ALKUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544. 2024.*16* (2), 79–88.

Original Research Paper

The correlation of interleukin 10 gene expression with hormones changes in polycystic ovary syndrome

Rana jaleil AL-Khafaji [1]
Basaad Abed zaid Al-Fatlawi [2]
Abdulnabi jwaid AL-mamory [3]

[1,2] *Department of Biology, Faculty of Science, University of Kufa, Iraq*
[3] *Department of Biology, Faculty of Science for Women , University of Babylon ,Iraq*

Article history
Received: 30/03/2024
Revised: 02/06/2024
Accepted: 12/06/2024

*Corresponding Author: *Rana jaleil AL-Khafaji*
Department of Biology, Faculty of Science, University of Kufa, Iraq
Rana987edu@gmail.com

Abstract: *The current study was done to determine the molecular status and hormone changes in polycystic ovary syndrome and polycystic ovary disease. The study included 155 clinical specimens that included age (20-45) years, which were collected from November 2022 to June 2023. The specimens included blood specimens for patients visiting Imam Al-Sadiq Hospital and Babylon Teaching Hospital for Maternity and Children in Babylon Governorate. The results of hormones included elevation in levels of prolactin and Free Testosterone were obtained in PCOS, PCOD patients group compared with healthy women. Also, the study of some genes, including a study of genetic polymorphisms of the cellular motor gene IL10.*

Keywords: *PCOS, PCOD, IL-10 gene, Prolactin, Free testosterone*

DOI: 10.36320/ajb/v16.i2.15683

1. Introduction

Women with polycystic ovary syndrome (PCOS) have irregular periods (oligomenorrhea or amenorrhoea), high amounts of androgenic hormones (hyperandrogenism), and many cysts on their ovaries (polycystic ovaries). Male pattern hirsutism, acne, increased skin pigmentation with tumours occasionally, and obesity are additional characteristics [1]. In addition to obesity, insulin resistance, and dyslipidemia, PCOS significantly increases the risk of developing metabolic and cardiovascular complications, such as diabetes and metabolic syndrome [2] . The normal ovulatory menstrual cycle depends on well-harmonized hormonal feedback loops and a completely established hypothalamic-pituitary-ovarian axis. The typical menstrual cycle, which includes three stages (the luteal phase, the ovulatory phase, and the follicular phase), results in the development of a mature follicle and the release of an egg at each cycle; menses would occur in the absence of fertilization [3]. At the luteal-follicular transition, every woman of reproductive age has an increase in FSH levels, which stimulates a group of follicular growth in the early follicular phase. A week before ovulation, the dominant follicle is identified in the mid-follicular phase. As it expands, it increasingly secretes oestradiol and inhibin A, followed by an LH surge and an increase in oestradiol. In response to LH pulses, the corpus luteum secretes progesterone, estrogen, and inhibin A, which reach their peak in size, vascularization, and secretions [4].

2. Methodology

Collection of specimens

The study included 155 clinical specimens, which were collected from November 2022 to June 2023. The specimens included blood specimens. About 75 specimens were collected from women with polycystic ovarian syndrome (PCOS), and 30 specimens were collected from women with polycystic ovarian disease (PCOD). They visit Imam Al-Sadiq Hospital and Babylon Teaching Hospital for Maternity and Children in Babylon Governorate. At the same time, 50 specimens were collected from women without polycystic ovaries as a healthy group.

blood specimens

I. The blood was collected using sterile syringe 5ml, then separated to 1ml with anticoagulant tube and 4 ml without anticoagulant tube.
II. The blood without an anticoagulant tube allowed clotting at room temperature.
III. The serum was separated by centrifugation at about 3000 rpm for 5min within 2-3 hours after collection [5].

Genomic RNA Extraction

Whole blood samples were obtained in EDTA tubes, then utilizing RNA Extraction TransZol Up and following the manufacturer's Biotech(China).

