

Assessment of CYP19A1 Gene Expression and its Correlation with Hormonal Profile in Patients with Polycystic Ovary Syndrome

Zainab Ameen Khadhum ¹, Prof. Dr. Mahdi Saber Al-deresawi ¹

¹ Wasit University Collage of Science Iraq

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is the most prevalent endocrine disorder affecting reproductive-aged women, characterized by hormonal imbalances and metabolic dysregulation. The CYP19A1 gene encodes aromatase, an enzyme critical for estrogen biosynthesis, and its expression may play a role in the pathophysiology of PCOS. **Objective:** This study aimed to evaluate CYP19A1 gene expression in PCOS patients and assess its relationship with key reproductive hormones, including luteinizing hormone (LH), prolactin (PRL), testosterone (T), follicle-stimulating hormone (FSH), and estradiol (E2). **Methods:** A total of 50 women diagnosed with PCOS based on the Rotterdam criteria and 20 healthy controls were recruited from Al-Zahra Teaching Hospital and Al-Karama Teaching Hospital in Wasit Province, Iraq. Hormonal profiling was performed using the Addendum-Mini VIDAS automated system, and CYP19A1 gene expression was quantified using real-time polymerase chain reaction (qRT-PCR). **Results:** PCOS patients exhibited significantly elevated levels of testosterone (1.58 ± 0.17 ng/mL, $p < 0.05$), prolactin (18.42 ± 1.12 ng/mL, $p < 0.05$), LH (10.98 ± 0.89 IU/L, $p < 0.05$), and FSH (8.35 ± 0.42 IU/L, $p \leq 0.01$) compared to the control group (T: 0.412 ± 0.04 ng/mL, PRL: 13.12 ± 1.13 ng/mL, LH: 5.06 ± 0.40 IU/L, FSH: 6.83 ± 0.34 IU/L). Conversely, estradiol levels were significantly reduced in PCOS patients (39.73 ± 2.76 pg/mL) compared to controls (49.73 ± 3.43 pg/mL, $p < 0.05$). CYP19A1 gene expression was significantly upregulated in PCOS patients ($p < 0.05$). Correlation analysis revealed no significant association between CYP19A1 expression and E2, PRL, FSH, or T levels. However, a significant positive correlation was observed between CYP19A1 gene expression and LH levels ($p \leq 0.05$). **Conclusion:** PCOS patients exhibit hormonal imbalances, including elevated LH, PRL, T, and FSH levels, alongside decreased E2 levels. The study also found increased CYP19A1 gene expression in PCOS patients, with a significant correlation between CYP19A1 expression and LH levels, suggesting a potential role of this gene in PCOS pathogenesis. These findings highlight the need for further research into the molecular mechanisms underlying PCOS and the potential of CYP19A1 as a biomarker for disease severity and progression.

Keywords: CYP19A1, Reproductive Hormones, Real Time PCR, Mini VIDAS

INTRODUCTION

Leventhal and Stein started the PCOS research in 1935 [1]. Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting reproductive -aged women, with a prevalence of between 8 and 13% depending on the population studied and definitions used [2, 3]. Along with various long-term and metabolic challenges, it is also diverse and is further exacerbated by obesity [4]. Depending on the diagnostic criteria applied, (5–20%) of women who are of reproductive age have PCOS; these standards demonstrate a clinical predominance of hirsutism and menstrual dysfunction [5]. It is estimated that PCOS affects (5–15) of women and is a leading cause of infertility by the lack of ovulation affects approximately 73% of women with [1]. The molecular study included *CYP19A1* gene, A frequent single nucleotide polymorphism (SNP50) in the *CYP19A1* gene has been found to be strongly correlated with androgen excess in girls [6]. During fetal development, aromatase converts androgens to estrogens in the placenta, this conversion in the placenta prevents androgens from directing sexual development in female fetuses [7]. *CYP19A1* gene mutations that cause aromatase deficiency decrease or eliminate aromatase activity, there is a decrease in estrogen production and an increase in the levels of androgens, including testosterone [8]. This study was aimed to Identification the hormonal disturbance in PCOS women (T, E2, PRL, LH, and FSH), determine the *CYP19A1* gene expression, find the relationship between

hormonal disturbance and genes expression levels and statistical analysis of results.

MATERIALS AND METHODS

Subjects

Fifty blood samples were collected from patients with polycystic ovarian syndrome (PCOS) were divided into two groups (fifty patients and twenty persons as control). The period of the sample collection was extended from September ,2023 to December ,2023, two study groups have been investigated:

Healthy Control Group

Healthy control group consists of twenty healthy individuals of different age ranged (15–45). All of them chosen dependent on the next criteria [9]. 1) Regular menstrual cycle (26 to 30 days), 2) blood sampling was collected during the follicular phase (3, 4, or 5 day).

