Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Genetic polymorphisms and immune cytokine profiles in the cellular pathogenesis of polycystic ovary syndrome among Iraqi women

Orass Madhi Shaheed Al-Taei[1*], Maather Baqer Hussein Al-Harmooshee[2]

[1] *Department of Microbiology, College of Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq*
[2] *Department of Bathology, College of Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq*

Article Info

Article history:

Received: April 28, 2025
Accepted: August 04, 2025
Published: September 30, 2025

Use your device to scan and read the article online

Abstract

Immune dysregulation and genetic polymorphisms are recognized as critical contributors to the pathogenesis of polycystic ovary syndrome (PCOS). The present study aimed to investigate the roles of specific cytokines and the CYP11A1 (rs4077582) polymorphism in Iraqi women diagnosed with PCOS. The current study included collecting samples from 100 women with PCOS and 100 healthy women as a control group. Clinical and laboratory tests were conducted at Diwaniyah General Hospital. The concentration of interferon-gamma, interleukin-2, interleukin-4 and interleukin-10 in the serum was determined utilizing the ELISA technique, while genetic CYP11A1(rs4077582) polymorphisms were determined using the PCR-RFLP technique. ELISA results demonstrated the concentration of interferon-gamma, interleukin-2 in FF in PCOS are significantly increased (123.8 ± 33.6, 45.77 ± 8.92 ng/ml respectively) compared with those in controls (23.5 ± 4.29, 7.31 ± 1.07 ng/ml respectively) while concentration of IL-4 and IL-10 reduced in patients (22.62 ± 6.24 and 5.81 ± 1.11 ng/ml respectively) compared to control (167.1 ± 18.62 and 37.54 ± 7.11ng/ml respectively). The results showed an increase in rate of mutant genotype CC and allele C in patients (34% and 64% respectively) compared to controls (12% and 25% respectively), while a decrease in the proportion of normal genotype TT and allele T in patients (6% and 36% respectively) compared with healthy subjects (62% and 75% respectively). immune function of IFN-γ, IL-2, IL-4 and IL-10 and CYP11A1(rs4077582) polymorphisms significantly associated with PCOS.

Keywords: CYP11A1, Polymorphism, PCOS, Hormones, Cytokines.

1. Introduction

Polycystic ovary syndrome (PCOS) is a multifactorial endocrine disorder characterized by oligo- or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic morphology of the ovaries as detected by ultrasound. It is one of the leading causes of female infertility and is commonly associated with metabolic disturbances [1]. It is important to appreciate that PCOS is a syndrome, not a disease, reflecting multiple potential etiologies and variable clinical symptoms [2]. The three most significant menstrual abnormalities associated with polycystic ovarian syndrome are oligomenorrhea (less than nine menstrual periods annually), amenorrhea (more than three months without menstruation), and erratic bleeding (loss of the cyclic menstrual pattern). About 85–90% of women with oligomenorrhea also have PCOS, and 30% to 40% of women with complete amenorrhea will also have PCOS. Additionally, 30% of women with PCOS will have regular menstruation [3]. A set of physiological alterations known as endometrial receptivity occurs when the endometrium offers the optimal conditions for embryo localization, adhesion, invasion, and implantation. Importantly, successful implantation of a fertilized ovum depends on the synchronized development of the embryo and the endometrium's ability to achieve an optimal state of tolerance. Endometrial receptivity refers to a series of physiological and molecular changes within the endometrium that create the appropriate conditions for embryo localization, adhesion, invasion, and subsequent implantation. Importantly, the key to figuring out whether the fertilized ovum can be successfully implanted is whether its timing and level of tolerance change in tandem with its growth [4]. During the implantation window, which usually lasts 30 to 36 hours after ovulation, the endometrium typically exhibits its maximum receptivity 6 to 9 days later [5]. In addition to being influenced by a number of genes, proteins, cytokines, and adhesion molecules, it needs the support of the estrogen and progesterone that the corpus luteum secretes. For blastocyst adhesion and implantation into the receptive endometrium, this time frame is essential. A close "dialogue" mechanism can then be established to finish the implantation once the embryo's invasive ability precisely matches the endometrium's receptivity [6Specifically, certain CYP gene SNPs may impact sex steroids, resulting in pathological or physiological alterations in people. The synthesis of sex hormones depends on the cholesterol side chain cleavage enzyme (CYP11A1) gene, which

* Corresponding author.
E-mail address: orass.shaheed@qu.edu.iq (O. M. Shaheed Al-Taei).
Doi: http://dx.doi.org/10.14715/cmb/2025.71.9.7

has also been linked to the pathophysiology of polycystic ovary syndrome. Cytochrome P450 family 11, subfamily A, includes CYP11A1. Cytochrome p450's superfamily is encoded. It can be found in the mitochondrial inner membrane [7].