Gene Expression Study for IL-10 Gene By qRT-PCR

Table (1). PCR reaction conditions

Step	Temperature (°C)	Time	Repeat cycle
Reverse transcription	37°C	15min	45 cycle
RT inactivation/Hot-start activation	95°C	10min	
Denaturation	95°C	30sec	
Annealing	57°C	20 sec	
Extension	72°C	30sec	

Measurements of reverse transcriptase to measure relative quantification (gene expression analysis), real-time PCR was used. The experiment was designed to compare the gene expression between three groups of specimens belonging to blood: the first group represents PCOS, the second group represents PCOD and the third group is considered as control group. This experiment screened gene expression of the IL-10 gene that contains an RNA genome for(45 blood specimens). After being separated and purified, total RNA was reverse-transcribed into cDNA. The IL10 gene (1082 bp) was intended to be amplified by gene fragments using primers intended for quantitative real-time PCR (qPCR) (Forward: **5'**CCAAGAC **3'**AACACTACTAAGGCTCCTTT**3')** **(**Reverse:**5'** GCTTCTTATATGCTAGTCAGGT **3'**), housekeeping gene (*rpoS* gene) 135 bp (Forward: GTTTTGTAGTTTTTGGAGTTAGTGTTGTGT; 54 Reverse:CTCAACCTACAATCAAAAACAACACAA ACA). The *rpoS* reference gene was amplified as described previously. As described previously, the qRT-PCR reaction mixtures, amplification parameters, and melt curve analyses were executed. The expression levels of oprM were normalized to that of the reference gene *rpoS* using the ΔΔC(t) method and 2- ΔΔC (fold change).

Quantitative Real-Time PCR (qPCR)

This method was carried out by *TransScript*® II Green One-Step qRT-PCR SuperMix for the genes and the

DOI: 10.36320/ajb/v16.i2.15683

components' reaction and their volumes as in Table (1).

Table 1: master mix component used to prepare qRT-PCR reaction

Component	Volume
Master Mix	10µl
Forward primer	2µl
Reverse primer	2µl
RT Mix for 1-Step RT-qPCR	0.4µl
RNA Template	3.7µl
MgCl2	1.6µl
CXR Reference Dye	0.3µl
Total	20 µl

Quantification Real-Time PCR (qRT-PCR) protocol

The qPCR plate was exposed to specified thermocycler conditions, as shown below (Mitchell *et al*., 2008).

Table 2: The program employed in the qPCR for IL-10 gene and *rpoS* (housekeeping gene) .

Statistical analysis

Number and percentage were used to express categorical variables. Parametric variables were given as mean ± standard deviation (SD) and significant differences were assessed using the least significant difference (LSD) test. Nonparametric variables were expressed as the median and interquartile range (IQR), and the Mann-Whitney U test (to compare two groups) and the Kruskal-walls test (to compare three or more groups) were used to assess significant differences between medians. The statistical analysis was performed using GraphPad Prism version 9.5.0 (San Diego, California USA).

The data results of qRT-PCR for target and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) (The ΔCt Method Using a reference gene) described by (Livak and Schmittgen ,2001) as the 60 following equation 3.1 ΔCt (contorl) = Ct (ref,control) – Ct (target,control) 3.1 Where Ct is the cycle threshold, control means the control measured Ct, ref refers to reference gene measured Ct. 2- ΔΔC (fold change).

3. Results and discussion

Genotype Study Results

The impact of hereditary factors on the development of PCOS is believed to be significant. This research aims to examine the genetic link between IL-10 and PCOS and PCOD by investigating the rs1082 A/G variant. Single nucleotide polymorphism (SNP) of the *IL-10* gene

Real-Time PCR for interleukin 10 Gene (rs 1082 A/G) Polymorphism

For 45 blood specimens out of 155 only included 20(48.78%) specimens for polycystic ovary syndrome (PCOS) and 15 (36.58%) specimens for polycystic ovary disease (PCOD), whereas 10 (14.63%) specimens for healthy controls results. Table (3) and Figure (1).