Patients Group:

This study included 50 infertile Iraqi women with PCOS. Patients were selected from the from Al-Zahra Teaching Hospital and AL-karama Teaching hospital, in Wasit provinces / Iraq. The period of the sample collection was extended from September 2023 to December

2023. Criteria used for the diagnosis PCOS subjects. To enroll the subjects with PCOS should include at least two of the following three features [10]: 1) oligo- and/or anovulation. 2) clinical and/or biochemical signs of hyperandrogenism. 3) polycystic ovaries.

Blood Sampling

Trained nurses collected venous blood sample (5 ml) from each individual of both PCOS and healthy control. Each blood sample was divided into two tubes, (2μ EDTA tubes) for Molecular analysis, (3μ of blood) for hormones test the serum was obtained by put the blood samples in plain tube and allowed to clot at 37°C for 30 minutes before centrifugation. Centrifuged at 5000 rpm for 5 minutes, the serum was collected and kept in freezer until used.

Hormonal Assay

Hormonal analysis for (E2, FSH, LH, PRL and T) was performed by using Addendum-Mini VIDAS apparatus (VIDAS) 12 mode I0, 1992, Biomerieux company, France, through an enzyme linked fluorescent assay (ELFA) technique.

Gene Expression

Total RNA of all samples was extracted utilizing the TRIzol® LS reagent as indicated by manufacturer's directions. Total RNA was conversely translated to complimentary DNA utilizing WizScript™ RT FDMix Kit. The system was completed in a response volume of 25 μl. Three main steps were needed to conversion by thermocycler (step 1:

5°C for 10 min, step 2: 42°C for 30 min, and 85°C for 5 min: one step). The expression levels of *CYP19A1* gene were assessment by quantitative real-time polymerase chain reaction (qRT-PCR)/Rotor-Gene/QIAGEN. To confirm the expression of test gene, EvaGreen was used. EvaGreen is a newly developed DNA-binding dye that has recently been used in quantitative real-time PCR. The mRNA levels of reference gene Cytochrome p450 family 19 subfamily A member 1 (*CYP19A1*) were amplified and used to normalize the mRNA levels of the *CYP19A1* gene. qRT-PCR reaction was performed using specific primers which were designed using Primer3 program. All primers were supplied by

CinnaGen Company, Iran, as a lyophilized product of different picomole concentrations. Lyophilized primers were dissolved in a free DNase/RNase water to give concentration of 100 pmol/μl as stock solution, to prepare 10 μM concentration as work solution suspended 10 pmol/μl in 90 μl of deionized water to arrive at a final concentration of 10 μM as work solution, the program of the reaction was: Initial denaturation: 95°C for 5 min/one cycle, denaturation: 95°C for 40 s, annealing: 57°C for 40 s, and extension: 72°C for 1 min, the run carried out with 35 cycles then holding with 4°C for 1 cycle. The sequences of *CYP19A1* F: 5'-cagaggccaagagtttgagg-3' and R: 5'-acactagcaggtgggttgg-3'.

Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability). Estimate of correlation coefficient between variables in this study.

Results and Discussions

Polycystic Ovary Syndrome and Hormonal Assay

Table (1) showed the mean distribution of estradiol hormone in the women with PCOS and the normal control groups. There was a significant decreased ($P < 0.05$) levels of E2 hormone in PCOS patients (39.73 ± 2.76 pg/mL) compared to controls group (49.11 ± 3.43 pg/mL). The mean PRL hormone assay recorded high significant ($P \leq 0.01$) in patient with PCOS (18.42 ± 1.12 ng/ml) while control group was (13.12 ± 1.13 ng/ml). LH hormone showed that there was high significant ($P \leq 0.01$) in PCOS patients (10.98 ± 0.89 μIU/ml) compared to control groups (5.06 ± 0.40 μIU/ml). The mean of follicle-stimulating hormone (FSH) in women with PCOS recorded that there was (8.35 ± 0.42 μIU/ml) but in control groups (6.83 ± 0.34 μIU/ml) However, the association was significant ($P \leq 0.05$) between PCOS patients and control groups.