Immune dysregulation is an important factor in the development of polycystic ovary syndrome, and chronic inflammation might be a critical underlying mechanism for polycystic ovary syndrome risk [8]. Immunomodulatory proteins called cytokines are crucial for managing immune system cell activity and function [9]. The pathophysiology of polycystic ovary syndrome has been thought to involve low levels of chronic inflammation and an imbalance between pro- and anti-inflammatory cytokines [10]. There is growing evidence that the etiology of PCOS may involve immune regulation and inflammation. But the underlying mechanisms are still unknown [7,10]. We attempted to explore the role of interferon-gamma, interleukin-2, interleukin-4 and interleukin-10 and *CYP11A1* gene polymorphism in development of PCOS.

2. Materials and methods

2.1. Study design

In this case-control study, 100 women with PCOS (ages 22–42) were enrolled from among the 200 women who were referred to the Consultant Clinic at the Department of Gynecology and Obstetrics in Al-Diwaniyah Teaching Hospitals. Of those patients, 50 were pregnant and 50 were not pregnant when they were enrolled in the study. On the other hand, 100 women who appeared to be in good health and were almost the same age as the patients made up the control group. The Iraqi Council for Medical Specializations has approved the study, and the included individuals have provided written consent. The clinic's consulting medical staff used international criteria based on laboratory and sonography evaluations to diagnose PCOS in the patients.

2.2. Collection of blood samples

Six milliliters of blood were collected from each participant in both the patient and control groups via venipuncture. Of this, 3 mL was drawn into a K3-EDTA anticoagulant tube and immediately transported to the laboratory, while the remaining 3 mL was collected in a plain tube for serological analysis.

2.3. Hormone levels

FSH, LH, Estrogen, Progesterone, Prolactin and Testosterone were also estimated automatically by GTQ system (USA).

2.4. Polymorphism genotyping analysis

Genomic DNA was extracted from leukocytes using the UltraPure™ Genome DNA Kit (SBS Genetech, Shanghai, China) and stored at -80°C until further analysis. Genotyping of the CYP11A1 (rs4077582) polymorphism was performed using the polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) technique. The sequences of the primers were 5'-GCC AGT CAG ACA AGG GCACAG GA-3' (forward) and 5'-GTG GCC GAC TAT GTAAAC CAG-3' (reverse) for rs 4077582. A total reaction volume of 25 µl, containing 50 ng of genomic DNA, 7.5 pmol of each primer, 2.5 µl of 10× STR buffer (Promega, Madison, WI, USA), and 0.75 U of GoTaq DNA polymerase (Promega, Madison, WI, USA), was used for PCR amplification. The PCR was performed in a MultiGene Gradient Thermal Cycler (Labnet, Edison, NJ, USA) as follows: 30 cycles including 1 min of denaturation at 94 °C, 1 min of annealing at 60 °C, and 1 min of extension at 72 °C. An initial denaturation step of 2 min at 96 °C and a final extension of 15 min at 72 °C were added. PCR products were digested with Mly I at 37 °C overnight. DNA fragments were then separated using electrophoresis on a 3% agarose gel that had been stained with ethidium bromide [11]. Images were taken, and image processing software was used to measure the lengths of the digits.

2.5. Serological test

Enzyme-linked immunosorbent assay (ELISA) was performed to quantitatively measure the levels of IFN-γ, IL-2, IL-4, and IL-10 in the follicular fluid (FF) of patients and healthy control samples, following the manufacturer's instructions (BT-LAB, USA).

2.6. Statistical analyses

The mean differences were statistically analyzed using unpaired, two-tailed tests. To compare differences in the case that the data were not normally distributed, a nonparametric test was employed. When doing statistical analysis using GraphPad Prism version 5.0 and SPSS Statistics 17.0, P-values of 0.05 were considered significant [12].

3. Results

A total of 200 women were enrolled in the present study, including 100 patients with PCOS and 100 healthy control subjects. The age of the patients ranged from 22 to 46 years, with a mean age of 34.75 ± 6.60 years, while the mean age of the control group was 36.83 ± 3.10 years (P = 0.6045).