Table (3): gene expression of *IL-10* gene (rs1082 A/G)

rs1082 A/G gene expression	PCOS group Mean ± SD	PCOD group Mean ± SD	Control group Mean ± SD	P-value
average Fold change	24.048 ±5.27	19.69± 3.72	16.24 ±1.88	0.005

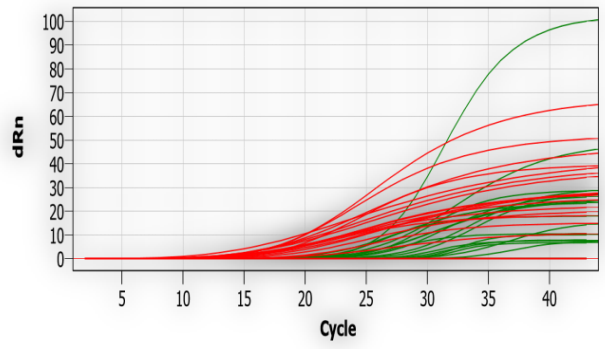


Figure 1: Amplification of RT-PCR of the *IL-10* gene in blood isolates for PCOS, PCOD and controls melting curve (green trace for gene, red trace for housekeeping gene.

The results of the study revealed that showed an average of the fold change elevation in both PCOS, PCOD patients group but the level is higher in PCOS more than PCOS with significant difference (P <0.005) when compared with healthy control group**.** Quantification of gene expression contributes to the study of PCOS,PCOD,responses to changes in circumstances and provides an understanding of resistance mechanisms. To obtain reliable gene expression results.Gene expression analysis has become increasingly significant in biological research.

DOI: 10.36320/ajb/v16.i2.15683

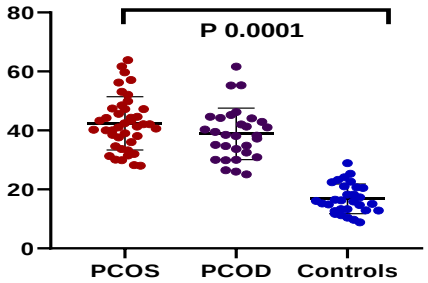
Today, biomedicine is greatly facilitated by revolutionary developments in microarray technology and bioinformatics, which allow us to explore disease-related alterations in transcriptional and related biological pathways. Several published studies have used gene expression datasets obtained from the Gene Expression Omnibus (GEO) database to illuminate the biologic mechanisms underlying the pathogenesis of PCOS .The results of those bioinformatic analyses provide ponderable hints to researchers for comprehending the pathogenesis of PCOS from different perspectives.

The Biochemical Parameters of PCOS, PCOD and Control Group

Table (4) and Figure (2) provide comparisons between the biochemical parameters of the research groups. Prolactin (P<0.0001) and free testosterone (P<0.0001) levels were significantly higher in the PCOS, PCOD patient group than in the healthy women group.

Table (4): Biochemical characteristics of the patients (PCOS, PCOD) and control groups.

Parameter	**PCOS group Mean ± SD**	**PCOD group Mean ± SD**	**Control group Mean ± SD**	**P- value**
Prolactin (ng/mL)	41.61± 8.66	38.48± 8.92	16.34 ±5.18	0.0001
Free Testosterone (pg/Ml)	16.20 ±3.8	14.98± 3.44	3.17± 1.8	0.0001



A- Prolactin (ng/mL)

DOI: 10.36320/ajb/v16.i2.15683

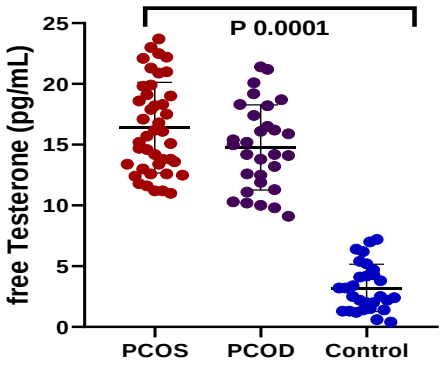


Figure 2: Difference between levels of (A) Prolactin and (B) Free Testosterone between PCOS, PCOD patients and healthy control group