Table 1: Comparison between Patients and Control Groups in Hormones Level

Group	Mean ± SE				
	Estradiol (pg/ml)	Prolactin (ng/ml)	Testosterone (ng/ml)	LH (μIU/ml)	FSH (μIU/ml)
Patients	39.73 ± 2.76	18.42 ± 1.12	1.58 ± 0.17	10.98 ± 0.89	8.35 ± 0.42
Control	49.73 ± 3.43	13.12 ± 1.13	0.412 ± 0.04	5.06 ± 0.40	6.83 ± 0.34
T-test	7.681 *	3.836 **	0.528 **	2.859 **	1.416 *
P-value	0.050	0.0076	0.0001	0.0001	0.0365

* ($P \leq 0.05$), ** ($P \leq 0.01$).

Significant * ($P \leq 0.05$), highly significantly ** ($P \leq 0.01$) LH: Luteinizing hormone, PRL: Prolactin hormone, T: Testosterone hormone, FSH: Follicle Stimulating hormone, E2: estradiol, SE: Standard error.

In the present study, the estradiol (E2) level is decreased in PCOS patients compare with control patient. The current result disagrees [11] in Chinese population which they showed that no significant differences in E2 level. The results agree with Khandgawi *et al.* [12], Saedii *et al.* [13] and AL-deresawi *et al.* [14] which showed that there was a significant decrease in E2 level in PCOS group when compared to control group, the decrease E2 level in PCOS women often result from a reduced level of FSH that relative to LH, the granulosa cells of the ovaries cannot aromatize the androgens to estrogens. [15] reported the decreased of E2 levels cause the pituitary to secrete more gonadotropin as well as FSH, which stimulates ovulate process by preventing the E2 negative feedback on the hypothalamic-pituitary axis. [16] recorded that a reduction in estradiol levels, as found in the

menopause, would therefore be expected to result in increased food intake (with the estradiol activity lost). Thus, a loss of estradiol after menopause, may contribute to the development of obesity, and systemic and cerebral insulin resistance. These result of PRL hormone is consistent with a study by Tawfiq, [17] and Nissreen *et al.* [18] who observed that women with infertility due to ovulation disorder increased their proportion of prolactin (PRL) due to insufficient progesterone release from corpus luteal. So when there are high levels of (PRL) hormone in the blood, women will not bleed and this non-ovulation can also cause irregular menstrual cycles, and this will lead to infertility [19]. It is possible that an increase in the incidence of polycystic ovary syndrome may lead to a state of psychological and nervous disorder in women who suffer from this syndrome, and this

leads to them suffering from severe anxiety or what is called psychological block [20]. This result was consistent with the studies conducted by Gilling *et al*, [21] and Sporanda and Papić, [22]. Testosterone (T) is a steroid hormone that causes the polycyclic ovary syndrome. The hormone is produced in females from the ovaries and adrenal gland [23]. The reason for the rise of the hormone (T) is due to the fact that the outer layer of ovary and tissue within the ovary heart was thick in women with PCOS. The dense pulp of the ovary inside contains theca cells that produce additional amounts of testosterone (T) and this result was consistent with Nelson *et al*, [24] and AL Deresawi *et al*, [25]. In the present study, the higher LH level was encountered in PCOS patients. The current result disagrees with Mohammed *et al*, [26] who's shows that no significant differences ($P > 0.01$) in LH level in Iraqi population. The result agrees with other studies in Iraqi population achieved by Omear *et al*, [27], Gattia *et al*, [28] and Jabbar *et al*, [29] which revealed that PCOS women had a higher concentration of LH than healthy women. Wang results also coincided with the present findings in Chinese population [11].

Hyperandrogenism is the key feature of PCOS, resulting primarily from excess androgen production in the ovaries and, to a lesser extent, in the adrenals. The primary mechanisms driving increased ovarian androgen production in PCOS include hypersecretion of LH and increased LH bioactivity, hyperinsulinemia due to insulin resistance and increased volume of theca cells in an expanded ovarian stroma [30]. Some studies referred to the increasing level of LH hormone in PCOS patients as a result of the presence of hypothalamic dysfunction. Additionally, there have been additional possible causes of luteinizing hormone hypersecretion such as aromatization of Androgens to

Estrogens which cause permanent estrogen overproduction that favors luteinizing hormone hypersecretion, direct leptin-induced GnRH modulation, and an insulin-mediated increase in serum LH pulse amplitude [31]. The normal gonadotrophin axis is disturbed in PCOS women, therefore LH levels increase, and FSH levels decrease, leading to a reversal of LH/FSH ratio [32]. The results are not concordant with the study of who found significant differences between obese and non-obese women regarding LH, FSH, and LH/FSH ratio. Upon these findings, they advised obese women who shared in the study to try to lose weight and change their lifestyle [33].

