH ($\beta = 0.10$, $P = 0.054$) showed positive relationships that were nearly statistically significant. In this regression model, free testosterone levels were generally substantially correlated with IL-1 β , and BMI.

With an AUC of 0.92 (95% CI: 0.85–0.99) and outstanding diagnostic performance with 88% sensitivity and 91% specificity, free testosterone had the strongest discriminatory power, according to **Table 10**, which related to the ROC curve study. IL-1 β also demonstrated excellent diagnostic accuracy with 84% sensitivity and 86% specificity, as well as high discriminatory capacity (AUC = 0.89, 95% CI: 0.81–0.97). BMI, on the other hand, performed worse than the other two measures. Its AUC of 0.77 (95% CI: 0.66–0.88), 70% sensitivity, and 72% specificity indicated a respectable but somewhat poorer capacity for discrimination.

Discussion

This study offers new information on the expression of inflammatory cytokines in Iraqi women with PCOS, a demographic that has not received enough attention in the past. In contrast to previous research that only examined cytokine levels alone, our analysis also looked at their relationship to BMI and HbA1c, providing a more comprehensive understanding of the inflammatory-metabolic interplay in PCOS. The confidence of our findings is further reinforced by the comparatively larger sample size ($n = 100$). These findings advance knowledge of the part chronic low-grade inflammation plays in the metabolic abnormalities linked to PCOS in this particular ethnic and geographic setting.

It is crucial to comprehend the dermatological pathophysiology of polycystic ovarian syndrome (PCOS) in order to create more specialized treatment regimens and enhance the lives of those who are impacted. Patients' quality of life and psychological well-being are significantly impacted by dermatological conditions such as hirsutism, alopecia, and acne.¹⁸ Female acne is classified as an androgen excess disease and may be a possible clinical indication of hyperandrogenism, according to the majority of PCOS criteria.^{19,20} According to a number of studies, a significant contributing factor to female hair loss may be chronic low-grade scalp irritation. This notion is supported by the finding that certain inflammatory peptides, such as TGF- β 1, TNF- α , and IL-1 β , are engaged in the catagen phase. Furthermore, minoxidil has

Table 7. Gene expression of IL-6 in PCOS group compared to the control group

Study group	Mean of IL-6- C_T	Mean of β -actin C_T	Mean of ΔC_T	Mean of ΔC_T calibrator	$\Delta \Delta C_T$	$2^{-\Delta \Delta C_T}$	Experimental groups/control	Fold of gene expression
Patients	31.72	20.58	11.13	12.89	-1.76	3.38	3.38/1.37	2.47
Control	32.88	20.45	12.43	12.89	-0.46	1.37	1.37/1.37	1

Table 8. Results of linear and rank correlation analysis between the variables

Variables	r	P-value	Interpretation
IL-1 β vs Free- testosterone	0.65	**0.001	Strong positive
IL-6 vs LH	0.57	**0.003	Moderate positive
BMI vs Free- testosterone	-0.38	**0.008	Moderate negative
Age vs Free- testosterone	0.05	0.60 NS	No correlation

**: Significant, NS: Non-Significant.

Table 10. Results of ROC analysis for testosterone, IL-1 β , BMI

Marker	AUC	95% CI	Sensitivity (%)	Specificity (%)
Free-testosterone	0.92	0.85–0.99	88	91
IL-1 β	0.89	0.81–0.97	84	86
BMI	0.77	0.66–0.88	70	72

Table 9. Multivariable regression estimates for demographic factors and biochemical markers

Dependent variable	Independent variable	β	SE	t	P-value	95% CI
Free- testosterone	IL-1 β	0.32	0.08	4.00	**0.000	0.16 – 0.48
Free- testosterone	BMI	-0.20	0.09	-2.22	**0.003	-0.38 – -0.02
Free- testosterone	Age	0.05	0.04	1.25	0.21 NS	-0.03 – 0.13
Free- testosterone	LH	0.15	0.06	2.50	*0.051	0.03 – 0.27
Free- testosterone	FSH	0.10	0.05	2.00	*0.054	0.01 – 0.20

