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Comparative Study of Some Physiological and Immunological Biomarkers in Women with Polycystic Ovary Syndrome (PCOS) with and without Insulin Resistance (IR)

Tamarah H. Ahmed[1], Noor H. Al-Mousawi[2]

[1]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Wasit, Wasit, Iraq
[2]Department of Pathological Analysis, College of Science, University of Wasit, Wasit, Iraq

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Abstract

Background: Polycystic ovarian syndrome (PCOS) is one of the most prevalent endocrine disorders and metabolic ailments in women of childbearing age. Also, now, closely related to the biology and clinical severity of PCOS is the insulin resistance (IR).**Objectives**: This study was done to investigate the immunological, hormonal, and metabolic indicators between the healthy controls, patients with PCOS and IR, and patients with PCOS only**. Methods:** A case-control study involving 50 patients with PCOS having IR ,and 50 patients with PCOS without IR and 50 as a healthy controls. Fasting blood glucose (FBG), HOMA-IR, insulin, LH, FSH, testosterone, IL-18, TGF-β, TLR4 and adiponectin were measured and statistically analyzed. **Results:** FBG (116.84 ± 14.3 mg/dL), HOMA-IR (5.47 ± 1.05) also insulin (23.87 ± 4.28 mIU/L) were significantly higher with PCOS patients having IR compared to PCOS patients only (FBG 98.88 ± 7.67, HOMO-IR 2.09 ± 0.88 ,insulin 11.83 ± 2.97), and control (FBG 88.20 ± 7.61, HOMO-IR 1.42 ± 0.45 ,insulin 6.54 ± 2.01) .While the hormonal analysis showed that PCOS - IR exhibited a higher level of LH (16.02 ± 4.6 mIU/mL and testosterone 116.52 ± 15.0 ng/dl) compared to PCOS patients (LH 13.84 ± 3.78, testosterone 84.76 ± 10.7) and controls (LH 4.69 ± 0.61, testosterone 44.72 ± 9.7).While there are no significant found in FSH between all groups .Also, The largest inflammatory load was found in the PCOS -IR group in TLR4, TGF-β and IL-18. While, adiponectin was reduced in PCOS with IR than in PCOS and controls. **Conclusion:** Such findings illustrate the interactions between metabolic dysfunction, hyperandrogenism and inflammation in the pathophysiology of PCOS, especially in insulin-resistant patients. The biomarkers may be convenient therapy indicators and the severity of the disease.

Keywords: Insulin resistance; PCOS; IL-18; TGF-β; TLR4; Adiponectin

Introduction

The prevalence of polycystic ovarian syndrome (PCOS), one of the most common endocrine and metabolic diseases in women of childbearing age, was between 6 and 20% of women in the world depending on the diagnostic criteria used (Joham *et al* ,. 2022 ; Bozdag *et al* ,. 2016). Also, polycystic ovarian morphology, hyperandrogenism, and chronic anovulation were the three clinical and biochemical abnormalities that characterize the syndrome (Rotterdam, 2020). Another pathogenic agent of PCOS was insulin resistance (IR), and it was prevalence was recognized as a system-wide metabolic disease that was not limited to its reproductive counterparts (Dunaif, 2008). So, irrespective of body mass index, half to two-thirds of PCOS patients suffer IR that was close to hyperinsulinemia, dysglycemia, and cardiometabolic risk (Escobar-Morreale, 2018; Diamanti-Kandarakis & Christakou, 2009). Overall, this has been popularized more in recent years that PCOS was low grade inflammatory chronic disease. It has been demonstrated by many studies that pro-inflammatory cytokines including transforming growth factor- β (TGF- β) and interleukin- 18 (IL- 18) were elevated in afflicted women(Chen *et al* ,. 2021 ; Zheng, & Zhao ,2024). So, these cytokines enhance the metabolic-reproductive interaction inherent in the syndrome due to oxidative stress, insulin dys-signaling and ovarian dysfunction (Bai *et al* ,. 2024). As well as, in PCOS, adipose tissue inflammation and impaired glucose homeostasis have been associated with the activation of an innate immune signaling protein Toll-like receptor 4 (TLR4) (Hu *et al* ,. 2021; Liu *et al* ,. 2024). Women with PCOS and especially with IR constantly experience lower concentrations of adiponectin, an anti-inflammatory adipokine which was also significant in the breakdown of fatty acids and insulin sensitivity (Shirazi *et al* ,. 2021; Mashhadi *et al* ,. 2025). Thus ,adiponectin was a potential biomarker associated with the severity of metabolism in such patients as a low level exacerbates the metabolic abnormalities and was connected with high levels of androgens and dyslipidemia (Nguyen, 2020). Likewise , hormonal imbalances were another noteworthy cause of pathophysiology of PCOS. The features of anovulation and follicular standstill were high testosterone levels and luteinizing hormone (LH) (Rosenfield, 2024; Penzias *et al* ,. 2021). So ,these hormonal abnormalities were aggravated by insulin resistance implying a mutual relationship between endocrine and metabolic dysfunction (Shaaban *et al* ,. 2021). Due to the multifaceted nature of PCOS, the interaction of metabolic index (FBG, HOMA-IR, insulin), hormonal (LH, FSH, testosterone), and inflammatory also (IL-18, TGF-B, TLR4, adiponectin) markers is vital in enhancing the diagnosis, risk stratification and targeted therapy. SO, to give a comprehensive analysis of the metabolic-immune-hormone interface that characterizes PCOS, this study aims at analyzing such biomarkers in patients with PCOS with and without insulin resistance and comparing them to healthy control groups.