A general clinical examination was conducted prior to oocyte retrieval. The baseline levels of FSH, LH, estrogens, prolactin, progesterone, and testosterone were among the plasma hormones that were measured. FSH levels were significantly lower in pregnant women compared to non-pregnant women in both the PCOS and control groups (4.49 ± 0.36 and 3.12 ± 0.16 IU/L for pregnant PCOS and control, respectively, versus 6.65 ± 0.16 and 5.43 ± 0.13 IU/L for non-pregnant PCOS and control, respectively (Table 1). Conversely, luteinizing hormone (LH) levels were higher in pregnant women in both groups (12.89 ± 8.04 and 7.60 ± 1.12 IU/L for pregnant PCOS and control, respectively) compared with non-pregnant women (6.32 ± 0.46 and 5.18 ± 1.23 IU/L for non-pregnant PCOS and control, respectively).

Estrogen levels in pregnant women were higher in the PCOS group compared to controls (47.2 ± 1.62 vs. 38.67 ± 1.3 pg/ml, respectively). In non-pregnant women, estrogen levels were slightly higher in controls than in the PCOS group (19.11 ± 0.9 vs. 18.27 ± 1.92 pg/ml, respectively). Prolactin concentrations were significantly elevated in pregnant women across both groups (47.2 ± 1.62 and 38.67 ± 1.3 mIU/L for PCOS and controls, respectively) compared with non-pregnant women (25.73 ± 1.38 and 20.13 ± 1.54 mIU/L for PCOS and controls, respectively). Notably, prolactin levels in pregnant women with PCOS were marginally lower than those in pregnant controls, but this difference was not statistically significant. As ex-

Table 1. Hormone levels (FSH, LH, Estrogens, Prolactin, Progesterone, and Testosterone) in patients and controls.

Level in serum	Pregnancy state	Groups		P value
		PCOS	Control	
		Mean ± SD	Mean ± SD	
FSH (IU/L)	**Pregnant**	4.49±0.36	3.12±0.16	0.644[NS]
	Non pregnant	6.65±0.156	5. 43±0.126	
LH (IU/L)	**Pregnant**	12.89±8.04	7.6±1.12	0.502 [NS]
	Non pregnant	6.32±0.46	5.18±1.23	
Estrogens (pg/mL)	Pregnant	48.77±2.22	23.45±1.36	0.0368 [S]
	Non pregnant	18.27±1.92	19.11±0.9	
Prolactin (mIU/L)	**Pregnant**	47.2±1.62	38.67±1.3	0.041[S]
	Non pregnant	25.73±1.38	20.13±1.54	
Progesterone (ng/ml)	**Pregnant**	27.2±1.52	16.48±1.3	0.044 [S]
	Non pregnant	14.2±1.44	10.22±1.14	
Testosterone (ng/dL)	**Pregnant**	12.49±0.49	6.67±0.37	0.078 [NS]
	Non pregnant	**22.42±0.47**	**7.57±0.46**	

PCOS: polycystic ovary syndrome. NOW: normally ovulating women. FSH: follicle stimulating hormone; LH: luteinizing hormone; SD: Standard Deviation; S: Significant difference at $p < 0.05$, NS: Significant difference at $p > 0.05$.

Table 2. Evaluation of FF level of IFN-γ, IL-2, IL-4 and IL-10 of patients and controls.

Conc.ng/ml	Mean ± SD		
	PCOS	Control	P value
IFN-γ	123.8 ± 33.6	23.5 ± 4.29	0.008 [S]
IL-2	45.77 ± 8.92	7.31 ± 1.07	0.012 [S]
IL-4	22.62 ± 6.24	167.1 ± 18.62	0.00012 [S]
IL-10	**5.81 ± 1.11**	**37.54 ± 7.11**	**0.024 [S]**

Table 3. Pearson correlation coefficient (r) between sex hormones and immunological markers.

Pearson correlation coefficient (r)	IFN-γ	IL-2	IL-4	IL-10
FSH	0.665	0.586	0.110	0.062
LH	0.583	0.499	0.044	-0.371
Estrogens	0.772	0.792	-0.133	-0.201
Prolactin	0.411	0.241	0.088	0.131
Progesterone	0.379	0.201	0.222	0.361
Testosterone	0.682	0.677	-0.261	-0.099

pected, progesterone levels were also higher in pregnant women compared to non-pregnant women in both groups (27.2 ± 1.52 and 16.48 ± 1.3 ng/ml in pregnant PCOS and controls, respectively; 14.2 ± 1.44 and 10.22 ± 1.14 ng/ml in non-pregnant PCOS and controls, respectively). In contrast to prolactin, testosterone concentrations were higher in non-pregnant women (22.42 ± 0.47 and 7.57 ± 0.46 ng/dL for PCOS and controls, respectively) than in pregnant women (12.49 ± 0.49 and 6.67 ± 0.37 ng/dL for PCOS and controls, respectively).