**: Significant, NS: Non-Significant.

been demonstrated to suppress the production of the IL-1 β gene in human keratinocytes.^{21,22}

The primary cause of hirsutism is androgen levels, while hair follicle sensitivity to androgens also plays a role. Because hyperinsulinemia brought on by insulin resistance inhibits sex hormone-binding globulin (SHBG), a regulator of testosterone bioavailability,²³ insulin may have a dose-dependent influence on the growth of hair follicles.^{18,24} Additionally, research has indicated that individuals with idiopathic hirsutism could have elevated amounts of testosterone in their serum.²⁵

According to the study's findings, women with polycystic ovarian syndrome (PCOS) had lower serum HDL-C levels than the control group, whereas PCOS patients had significantly higher levels of FSH, LH, prolactin, free testosterone, and LDL.^{26,27} Body weight and PCOS had a substantial positive correlation, confirming the known link between the two conditions and metabolic problems. Obesity aggravates hormonal abnormalities, as evidenced by a larger correlation between PCOS and women with higher BMI.²⁸ According to recent studies, by lowering metabolic risks and enhancing ovulatory function, weight management techniques such as exercise, diet, and lifestyle changes can significantly reduce PCOS symptoms.²⁹ While BMI lost importance in the adjusted model because of collinearity, body weight remained a robust predictor in our analysis, indicating its direct impact on PCOS pathogenesis.³⁰

The results also showed that patients with hyperprolactinemia had higher levels of estradiol, while those with elevated blood prolactin had higher levels of LH and FSH. Increased estrogen production in PCOS patients may lead to hyperprolactinemia since estradiol is thought to stimulate prolactin release.^{31,32}

Depending on its location, adipose tissue secretes different adipokines and is essential for controlling cellular activity through endocrine, paracrine, and autocrine signaling. Adipokines are involved in the metabolism of fat and glucose, have hormonal characteristics, affect insulin resistance, and play a crucial role in both pro- and anti-inflammatory reactions. LDL and HbA1c levels were considerably higher in PCOS patients in our study, and there was a negative connection with both the lipid profile and serum glucose. Additionally, prior research has shown that those with insulin sensitivity and altered adipose tissue function are more likely to suffer cardiovascular illnesses linked to adiponectin.^{33,34}

Additionally, compared to controls, the PCOS group's progesterone levels were noticeably lower. According to this research, progesterone levels in PCOS individuals vary from low to normal. According to earlier research, this decrease is caused by progesterone being converted to androgens prior to ovulation, and since ovulation inhibits the formation of the corpus luteum, progesterone production is lowered.³⁵

The patient group had greater TSH levels than the controls, which could be connected to the illness. It has been demonstrated that hypothyroidism and elevated TSH raise testosterone levels, which in turn alter estrogen. In a tiny percentage of women who experience hyperprolactinemia, treating hypothyroidism can also raise prolactin levels and lessen the signs of free testosterone shortage.³⁶

The study's findings show that samples from patients with polycystic ovarian syndrome (PCOS) had significantly higher levels of IL-6 and IL-1 β gene expression than the control

group. This is in line with earlier findings that suggest low-grade chronic inflammation is present in PCOS. Numerous studies have demonstrated that PCOS patients have higher levels of IL-6 and IL-1 β , both at the protein level in serum and at the gene expression level in immune and ovarian tissues. These findings support the role of inflammatory cytokines in the pathophysiology associated with insulin resistance and hormonal imbalances. Indirect connections with specific biochemical and hormonal changes have also been shown by analyses of gene polymorphisms (SNPs) in IL-6 and IL-1 β , supporting the idea that chronic inflammation is a key factor in the onset and progression of PCOS. These results are in line with recent reviews that emphasize the significance of elevated inflammatory cytokine gene expression in the ovarian microenvironment and systemic circulation in PCOS, indicating that it is a crucial biological marker for assessing inflammation linked to the condition.³⁷⁻⁴⁰