Materials and Methods

Study Design and Population

A case-control study was conducted in the Al-Zahraa Teaching Hospital and some of the private clinics in the Wasit province ,Iraq ,during the period of March 2025 to December 2025. The study involved 50 women diagnosed with PCOS with IR ,50 women diagnosed with PCOS only and 50 women as healthy

controls. The diagnosis of PCOS was based on the Rotterdam criteria 2003 while Insulin resistance was defined depended on HOMO-IR that a value of 2.5 up to 2.9 depicts IR in patient .

Sample Collection and Analysis

A Five milliliters of fasting venous blood were collected from each of the participants. The blood was transported into gel tube , left at room temperature after that centrifuged for 10 minutes at 3000 rpm .The serum was kept at -20C for measurement of parameters . A commercial kit by (Linear Chemicals, Spain) was employed to enzymatic colorimetric GOD / POD method to determine the fasting blood glucose (FBG) in serum. Insulin resistance was measured using the HOMA-IR formula. Whereas, the testosterone was confirmed through competitive ELISA kit (BioMerieux, France), as suggested by the manufacturer. Likewise ,serum LH and FSH were measured using ELISA kits of (Monobind Inc., USA). In addition ,Immunological markers, including (IL-18, TGF-b, TLR4 , adiponectin), and insulin were measured with the help of quantitative sandwich ELISA kits (BTLab, China).

Statistical Analysis

All statistical analyses were performed with the help of SPSS software (Version 26, IBM Corp., Armonk, NY, USA). Values of each parameter were presented in the form of Mean± Standard Deviation (Mean± SD).All study group were compared through One-Way Analysis of variance. Also, Post-hoc Multiple Range Test (DMRT) of Duncan was applied to the data when ANOVA produced statistically significant differences between the means (18). Superscript letters (A, B and C) in the tables indicated significant differences between groups. A statistically significant difference was considered as p P>0.05 and a highly significant difference was considered as $p \leq 0.001$.

Ethical approval

The ethical committee of the College of Science of the University of Wasit approved the protocol of the study (Approval No. BIO-2025-012).

Results

Patient and control metabolic parameters

The sample size of the study was 150 participants divided into three groups including first group that comprised of 50 participants with PCOS and IR; second group consisted of 50 participants with PCOS only; finally, third group comprised of 50 healthy people who were used as the control group. Some metabolic markers (FBG, HOMA-IR, and insulin) were compared between the patients and the healthy controls and the results are presented in table (1). PCOS patients who had IR, PCOS patients, and the healthy control population had a mean of (116.84 ± 14.3 , 98.88 ±7.76 , 88.20 ± 7.61mg/dl) fasting blood glucose, respectively; The mean levels of insulin in the patient groups (PCOS patients with IR and PCOS patients only) were higher as compared to the control group, and the difference was significant (P>0.05). Also, the mean value of two groups of patients was quite different (P > 0.05). The mean of HOMA-IR in PCOS patients with IR was significantly greater when compared with the two other groups (PCOS patients only and healthy control); nevertheless, no significant difference was noticed between the two other groups (P > 0.05)

Table (1): Comparing a few metabolic measures between patients and healthy controls, including FBG, HOMA-IR, and insulin