Additionally, the ELISA result showed that PCOS patients' FF had significantly higher levels of IFN-γ and IL-2 (123.8 ± 33.6, 45.77 ± 8.92 ng/ml respectively) compared with those in controls (23.5 ± 4.29, 7.31 ± 1.07 ng/ml respectively) while concentration of IL-4 and IL-10 reduced in patients (22.62 ± 6.24 and 5.81 ± 1.11 ng/ml respectively) compared to control (167.1 ± 18.62 and 37.54 ± 7.11ng/ml respectively) (Table 2).

Pearson's linear correlation showed that IFN-γ and IL-2 were associated with a clear positive linear relationship with hormones, especially FSH, estrogen, and testosterone. On the contrary, anti-inflammatory immune indicators (IL-4 and IL-10) were associated with a weak relationship with those hormones, especially FSH, estrogen, and testosterone (Table 3).

The results of our study showed an increase in the proportion of mutant genotype CC and allele C in patients (34% and 64% respectively) compared to controls (12% and 25% respectively), while a decrease in the proportion of normal genotype TT and allele T in patients (6% and 36% respectively) compared with healthy subjects (62% and 75% respectively) (Table 4). In Table 5, we found an increase in most hormones in patients carrying the mutant CC genotype, and a clear correlation appeared, resulting in a statistical difference ($P< 0.05$) when distributing the genotype according to the concentration of prolactin and estrogens.

The results presented in Table 6 show that individuals carrying the mutant CC genotype exhibited the highest concentrations of interleukin-2 and interferon-gamma (50.65 ± 8.11 ng/ml and 198.8 ± 22.6 ng/ml, respectively). In contrast, the highest level of interleukin-10 was observed in individuals with the heterozygous CT genotype (66.21 ± 12.09 ng/ml). Additionally, the lowest levels of

Table 4. Genotype frequencies of *CYP11A1* gene polymorphism among studied groups.

Genotype of CYP11A1	PCOS		control		P -value	OR	CI=95
	No.	%	No.	%			
CC	34(34%)		12(12%)		0.033*	1.23	0.44 – 6.77
CT	60(60%)		26(26%)		0.021 *	0.82	(0.37- 3.84)
TT	6 (6%)		62 (62%)		0.001*	2.86	(0.01- 0.20)
Total number	100		100				
Alleles of CYP11A1							
C	128 (64%)		50 (25%)		0.019*	2.86	0.67 -2.91
T	72 (36%)		150 (75%)		0.017*	0.18	0.77 – 0.91
Total	**200**		**200**				

No: number of cases; OR: Odds ratio; CI: Confidence interval;* significant difference at P<0.05.

Table 5. Evaluation of hormone levels according to *CYP11A1(rs4077582)* genotypes.

Level in serum	Pregnancy state	Groups			P value
		CC	CT	TT	
		Mean ± SD	Mean ± SD		
FSH (IU/L)	**Pregnant**	4.77±0.41	4.38±0.15	4.44±0.24	0.701
	Non pregnant	6.9±0.156	5. 74±0.14	5.11±0.22	
LH (IU/L)	**Pregnant**	16.89±8.04	8.45±1.12	8.41±1.21	0.215
	Non pregnant	18.53±1.33	18.18±1.61	11.20±2.09	
Estrogens (pg/mL)	Pregnant	52.8±6.31	33.72±5.53	20.6±7.51	0.027*
	Non pregnant	11.12±2.17	22.13±4.82	22.24±5.02	
Prolactin (mIU/L)	**Pregnant**	55.3±10.5	47.55±11.91	40.7±7.81	0.038*
	Non pregnant	33.14±5.34	33.10±6.29	20.81±4.25	
Progesterone (ng/ml)	**Pregnant**	30.2±4.11	27.48±6.72	20.99±4.14	0.064
	Non pregnant	15.2±3.28	15.7±4.21	13.72±3.66	
Testosterone (ng/dL)	**Pregnant**	14.44±7.22	15.8±3.77	7.51±1.66	0.113
	Non pregnant	**25.39±7.42**	**23.15±4.61**	**10.73±2.85**	

SD: Standard Deviation; * significant difference at P<0.05.

Table 6. Distribution of immunological markers according to *CYP11A1(rs4077582)* genotypes.