The most common causes of female infertility are hyperprolactinemia and polycystic ovary syndrome (PCOS) [6]. Diverse views exist about the correlation between PCOS and hyperprolactinemia: on the one hand, high prolactin levels are identified as a feature of PCOS, while on the other hand, hyperprolactinemia must be ruled out [7]. Prolactin production may increase in PCOS due to several potential reasons.

The impact of oestrogens on lactotropic pituitary cells is mediated by their stimulation of prolactin synthesis and secretion, in addition to cell proliferation. Elevated prolactin concentrations may result from elevated estradiol levels in PCOS [8]. Prolactin's function in stimulating insulin secretion in islets has been extensively documented. Research indicates that prolactin can affect the crucial enzymes and transporters in target organs that are involved in the metabolism of glucose and lipids, as well as maintaining metabolic balance [9]. Thus, there is a hypothesis suggesting a close connection between prolactin and conditions such as insulin resistance, hypertension, thromboembolic stroke, and coronary syndrome [10]. PCOS is a reasonably common condition among infertile women. PCOS is associated with hyperinflammation, insulin resistance, poor glucose tolerance, metabolic syndrome, hyperlipidemia, hypertension, and an increased risk of cardiovascular disease. Haiyan Yang's research indicates that PRL is significantly lower in PCOS patients than controls [11]. Others discovered that the upper reference limit for serum prolactin was nearly 1.5 times that of the control group. Possible explanation for the increase in Prolactin associated with PCOS: a decrease in central dopaminergic tone, which elevates both Prolactin and LH levels [12].

Elevated LH does not always indicate PCOS, despite being a prevalent indication of the condition. In individuals with polycystic ovary syndrome, luteinization and ovarian androgen production are known to be stimulated by LH, which is one of the main factors contributing to hyperandrogenism. The majority of androgen secretion is stimulated by luteinizing hormone in ovarian theca cells with LH receptors [13]. Severe forms of PCOS seem to be associated with higher LH concentrations. Follicle count and ovarian volume have been positively correlated in the past, and PCOS patients with greater LH concentrations have been linked to more severe cycle disruptions and a higher risk of infertility. In PCOS women, LH hypersecretion also indicates the severity of the illness [14].

Follicles inside the ovary's theca cells become largely pre-antral and antral phases stopped when LH stimulation is increased. This causes the theca cells to hyperplasia and follicular fluid to accumulate, forming cyst-like structures that give the ovary a string of pearls appearance. On the other hand, follicular development is hindered by a relative FSH deficiency (Ashraf *et al.*, 2019). Elevated LH pulse frequency hinders the synthesis of oestrogen and FSH, thereby impeding follicle development and ovulation; this ultimately contributes to the development of polycystic ovaries in patients with polycystic ovary syndrome [15]. It is hypothesized that consistently rapid (GnRH) pulsatility is a neuroendocrine characteristic of PCOS, as it favours pituitary LH synthesis over FSH production and results in elevated LH concentrations and altered LH/FSH ratios characteristic of the condition. Ovarian androgen production is stimulated by high LH levels, whereas follicular development is impeded by low FSH levels [16].

FSH levels in PCOS may continue to be elevated, decrease, or remain normal [17]. The variance in LH and FSH hormone levels among affected women is attributable to the hypothalamus-pituitary gland, which is responsible for the disorder in these hormones [18]. Polycystic ovarian illness has been linked to abnormalities in the hypothalamic-pituitary-ovarian or adrenal axis. The disparity in LH to FSH secretion can be attributed to an alteration in the production pattern of gonadotropin-releasing hormone (GnRH). FSH influences the ovarian granulosa cells by converting androgens synthesized in theca cells into oestrogens, predominantly estradiol, which are indispensable for follicle formation [13]. The abnormal feedback mechanism that enhanced LH release was caused by ovarian oestrogen. The LH to FSH ratios in healthy women often fall between the range of 1 to 2. In women with polycystic ovarian disorder, the ratio is reversed and may potentially rise to 2 or 3[19].