Groups		FBG (mg/dL)	HOMA-IR	Insulin (mIU/L)
PCOS and IR	Mean ± SD	116.84 ± 14.3^{A}	5.47 ± 1.05^{A}	23.87 ± 4.28^{A}
	Range	92.00-136.00	2.80-8.00	14.80-34.80
PCOS only	Mean ± SD	98.88 ± 7.67^{B}	2.09 ± 0.88^{B}	11.83 ± 2.97^{B}
	Range	80.90-109.00	1.00-7.00	6.30-16.00
Control	Mean ± SD	88.20 ± 7.61^{C}	1.42 ± 0.45^{B}	6.54 ± 2.01^{C}
	Range	75.00-98.00	0.70-2.10	2.40-12.00
p-value		0.001**	0.001**	0.001**

According to Duncan, multiple range comparisons (DMRTs) indicate that means with dissimilar letters after them are significantly different whereas means with similar letters after them are not.

SD: standard deviation; †: one way ANOVA; ******: significant at $P < 0.05$

SD: standard deviation; †: Independent T test; ******: significant at P > 0.05

Hormonal parameters of patients and controls

Patients were compared to healthy controls regarding some of the hormonal parameters (LH, FSH, and testosterone) and the results are displayed in table (2). The mean value of LH in PCOS patients with IR, PCOS patients only, and healthy control group was 16.02 ± 4.6 mIU/mL, 13.84 ± 3.78 mIU/mL and 4.69 ± 0.61 mIU/mL, respectively. The level of both groups of patients was higher than in the control group and significance (P>0.05) was attained. So, the two groups of patients did not, however, differ significantly in the mean values (P<0.05). The mean level of testosterone among patients with PCOS and IR was 116.52 ± 15.0 ng/dl, among patients with PCOS and isolated PCOS was 84.76 ± 10.7 ng/dl, and the normal control group was 44.72 ± 9.7 ng/dl with the difference important (P>0.05). as well as , the mean values in the two groups of patients differed significantly (P>0.05). While ,no significant difference was observed in all the study groups in relation to FSH levels (P > 0.05).

Table (2): LH, FSH, and testosterone levels in patients and healthy controls are compared.

Groups		LH (mIU/mL)	FSH (mIU/mL)	Testosterone (ng/dl)
PCOS and IR	Mean ± SD	16.02 ± 4.6^{A}	5.57 ± 0.99^{A}	116.52 ± 15.0^{A}
	Range	8.90-24.90	2.40-9.80	62.00-170.00
PCOS only	Mean ± SD	13.84 ± 3.78^{A}	6.01± 0.68^{A}	84.76 ± 10.7^{B}
	Range	6.80-19.80	3.20-8.60	52.00-118.00
Control	Mean ± SD	4.69 ± 0.61^{B}	6.60 ± 0.61^{A}	44.72 ± 9.7^{C}
	Range	1.90-7.00	4.30-9.20	18.00-64.00
p-value		0.001**	0.127	0.001**

According to Duncan, multiple range comparisons (DMRTs) indicate that means with dissimilar letters after them are significantly different whereas means with similar letters after them are not.

SD: standard deviation; †: Independent T test; ******: significant at P > 0.05

Immunological parameters of patients and controls

Table (3) compares immunological (IL-18 level, TGF- β, TLR4 and Adiponectin) of patients and controls. Compared to healthy controls, the levels of certain immunological markers (IL-18, TGF- β, and TLR4) were significantly increased in both groups of patients. The mean value of IL-18 (555.4± 46.5 pg/mL , 303.40 ±35.4 pg/mL and 127.80± 17.4 pg/mL in PCOS patients with IR, PCOS patients only, and healthy control group, respectively. Also, the mean values of TGF- β and TLR4 were found to be higher in both PCOS patients with IR and PCOS patients as compared to the control group and the difference was found to be significant (P>0.05). Nonetheless, the average value of adiponectin was smaller in the two groups of patients (PCOS patients with IR and PCOS patients alone) and the margin was high (P>0.05). Also, the mean values of two groups of patients were considerably different (P>0.05).

Table (3): Immunological markers (IL-18, TGF-β, TLR4, and adiponectin) in patients and healthy controls were compared.