Conc.ng/ml	Mean ± SD			
	CC	CT	TT	P value
IFN-γ	198.8 ± 22.6	143.5 ± 22.33	78.63 ± 14.5	0.023*
IL-2	50.65 ± 8.11	44.31 ± 9.10	44.11 ± 11.4	0.204
IL-4	108.1 ± 18.62	200.62 ± 6.3	116.6 ± 23.6	0.037*
IL-10	**40.67 ± 11.6**	**66.21 ± 12.09**	**58.65 ± 6.52**	**0.048***

SD: Standard Deviation; * significant difference at P<0.05.

interleukin-4 and interleukin-10 were found in cases with the mutant CC genotype (108.1 ± 18.62 ng/ml and 40.67 ± 11.6 ng/ml, respectively).

4. Discussion

Women with PCOS are experiencing pathological symptoms due to hormonal changes. It has been shown that PCOS causes a variety of hormone changes. According to the current study, PCOS women have abnormal levels of androgens, estrogens, and the luteinizing hormone/follicle-stimulating hormone (LH/FSH) ratio. Infertility, overweight and obesity, insulin resistance, diabetes, and irregular menstruation in PCOS patients are all associated with these hormones [13]. PCOS women are more likely to have diabetes and obesity due to the pathological alterations of these hormones, which include increased insulin, decreased GH, increased ghrelin, and leptin resistance. The high LH basal, increased LH/FSH ratio, high androgens, and low estrogen are demonstrated in PCOS and linked to infertility [14].

The present study hypothesized that, since FSH plays a crucial role in follicular development and ovulation processes suppressed during pregnancy, FSH levels would differ accordingly. Consistent with this, our results showed that non-pregnant women with PCOS exhibited higher FSH levels compared to the control group. This is a characteristic feature of PCOS, where chronic anovulation leads to elevated FSH levels to stimulate follicle development. LH appeared high in pregnant women across all groups (LH=12.89±8.04 and 7.6±1.12 IU/L for PCOS and control, respectively) compared with non-pregnant women (LH=6.32±0.46 and 5.18±1.23 IU/L for PCOS and control, respectively) due to increased placental production [15]. LH levels were significantly higher in non-

pregnant women compared to pregnant control groups, potentially reflecting underlying hormonal imbalances. Similarly, testosterone levels were elevated in non-pregnant groups (22.42 ± 0.47 ng/dl in PCOS and 7.57 ± 0.46 ng/dl in controls) compared to pregnant women (12.49 ± 0.49 ng/dl in PCOS and 6.67 ± 0.37 ng/dl in controls), whereas prolactin levels showed a different pattern. These findings are based on average values and individual variations exist within each group. Other factors, like menstrual cycle phase, can influence hormone levels [16,17]. Consulting a healthcare professional for personalized interpretation is recommended. There were differences between our data and earlier reports. The genetic and demographic distinctions between Asian and European or American people may be the cause of this disparity [16-18]. The immune regulatory mechanisms and immune barriers play a crucial role in follicle development, fertilization, and implantation of the fertilized egg in the uterus [19]. The present study evaluated the roles of various pro-inflammatory, anti-inflammatory, and dual-function cytokines that differ between patients and controls. We found the highest concentrations of interferon-gamma and interleukin-2 in individuals carrying the mutant CC genotype, whereas the highest levels of interleukin-10 were observed in those with the heterozygous CT genotype. Moreover, we found a lower level of interleukin-4 and interleukin-10 in cases with mutant CC genotype. However, interleukin-10 is a cytokine produced by T-H2 cells, macrophages and T cells [19]. interleukin-10 inhibits the action of such pro-inflammatory cytokines as interleukin-6, interleukin-1 and Tumor Necrotic Factor- alpha [20]. Numerous investigations have documented a connection between PCOS and IL-10 polymorphism [20,21]. Abraham Gnanadass *et al*. showed that abnormal levels of cytokines, such as IL-4, IL-8, and IL-10, result in ovarian dysfunction [21]. In a study by Talaat *et al*. (2016), interleukin-10 levels were lower in women with polycystic ovary syndrome [22]. A crucial type-2 T helper (Th2) cytokine, IL-4, is involved in inflammation, the regulation of antibody production, and the maturation of effector T-cell responses [23]. Regardless of BMI, IL-4 levels in PCOS patients were significantly lower than those in healthy women [24]. Serum levels of IL-4 were lower in PCOS patients than in controls, according to a different study that examined the profiles of the type-1 T helper (Th1) (interferon-gamma, IL-2) and Th2 (IL-4, IL-10) cytokines of CD3+CD4+ T lymphocyte subsets in follicular fluid [25].