Testosterone may coexist with other proteins like

albumin and SHBG or act alone. The following is the normal range for testosterone levels: Only 1% of it is unbound and free to circulate, while the remaining 80% is bound to the sex hormone-binding globulin and the remaining 19% to albumin. Instead of serum total testosterone, the Rotterdam Agreement recommends measuring circulating free testosterone (FAI) in women with PCOS to detect hyperandrogenism [13]. The increase in androgen is caused by two factors: the hyperplasia of theca cells in the ovaries of PCOS women and the dysregulation of enzymes in the steroid synthesis pathway. The frequency of GnRH pulses increases as elevated androgens prevent the hypothalamic-pituitary axis from receiving negative feedback. Elevated hypothalamic GnRH promotes LH production more than FSH production [13]. Reduced folliculogenesis is the primary effect of androgen excess in polycystic ovary syndrome. A surge in androgens during the first gonadotropin-independent stage causes an increase in the quantity of tiny antral follicles and stimulation of primordial follicle development [20]. In PCOS patients, elevated FSH may cause the ovary's cells to convert an excessive amount of androgen to oestrogen. At the same time, high LH stimulates the ovary to generate an excessive amount of androgens [16]. When there is uncertainty or absence of clinical signs of hyperandrogenemia, PCOS and/or phenotypic hyperandrogenism should be diagnosed.

In clinical practice, this may be achieved by measuring calculated free testosterone, calculated androgen index, or calculated bioavailable testosterone. Even though 78% of PCOS patients who consult clinics have biochemical hyperandrogenemia [21]. Consequently, an excess of androgen is thought to be the primary component responsible for the development of this disorder's symptoms and signs [22]. In women with PCOS, hyperandrogenism manifests clinically as acne, hirsutism, and androgenic alopecia. Enhanced androgen excess may also give rise to supplementary symptoms such as insulin resistance, weight gain, and irregular menstruation [23]. One of the most prominent and consistent diagnostic features of PCOS is hyperandrogenism. Formerly known as the Androgen Excess Society, the Androgen Excess and PCOS Society published the most recent addition to the PCOS diagnostic criteria in 2006. Considering the subjectivity of clinical assessments of hyperandrogenism, it is probable that biochemical hyperandrogenism would be more valuable in diagnosing polycystic ovary syndrome [24]. Elevated levels of serum LH are positively correlated with blood testosterone levels [24].

Conclusion

According to the current study, Polycystic ovary syndrome and Polycystic ovary disease appeared to increase in the level of hormones (Prolactin and Free Testosterone). Also, a Molecular study of blood showed that the IL-10 gene expression is a trace-related pathogenesis of patients with Polycystic ovary syndrome and Polycystic ovary disease.

Ethical approval

The agreement of the irrigation for minors and the patients' approval for adult patients were obtained by the study's specimens in accordance with ethical guidelines, legal requirements, and human rights organizations' directives.

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Conclusion

Our findings highlight the multifactorial nature of CKD pathology, emphasizing the importance of considering a panel of biomarkers to comprehensively assess renal function and disease progression. Notably, Vimentin emerged as a promising biomarker, demonstrating a significant association with macroalbuminuria in CKD patients. To gain further details and validate the significance of these criteria when coupled with other CKD Biomarkers, further investigation with an extensive group needs to carry out.

Ethical approval

The agreement of the irrigation for minors and the patients' approval for adult patients were obtained by the study's specimens in accordance with ethical guidelines, legal requirements, and human rights organizations' directives.

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