The study of FSH hormone are at odds with those of Mohammed *et al*, [26] and Jabbar *et al*, [29], who demonstrate a noteworthy variation in FSH levels between PCOS patients and controls in the Iraqi community. The findings are consistent with those De Silva *et al*, [34], and Omear *et al*, [27], who demonstrate that there are no appreciable variations in FSH levels between PCOS patients and controls. Nory *et al*, [35] and AL-Deresawi, [36] mentioned that the PCOS group had higher FSH levels. FSH stimulates oocyte growth, and when oocyte growth exceeds normal limits, ovarian function is compromised. This is because of its relationship to the hormone estrogen and the negative feedback process; as a result, in women with PCOS, the pituitary gland excretes a lot of FSH.

Gene Expression of *CYP19A1* in Patient with PCOS

Table (2) recorded that the gene expression of *CYP19A1* was significant ($P \leq 0.05$) in patients with PCOS (2.490 ± 1.41) compared to control group (1.00 ± 0.00).

Table 2: Fold change of *CYP19A1* gene expression in patients and control groups

Group	Ct <i>CYP19A1</i>	GAPDH	Δ ct	$\Delta\Delta$ ct	Folding $\pm$ SD
Patients	29.716	29.411	0.305	-1.074	2.490 ± 1.41
Control	26.440	25.060	1.38	0	1.00 ± 0.00
T-test (P-value)	---	---	---	---	$0.755^* (0.0392)$

* ($P \leq 0.05$).

CYP19A1: Cytochrome p450 family 19 subfamily A member 1, GAPDH: Glyceraldehyde -3-phosphate dehydrogenase.

Table (2) showed that the *CYP19A1* gene expression was significant ($P \leq 0.05$) in patients with PCOS, this result agree with Xu *et al*, [37] demonstrated that PCOS patients had a greater frequency of (TT/TA) alleles in *CYP19A1* gene intron 4. Munawar *et al*, [38] concluded the *CYP19A1* gene expression was significantly associated with susceptibility to PCOS in Pakistani women having the traits of infertility and family history of hypertension. This result disagree with study Yu *et al*, [39] the methylation status of the *CYP19A1* promoter in Chinese PCOS and control groups. The findings showed that promoter hypermethylation frequently causes *CYP19A1* to be repressed in PCOS ovaries. Kaur *et al*, [40] Reported the *CYP19A1* did not show significant association with PCOS in present study, because; Aromatase

activity is reduced in PCOS follicular fluid, which might be due to genes involved or when follicular stimulating hormone (FSH) activity is low as compared to LH, and due to reduced levels of FSH, aromatase activity is lowered and the androstenedione and testosterone are not completely aromatized to estrogens.

Correlation between *CYP19A1* with Hormonal Profile

The result showed in table (3) there were non-significant relationship between *CYP19A1* gene expression and hormonal profile of (E2, PRL, FSH, and T) but there were a significant ($P \leq 0.05$) association between *CYP19A1* gene expression and level of LH hormone.

Table 3: Correlation between *CYP19A1* gene Expression and Hormonal Profile

Hormones	Correlation Coefficient-R With Fold of <i>CYP19A1</i> Gene	P-Value
Estradiol	0.09 NS	0.521
Prolactin	-0.08 NS	0.556
Testosterone	-0.14 NS	0.314
LH	0.27 *	0.049
FSH	0.02 NS	0.903

* ($P \leq 0.05$), NS: Non-Significant.

This result was agree with Do *et al*, [41], LH excess associated with PCOS may be secondary to the peripheral events within the ovary, the mechanism of neuroendocrine dysfunction resulting in an elevated LH

in PCOS may be an uncoupling of hypothalamic estradiol inhibition by elevated ovarian androstenedione, while elevated LH may cause hyperandrogenemia. Jin *et al*, [42] reported the correlation between

serum LH and CYP19A1 gene polymorphisms and found that in PCOS patients the serum LH of AA type was lower than that of GA or GG type. As hyperandrogenemia is a key characteristics seen in PCOS patients, we focused on the CYP19A1 gene polymorphisms that affect androgen synthesis and the SHBG gene polymorphisms that affect SHBG synthesis to determine the relationship between gene polymorphisms and PCOS risk through a comprehensive literature search, so as to draw more comprehensive, accurate, and reliable conclusions.

Conclusions

High levels of the hormone (LH, PRL, T and FSH) and low levels of the E2 hormone in women with PCOS, high gene expression for gene *CYP19A1* in patient with PCOS, the study showed that there is positive correlation between the LH hormone and *CYP19A1* gene expression.

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