Additionally, because PCOS women have a large number of follicles without ovulation, their estrogen levels are elevated, which increases Th1 cell secretion of inflammatory cytokines like IL-2 and IFN-γ [26]. When compared to healthy controls, obese PCOS patients have significantly higher levels of IL-2 in both serum and FF, according to research on the relationship between PCOS and IL-2 [27, 28]. They have also found that polycystic ovary syndrome patients exhibit decreased levels of regulatory T cells due to an inherent hypo-responsiveness to interleukin-2 [29]. Our findings support the findings of previous clinical analysis that women with PCOS have higher levels of circulating VEGF and IL-2, but more research is required to completely comprehend the underlying molecular pathways [30].

A crucial marker in the steroid synthesis pathway, CYP11A1's altered expression has been shown to interfere with steroid synthesis, increasing the risk of PCOS development. The current study found a significant correlation between PCOS and the CC genotype and the C allele. A linkage analysis conducted by Urbanek et al. [37] involving 37 candidate genes also identified a possible association between PCOS and both CYP11A1 and CYP17. Therefore, these important genes in the androgen biosynthesis pathway may be suitable candidates to help identify the genetic cause of the hyperandrogenism linked to PCOS. These findings contradict the observation of Diamanti-Kandarakis *et al.* [32], who failed to find any correlation between this polymorphism and hyperandrogenism or cystic ovaries. Additionally, a previous extensive examination of the CYP11A1 promoter in roughly 500 PCOS cases did not find any evidence linking the CYP11A1 polymorphism to the PCOS phenotype, especially hyperandrogenism [30]. Our findings are supported by a study by Zang et al. that found that people with different rs4077582 genotypes may respond differently to LH stimuli due to differences in LH levels between genotypes, which influence downstream steroid hormone levels [11]. The heterozygous CT genotype's testosterone level was significantly higher than the TT genotype's, as predicted, and was most likely positively regulated by LH. However, no difference in LH levels was found between the three rs4077582 genotypes in PCOS patients, most likely as a result of the abnormal internal LH secretion in PCOS patients [33].

We conclude from previous research that *CYP11A1* polymorphism is linked to the regulation of sex hormones, which in turn directly affects the immune response, and this explains the association of the immune indicators in our study with *CYP11A1(rs4077582)* genetic variation, as hormonal disturbance directly affects the occurrence of inflammation and cysts of reproductive cells in the ovaries, which leads to an imbalance in the level of some cytokines as interferon-gamma, interleukin-2, interleukin-4 and interleukin-10 Studies have been shown that polycystic ovary syndrome is a chronic low-level inflammation and this chronic disease can be a potential cause of the long-term consequence of polycystic ovary syndrome [34].In vitro studies suggest that pro/anti-inflammatory cytokines are capable of up-regulation of the genetic basis as *CYP11A1* SNPs for steroidogenic enzymes for the production of androgens in theca cells of the ovary (hyperandrogenism). This concept raises the possibility that the *CYP11A1* gene may be capable of directly inducing hyperandrogenism in polycystic ovary syndrome through hormones or indirectly by inducing the immune system [35].

The findings of our investigation revealed a clear association of the interferon-gamma, interleukin-2, interleukin-4 and interleukin-10 and CYP11A1 with development or occurrence of polycystic ovary syndrome. We found a high concentration of the studied hormones in patients carrying the mutant *CYP11A1(rs4077582)* CC genotype. We also found the highest concentration of interferon-gamma and interleukin-2 in people carrying the *CYP11A1(rs4077582)* mutant genotype (CC), while the lower levels of interleukin-4 and interleukin-10 were determined in cases with the CC genotype. From this, we conclude that *CYP11A1(rs4077582)* genetic variation and the immune levels of IFN-γ, IL-2, IL-4, and IL-10 may be a target for treatments or indicators for diagnosing the polycystic ovary syndrome.

Ethical considerations

The research was approved by the Research Ethical Committees of Al-Qadisiyah University. Informed consent was obtained from all participants and/or their legal guardians.

Acknowledgements

Special thanks to the specialists and all participants who contributed to the collection of blood samples and provided the required data.

Funding

No external funds were received (private funding).

Competing interest

The authors have disclosed that they have no struggles of attention.

Data availability

All data generated or analyzed during this study are included in this published article. Additional information is available from the corresponding author upon reasonable request.

Use of artificial intelligence tools

Some sentences in this manuscript were revised using artificial intelligence language models to enhance clarity and readability. All final content decisions and intellectual contributions remain solely the responsibility of the authors.