Groups	IL-18 (pg/mL)	TGF-β (pg/mL)	TLR4 (pg/mL)	Adiponectin (µg/mL)
PCOS and IR	**555.4** ± 46.5^{A}	**35.91** ± 4.99^{A}	**702.40** ± 74.8^{A}	**4.14** ± 1.01^{A}
	310.00-719.00	24.80-44.10	410.00-876.00	2.10-6.10
PCOS only	**303.40** ± 35.4^{B}	**29.81**± 4.29^{A}	**404.44** ± 52.5^{B}	**6.24** ± 1.11^{B}
	185.00-425.00	19.20-35.90	235.00-525.00	4.60-8.20
Control	**127.80** ± 17.4^{C}	**14.82** ± 3.56^{B}	**189.90** ± 22.80^{C}	**8.88** ± 1.82^{C}
	80.00-180.00	4.30-9.20	140.00-280.00	6.10-12.10
p-value	**0.001****	**0.001****	**0.001****	**0.001****

According to Duncan, multiple range comparisons (DMRTs) indicate that means with dissimilar letters after them are significantly different whereas means with similar letters after them are not.

SD: standard deviation; †: Independent T test; ******: significant at P > 0.05

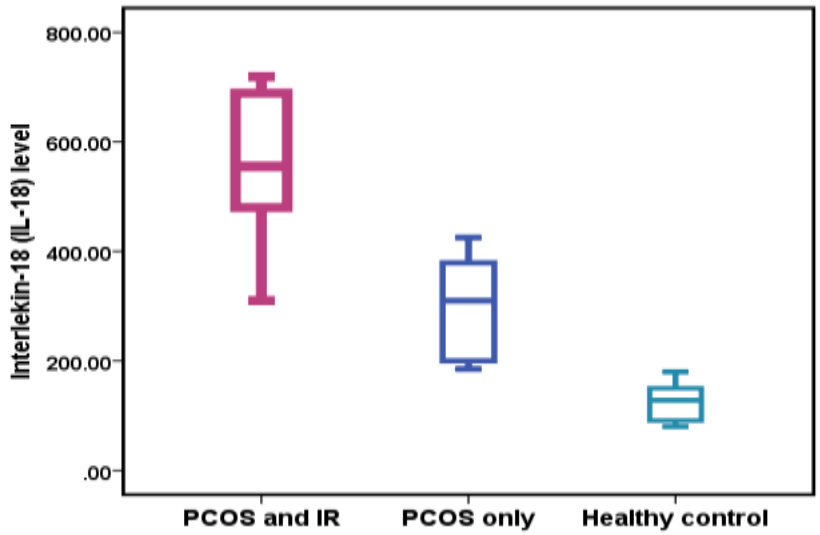

Figure (1): The means level of IL-18 in patients and control groups

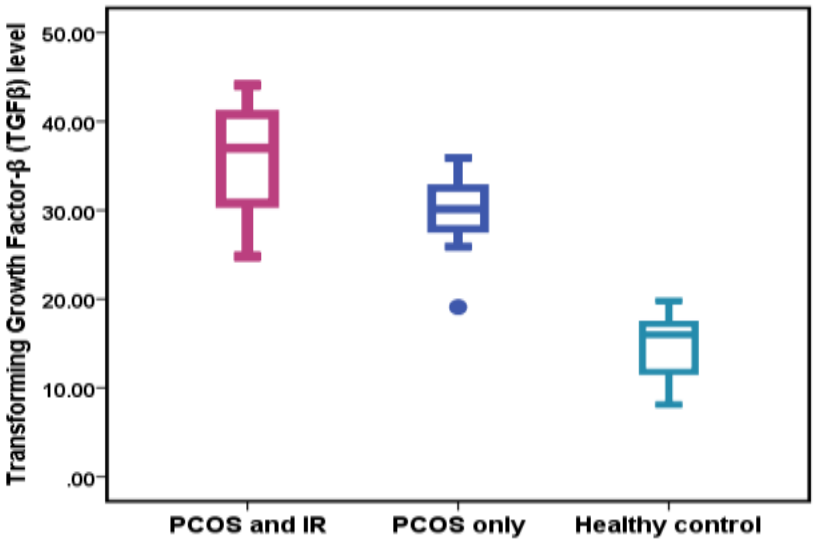

Figure (2): The means level of TGF-β in patients and control groups

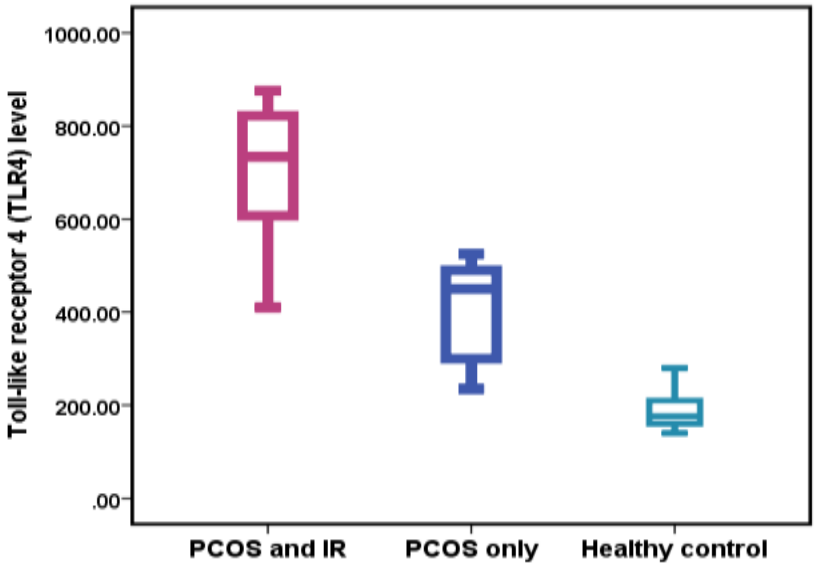