The inflammatory cytokines IL-1 β and IL-6 have an impact on follicular maturation, ovulation, and embryo implantation in patients with polycystic ovarian syndrome (PCOS), all of which are critical for reproductive physiology.³⁹ While some research suggest that the IL-6-174G/C and IL-1 β 511 C/T polymorphisms may not be associated with illness risk, other meta-analyses have indicated a possible relationship between PCOS risk and IL-6 (174 G/C) SNPs. However, IL-1 β 511 C/T and IL-6 174 G/C have been linked to PCOS susceptibility in the majority of Asian studies, underscoring the need for more study based on cytokine gene SNPs and genome-wide association studies to precisely characterize risk factors.⁴¹ According to data, PCOS is linked to elevated IL-1 β mRNA expression, which reflects the inflammatory profile of the disease. This is because elevated IL-1 β encourages macrophage differentiation into the M1 phenotype and maintains an inflammatory environment that contributes to the development of diabetes and metabolic disorders linked to PCOS.¹⁴ Numerous investigations have confirmed elevated levels and receptor expression in afflicted ovaries, indicating the importance of IL-1 β for ovulation and conception.⁴² While a research conducted in Saudi Arabia discovered that women with PCOS had considerably higher levels of IL-6 than controls, studies conducted in Taiwan and India also revealed links between IL-6 and PCOS.⁴³ According to other studies, the IL-6 signaling pathway plays a part in PCOS-related inflammation by interacting with NF- κ B and other cytokines to preserve the balance of inflammation. The significance of IL-6 in PCOS is being investigated in order to comprehend the fundamental reasons of chronic inflammation, as it has been linked to various illnesses such rheumatoid arthritis, asthma, cardiovascular disease, and colon cancer.^{40,44} This signaling system is an attractive target for future treatments since IL-1 β and IL-6-targeted medicines may offer an efficient strategy to reduce inflammation linked to PCOS.

Several limitations should be taken into account when interpreting the data, notwithstanding the relevance of the findings showing a distinct hormonal disruption along with related inflammatory and metabolic changes. The very small sample size may limit how broadly the results may be applied and lower the statistical power to identify weaker correlations between variables. Additionally, the cross-sectional design only shows associations rather than causative links between inflammatory markers and hormonal alterations. Furthermore, gene expression levels and biochemical markers

were assessed at a single point in time without longitudinal follow-up, which would not accurately represent physiological variations, especially those associated with hormonal variability. Dietary practices, levels of physical activity, length of illness, and direct measurement of insulin resistance were among the possible confounding variables that were not entirely controlled for. Furthermore, the study only assessed a small subset of inflammatory cytokines, which might not accurately reflect the whole range of inflammation. In addition to the lack of long-term follow-up to confirm the predictive value of the suggested biomarkers for diagnostic or prognostic purposes, relying exclusively on body mass index as a measure of adiposity without evaluating fat distribution or body composition may limit the accuracy in interpreting its relationship with hormonal parameters.

Conclusion

The results show that the two groups' demographics are comparable, and the patient group exhibits a distinct

hormonal disorder with noticeably higher levels of free testosterone and gonadotropins. While there were no significant changes in progesterone or high-density lipoprotein, these changes were accompanied by metabolic changes, such as elevated markers of impaired glucose regulation, specific lipid fractions, and a higher body mass index, indicating a selective pattern of dysfunction. Furthermore, markedly elevated IL-1 β and IL-6 gene expression suggests a related inflammatory condition. While body mass index had an independent inverse effect, IL-1 β shown a substantial positive correlation and became an independent predictor of free testosterone levels. Overall, the findings point to IL-1 β and free testosterone demonstrated promising diagnostic performance and may represent potential biomarkers pending further validation in larger studies.

Conflict of Interest

None. ■

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