References

1. Saleh FL, Starkman H, Furness A, Pfeifer SM, Kives S (2024) Polycystic ovary syndrome in adolescents. Obstet Gynecol Clin North Am 51(4):679-693. doi:10.1016/j.ogc.2024.08.005
2. Christodoulopoulou V, Trakakis E, Pergialiotis V, Peppa M, Chrelias C, Kassanos D, Papantoniou N (2016) Clinical and biochemical characteristics in PCOS women with menstrual abnormalities. J Family Reprod Health 10(4):184-190
3. Łagowska K, Pieczyńska JM (2022) Not only diet quality and physical activity but also snacking and skipping meals could be related with menstrual disorders in PCOS. Women Health 62(8):667–677. doi: 10.1080/03630242.2022.2106529
4. Lessey BA, Young SL (2019) What exactly is endometrial receptivity? Fertil Steril 111:611–7
5. Paulson RJ (2019) Introduction: Endometrial receptivity: evaluation, induction and inhibition. Fertil Steril 111:609–10
6. Ruiz-Alonso M, Valbuena D, Gomez C, Cuzzi J, Simon C (2021) Endometrial receptivity analysis (ERA): data versus opinions. Hum Reprod Open 2021(2):hoab011
7. Hu Y, Wang L, Yang G, Wang S, Guo M, Lu H, Zhang T (2023) VDR promotes testosterone synthesis in mouse Leydig cells via regulation of cholesterol side chain cleavage cytochrome P450 (Cyp11a1) expression. Genes Genom 45(11):1377–1387. doi: 10.1007/s13258-023-01444-z
8. Qin L, Xu W, Li X, Meng W, Hu L, Luo Z, Wang Y, Luo S, Li S (2016) Differential expression profile of immunological cytokines in local ovary in patients with polycystic ovarian syndrome: analysis by flow cytometry. Eur J Obstet Gynecol Reprod Biol 197:136–141. doi: 10.1016/j.ejogrb.2015.12.003
9. Qin L, Xu W, Li X, Meng W, Hu L, Luo Z, Wang Y, Luo S, Li S (2016) Differential expression profile of immunological cytokines in local ovary in patients with polycystic ovarian syndrome: analysis by flow cytometry. Eur J Obstet Gynecol Reprod Biol 197:136–141
10. Deng H, Chen Y, Xing J, Zhang N, Xu L (2024) Systematic low-grade chronic inflammation and intrinsic mechanisms in polycystic ovary syndrome. Front Immunol 15:1470283. doi: 10.3389/fimmu.2024.1470283
11. Shyma KP, Gupta SK, Gupta JP, Singh A, Chaudhari SS, Singh V (2016) Restriction site detection in repetitive nuclear DNA sequences of Trypanosoma evansi for strain differentiation among different isolates. J Parasit Dis 40(3):1087–1090. doi: 10.1007/s12639-014-0582-8
12. Riffenburgh RH (2012) Statistics in medicine. Academic Press, London
13. Yang J, Chen C (2024) Hormonal changes in PCOS. J Endocrinol 261(1):e230342
14. Kambale T, Sawaimul KD, Prakash S (2023) A study of hormonal and anthropometric parameters in polycystic ovarian syndrome. Ann Afr Med 22(1):112-116
15. Cao NT, Le MT, Nguyen VQH, Pilgrim J, Le VNS, Le DD, Pham CK, Aharon D, Hill MJ (2019) Defining polycystic ovary syndrome phenotype in Vietnamese women. J Obstet Gynaecol Res 45(11):2209-2219
16. Al-Harmooshee MB, Shaheed OM (2020) Germline mutation of RAD51 single nucleotide polymorphisms as susceptibility factor for breast and ovarian cancer, Iraq. Sys Rev Pharm 11(10):100-108
17. Lie Fong S, Laven JSE, Duhamel A, Dewailly D (2017) Polycystic ovarian morphology and the diagnosis of polycystic ovary syndrome: redefining threshold levels for follicle count and serum anti-Müllerian hormone using cluster analysis. Hum Reprod 32(8):1723-1731
18. Rudnicka E, Kunicki M, Calik-Ksepka A, Suchta K, Duszewska A, Smolarczyk K, Smolarczyk R (2021) Anti-Müllerian hormone in pathogenesis, diagnostic and treatment of PCOS. Int J Mol Sci 22(22):12507
19. Bülow NS, Warzecha AK, Nielsen MV, Andersen CY, Holt MD, Petersen MR, Sopa N, Zedeler A, Englund AL, Pinborg A, Grøndahl ML, Skouby SO, Macklon NS (2023) Impact of letrozole co-treatment during ovarian stimulation on oocyte yield, embryo development, and live birth rate in women with normal ovarian reserve: secondary outcomes from the RIOT trial. Hum Reprod 38(11):2154-2165
20. Vasyukova E, Zaikova E, Kalinina O, Gorelova I, Pyanova I, Bogatyreva E, Vasilieva E, Grineva E, Popova P (2023) Inflammatory and anti-inflammatory parameters in PCOS patients depending on body mass index: a case-control study. Biomedicines 11(10):2791
21. Abraham Gnanadass S, Divakar Prabhu Y, Valsala GA (2021) Association of metabolic and inflammatory markers with polycystic ovarian syndrome (PCOS): an update. Arch Gynecol Obstet 303(3):631–43
22. Talaat RM, Mohamed YA, Mohamad EH, Elsharkawy M, Guirgis AA (2016) Interleukin 10 (- 1082 G/A) and (- 819 C/T) gene polymorphisms in Egyptian women with polycystic ovary syndrome (PCOS). Meta Gene 9:254-8
23. Brown MA, Hural J (1997) Functions of IL-4 and control of its expression. Crit Rev Immunol 17(1):1-32
24. Burgert T P (2024). PCOS and common androgen abnormalities in adolescents. N. K. Tyson, NASPAG Essentials of Pediatric & Adolescent Gynecology (s. 190-204). Elsevier
25. Qin L, Xu W, Li X, Meng W, Hu L, Luo Z, Wang Y, Luo S, Li S (2016) Differential expression profile of immunological cytokines in local ovary in patients with polycystic ovarian syndrome: analysis by flow cytometry. Eur J Obstet Gynecol Reprod Biol 197:136-141