Figure (3): The means level of TLR-4 in patients and control groups

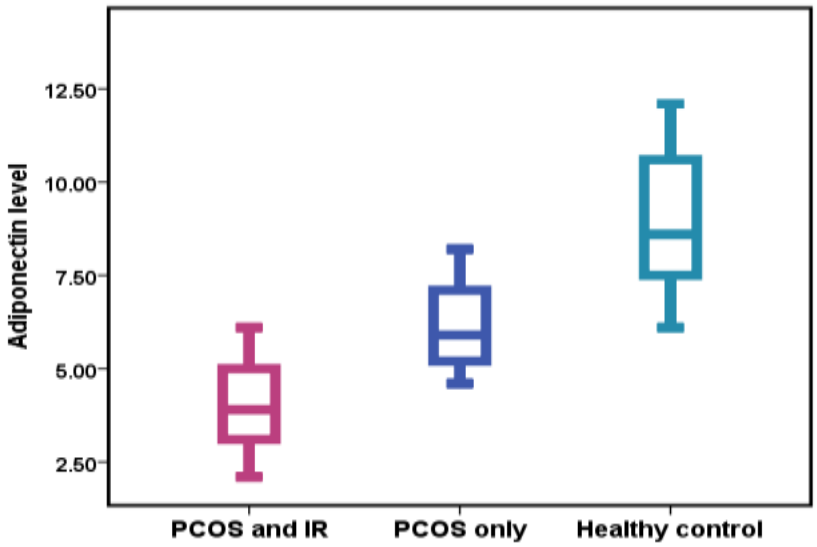

Figure (4): The means level of TLR-4 in patients and control groups

Correlation between Immunological parameters (IL-18, TGF-β, TLR4 and Adiponectin) and other parameters in PCOS patients with IR

Table (4) showed the association between IL-18, TGF- β, TLR4 and adiponectin and other markers in PCOS patients with IR. There are positive correlation between IL-18 and TGF-B (r=0.746 and p=0.001), IL-18 and TLR-4 (r=0.662 and p=0.001), IL-18 and LH (r=0.320 and p=0.011), IL-18 and Testosterone (r=0.680 and p=0.001), TGF-β and TLR-4 (r=0.732 and p=0.001), TGF-β and LH (r=0.331 and p=0.019), TGF-β and Testosterone (r=0.449 and p=0.001), TGF-β and FBG (r=0.354 and p=0.013), TLR-4 and FSH (r=0.417 and p=0.001), Adeponectin and HOMA-IR (r=0.480 and p=0.001), and Adeponectin and Insulin (r=0.332 and p=0.018) in PCOS patients with IR. But there are were non-significant correlation between all other parameters.

Table (4): Immunological markers (IL-18, TGF-β, TLR4, and adiponectin) and other factors in PCOS patients with IR are correlated.

Parameters	Immunological parameters							
	IL-18		TGF-β		TLR4		Adiponectin	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
IL-18	1							
TGF-β	0.746	0.001*	1					
TLR4	0.662	0.001*	0.732	0.001*	1			
Adiponectin	-0.106	0.604	-0.095	0.653	-0.052	0.806	1	
LH	0.320	0.021*	0.331	0.019*	0.250	0.228	0.094	0.517
FSH	-0.249	0.131	-0.094	0.665	0.417	0.001*	0.189	0.188
Testosterone	0.680	0.001*	0.449	0.001*	0.148	0.479	0.008	0.957

FBG	0.089	0.674	0.354	0.013*	0.069	0.744	0.110	0.446
HOMA-IR	0.165	0.429	-0.007	0.972	0.263	0.104	0.480	0.001*
Insulin	-0.180	0.390	0.178	0.396	-0.084	0.690	0.332	0.018*

r: correlation coefficient.

Correlation between Immunological parameters (IL-18, TGF-β, TLR4 and Adiponectin) and other parameters in PCOS patients only.

The correlations between (IL-18, TGF-β, TLR4 and Adiponectin) and other parameters in PCOS patients only were shown in tables (5). Also, there are positive correlation between IL-18 and TGF-β (r=0.61 and p=0.010), IL-18 and TLR-4 (r=0.558 and p=0.001), IL-18 and Testosterone (r=0.401 and p=0.001), IL-18 and FBG (r=0.549 and p=0.001), IL-18 and HOMO-IR (r=0.577 and p=0.001), IL-18 and insulin (r=0.717 and p=0.001), TGF-β and TLR-4 (r=0.463 and p=0.001), TGF-β and Testosterone (r=0.398 and p=0.004), TGF-β and FBG (r=0.389 and p=0.005), TGF-β and HOMO-IR (r=0.383 and p=0.006), TGF-β and insulin (r=0.395 and p=0.005), TLR-4 and Testosterone (r=0.331 and p=0.019), TLR-4 and FBG (r=0.564 and p=0.001), TLR-4 and HOMA-IR (r=0.643 and p=0.001), TGF-β and insulin (r=0.685 and p=0.001), in PCOS patients only. Furthermore, there was significant negative correlation between Adiponectin and IL-18(r=-0.466 and p=0.001),Adiponectin and TGF-β (r=-0.297 and p=0.037), Adiponectin and TLR-4 (r=-0.428 and p=0.001), Adiponectin and Testosterone (r=-0.346 and p=0.013), Adiponectin and HOMO-IR (r=-0.417and p=0.001) in PCOS patients only. But there are were non-significant correlation between all other parameters.

Table (5): correlation between other measures and immunological parameters in PCOS patients only (IL-18, TGF-β, TLR4, and adiponectin).

Parameters	**Immunological parameters**							
	IL-18		**TGF-β**		**TLR4**		**Adiponectin**	
	r	***P***	***r***	***P***	***r***	***P***	***r***	***P***
IL-18	1							
TGF-β	0.361	0.010*	1					
TLR4	0.558	0.001*	0.463	0.001*	1			
Adiponectin	-0.466	0.001*	-0.296	0.037*	-0.428	0.001*	1	
LH	0.243	0.089	0.163	0.258	0.168	0.244	-0.066	0.651
FSH	0.130	0.370	0.120	0.406	0.038	0.798	0.245	0.088
Testosterone	0.401	0.001*	0.398	0.004*	0.331	0.019*	-0.346	0.014*
FBG	0.549	0.001*	0.389	0.005*	0.564	0.001*	-0.278	0.054
HOMA-IR	0.577	0.001*	0.383	0.006*	0.643	0.001*	-0.417	0.001*

Insulin	0.717	0.001*	0.395	0.005*	0.685	0.001*	-0.412	0.001*

r: correlation coefficient.