26. Hu C, Pang B, Ma Z, Yi H (2020) Immunophenotypic profiles in polycystic ovary syndrome. Mediators Inflamm 2020:5894768
27. Shabbir S, Khurram E, Moorthi VS, Eissa YTH, Kamal MA, Butler AE (2023) The interplay between androgens and the immune response in polycystic ovary syndrome. J Transl Med 21(1):259
28. Šimková M, Vítků J, Kolátorová L, Vrbíková J, Vosátková M, Včelák J, Dušková M (2020) Endocrine disruptors, obesity, and cytokines – how relevant are they to PCOS? Physiol Res 69(Suppl 2):S279-S293
29. Dambala K, Paschou SA, Michopoulos A, Siasos G, Goulis DG, Vavilis D, Tarlatzis BC (2019) Biomarkers of endothelial dysfunction in women with polycystic ovary syndrome. Angiology 70(9):797-801
30. Chang K J, Chen J H, & Chen K H (2024). The Pathophysiological Mechanism and Clinical Treatment of Polycystic Ovary Syndrome: A Molecular and Cellular Review of the Literature. International journal of molecular sciences, 25(16), 9037. https://doi.org/10.3390/ijms25169037
31. Gaasenbeek M, Powell BL, Sovio U, Haddad L, Gharani N, Bennett A, Groves CJ, Rush K, Goh MJ, Conway GS, Ruokonen A, Martikainen H, Pouta A, Taponen S, Hartikainen AL, Halford S, Järvelin MR, Franks S, McCarthy MI (2004) Large-scale analysis of the relationship between CYP11A promoter variation, polycystic ovarian syndrome, and serum testosterone. J Clin Endocrinol Metab 89(5):2408-13
32. Urbanek M, Legro RS, Driscoll DA, Azziz R, Ehrmann DA, Norman RJ, Strauss JF 3rd, Spielman RS, Dunaif A (1999) Thirty-seven candidate genes for polycystic ovary syndrome: strongest evidence for linkage is with follistatin. Proc Natl Acad Sci U S A 96(15):8573-8
33. Repaci A, Gambineri A, Pasquali R (2011) The role of low-grade inflammation in the polycystic ovary syndrome. Mol Cell Endocrinol 335:30–41
34. Shorakae S, Teede H, de Courten B, Lambert G, Boyle J, Moran LJ (2015) The emerging role of chronic low-grade inflammation in the pathophysiology of polycystic ovary syndrome. Semin Reprod Med 33:257–269
35. Diamanti-Kandarakis E, Bartzis MI, Zapanti ED, Spina GG, Filandra FA, Tsianateli TC, Bergiele AT, Kouli CR (1999) Polymorphism T→C (-34 bp) of gene CYP17 promoter in Greek patients with polycystic ovary syndrome. Fertil Steril 71(3):431-5