Discussion

The findings of this paper indicate that extreme metabolic, hormonal and immunological disorders are common in women with polycystic ovarian syndrome (PCOS), particularly in women with insulin resistance (IR). PCOS patients with IR showed high levels of fasting blood glucose (FBG), HOMA-IR, and insulin in comparison to PCOS-only and healthy patients, which demonstrates the important role of IR in PCOS pathogenesis. So, the results are conciliated with the findings of other studies that demonstrate that IR impacts up to 70% of PCOS-affected women and is a key causative factor of hyperinsulinemia and metabolic dysfunction (Escobar-Morreale, 2022; Elsevier.Dunaif, 2016). As well as , the present research established that the PCOS patients had significantly greater levels of testosterone and LH with the IR group recording the highest levels. PCOS was typified by hyperandrogenism which was compounded by hyperinsulinemia which stimulates theca cells and suppresses sex hormone-binding globulin (SHBG) (Rosenfield, 2024; Azziz, 2021). So, the classical theory of the hypersecretion of LH in PCOS, which is caused by the imbalance of the hypothalamic-pituitary-ovarian axis, is justified by the fact that the levels of LH are increased in our study (Sánchez-Garrido *et al* ,. 2022). According to many studies, there exist comparable hormonal responses that relate elevated testosterone and LH with follicular stagnation and anovulation among patients of PCOS (Yang & Chen 2024; Malini & George 2018). And, one of the key conclusions of the study is the drastic increase of immunological and inflammatory markers of the PCOS patients, especially those having IR. Consequently , the levels of IL-18 in the IR group was significantly higher, and it corresponds to the previous literature that illustrates the function of IL-18 in oxidative stress, systemic inflammation, and insulin signaling impairment (Kabakchieva *et al* ,. 2022 ; Chen *et al* ,. 2023). As a result, the increase in the IL-18 inflammatory factor, which occurs to be a significant predictor of metabolic syndrome and IR, was in favor of the inflammatory nature of PCOS (Liu *et al* ,. 2015). Likewise , TGF-β levels was significantly elevated in PCOS patients with IR. TGF-β plays a vital role in the remodeling of the tissues, aberrant folliculogenesis and ovarian fibrosis. Thus , high levels have been observed to be associated with reproductive dysfunction and impaired ovarian microenvironment in PCOS (Raja-Khan Liu *et al* ,. 2014 ; Shah & Jirge2024). This study is in agreement with findings on the TGF-β stimulation of granulosa cells of PCOS patients (Shen & Wang2019). Moreover, TLR4, which was play a significant role in the activation of innate immune, was also significantly elevated, especially in the IR group. And , TLR4 activation of ovarian cells and adipose tissue was shown to enhance androgen production, hinder insulin receptor supply, and also facilitate the occurrence of inflammation (Hu *et al* ,. 2021). Therefore ,it was already reported that there is an increase in TLR4 expression in PCOS women with IR, which is closely linked to fat-related inflammation (Yuan *et al* ,. 2022; Behboudi-Gandevani *et al* ,. 2023). So , this makes the suggestion of metabolic inflammation being one of the primary causes of PCOS issues plausible. In contrast, adiponectin levels were significantly reduced in the two groups of PCOS patients. Adiponectin was an anti-inflammatory adipokine and regulates lipid metabolism and enhances insulin sensitivity (Nguyen, 2020) . So , low level of adiponectin are one of the well-established metabolic dysfunctions of PCOS that are associated with IR, hyperandrogenism, and also increased risk of cardiovascular disease (Patel *et al* ,. 2017; Jiang *et al* ,. 2025). As well as , the role of adiponectin as a

metabolic marker is also justified by the fact that the study report indicates that there was a negative correlation between adiponectin and HOMA-IR. The correlation study in this paper showed strong positive correlations between IL-18, TGF-β and TLR4 and the metabolic (FBG, insulin, HOMA-IR) and hormonal (LH, testosterone) markers. Thus , the above findings imply that there were interactions between endocrine imbalance, metabolic impairment, and inflammation. The protective metabolic effect of adiponectin was demonstrated by the negative associations of the metabolic and hormonal indicators with adiponectin (Jiang *et al* ,. 2025; Li *et al* ,. 2018) . In the same way , the equivalent patterns of connection have been discovered by recent investigations which have established that inflammation oversups and worsens metabolic inefficiency and excess androgen in PCOS (Armanini *et al* ,. 2022 ; Sharma *et al* ,. 2022). Overall, the results of the study confirm the idea that the worst inflammatory, metabolic, and hormonal changes are observed in PCOS and insulin resistance. These findings indicate that IR and inflammation are valuable therapeutic targets. Consequently , the use of inflammatory markers such as TLR4, TGF-β, and IL-18 in the clinical evaluation of PCOS could contribute to the better management and classification of the disease.

Conclusion

In this study, the immunological, hormonal, and metabolic markers abnormalities were demonstrated to be the most severe in insulin-resistant with PCOS patients. In addition ,the IL-18, TGF-β, TLR4, and adiponectin low levels were significantly associated with hyperandrogenism and metabolic abnormalities. Thus ,these links highlight the importance of considering inflammatory markers in PCOS diagnosis and treatment programs in a bid to improve clinical outcomes.

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