

Estimation of *BMP-15* gene expression and its Association to Hormonal Profile in Iraq Patient with Polycystic Ovary Syndrome

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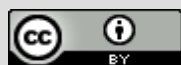
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ABSTRACT: Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting reproductive aged women. The objective of this study was determining the gene expression levels in patients with PCOS and its reflects in hormonal reproductive: Luteinizing hormone (LH), Prolactin hormone (PRL), Testosterone hormone (T), Follicle Stimulating hormone (FHS), estradiol (E2). Fifty patients with PCOS and twenty healthy control group recruited from the Al-Zahra Teaching Hospital and AL-karama Teaching Hospital, in Wasit provinces/Iraq, based on criteria of Rotterdam. The hormonal profile was carried out by Addendum-Mini VIDAS apparatus. *BMP-15* gene expression was estimated by quantitative real-time polymerase chain reaction (qRT-PCR). The mean of Testosterone (1.58 ± 0.17), prolactin (18.42 ± 1.12), Luteinizing hormone (10.98 ± 0.89) was a significant ($P < 0.05$) and follicle-stimulating hormone (8.35 ± 0.42) was high significant ($P \leq 0.01$) but the Estradiol hormone decrease in patient with PCOS (39.73 ± 2.76) was a significant ($P < 0.05$) when compared to healthy control group (0.412 ± 0.04), (13.12 ± 1.13), (5.06 ± 0.40), (6.83 ± 0.34), (49.73 ± 3.43) respectively. The result of *BMP-15* gene expression in patient with PCOS that were a significant ($P < 0.05$). The result showed there were non-significant correlation between *PMP-15* gene expression and hormonal profile of (E2, PRL, FSH, LH and T). The women with PCOS have high levels of the hormone (LH, PRL, T and FSH) and low levels of the E2 hormone and the gene expression of *BMP-15* was high level in patient with PCOS.

Keywords: *BMP-15*, reproductive hormones, Real time PCR, Mini VIDAS



1. 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 *BMP-15* gene, The *BMP-15* gene is located on the X-chromosome (6). *BMP-15* is translated as a preproprotein that is composed of a single peptide, which contains a proregion and a smaller mature region (7). Intracellular processing then leads to the removal of the proregion, leaving the biologically active mature region to perform the functions (8). Functions of *BMP-15* include Promotion of growth and maturation of ovarian follicles, regulation of

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the sensitivity of granulosa cells to follicle-stimulating hormone (FSH) action (9). This study was aimed to Identification the hormonal disturbance in PCOS women (T, E2, PRL, LH, and FSH), determine the BMP-15 gene expression, find the relationship between hormonal disturbance and genes expression levels and statistical analysis of results.

2. MATERIALS AND METHODS

2.1 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:

2.2 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 (10)

- Regular menstrual cycle (26 to 30 days)
- blood sampling was collected during the follicular phase (3, 4, or 5 day).

2.3 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 (11):

- oligo- and/or anovulation.
- clinical and/or biochemical signs of hyperandrogenism.
- polycystic ovaries.

2.4 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.

2.5 Hormonal Assay

Hormonal analysis for (E2, FSH, LH, PRL and T) was performed by using Addendum-Mini VIDAS apparatus (VIDAS) 12 mode l0, 1992, Biomerieux company, France, through an enzyme linked fluorescent assay (ELFA) technique.

2.6 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 *BMP-15* 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 *BMP-15* F: 5'- tggaggagttcatggaaagg d R: 5'- ttagaaaagggggagcaatg -3'.

2.7 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.

3. RESULTS AND DISCUSSION

3.1 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 $\pm$ SE				
	Estradiol (pg/ml)	Prolactin (ng/ml)	Testosterone (ng/ml)	LH (μ IU/ml)	FSH (μ IU/ml)
Patients	39.73 $\pm$ 2.76	18.42 $\pm$ 1.12	1.58 $\pm$ 0.17	10.98 $\pm$ 0.89	8.35 $\pm$ 0.42
Control	49.73 $\pm$ 3.43	13.12 $\pm$ 1.13	0.412 $\pm$ 0.04	5.06 $\pm$ 0.40	6.83 $\pm$ 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 (12) in Chinese population which they showed that no significant differences in E2 level. The results agree with (13, 14, 15) 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.

(16)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. (17)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 *et al*, (18) and Nissreen *et al*, (19)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(20). 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 (21) .

This result was consistent with the studies conducted by Gilling *et al*(22)and Spranda *et al*, (23).

Testosterone (T) is a steroid hormone that causes the polycyclic ovary syndrome. The hormone is produced in females from the ovaries and adrenal gland(24). 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(25, 26).

In the present study, the higher LH level was encountered in PCOS patients. The current result disagrees with Mohammed *et al*, (27)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 Omeear *et al*, (28), Gattia *et al*, (29), Jabbar *et al* (30) 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 (12).

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 (31)

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 (32).

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 (33). 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 (36).

The study of FSH hormone are at odds with those of Mohammed *et al*, (28) and Jabber (30), 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 Omeear *et al*, (28), who demonstrate that there are no appreciable variations in FSH levels between PCOS patients and controls.

Nory, Abadi (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.

3.2 Gene expression of *BMP-15* in patient with PCOS

Table (2) recorded that the gene expression of *BMP-15* was significant ($P \leq 0.05$) in patients with PCOS (2.455 ± 0.93) compared to control group (1.00 ± 0.00).

Table 2: Fold change of *BMP-15* gene expression in patients and control groups

Group	ct <i>BMP-15</i>	GAP	Δ ct	$\Delta\Delta$ ct	Folding $\pm$ SD
Patients	28.93	28.69	0.236	-1.204	2.455 $\pm$ 0.93
Control	26.44	25.00	1.44	0	1.00 $\pm$ 0.00
T-test (P-value)	---	---	---	---	0.861 * (0.0337)
* ($P \leq 0.05$).					

BMP-15: Bone morphogenetic protein 15 gene, GAPDH: Glyceraldehyde -3-phosphate dehydrogenase.

Table (2) showed that the *BMP-15* gene expression was significant ($P \leq 0.05$) in patients with PCOS than control group, this result agrees with Zhao *et al.*, (37), revealed increased expression *BMP-15* in MI-stage oocytes ($n = 10$) in women with PCOS, suggesting that exogenous rFSH use may correct dysfunction in *BMP-15* expression in PCOS oocyte, both oocytes and GCs from healthy ovulatory women can have their *BMP-15* levels elevated by controlled ovarian stimulation(38).

Gilchrist *et al.*,(39) that showed the *BMP-15* expression was reduced in oocytes of PCOS patients compared to normal oocytes, which may be associated with aberrant follicular development in PCOS. Therefore, the reduced expression of *BMP-15* in oocytes may have detrimental effects on follicular development in ovaries of PCOS patients, expression of *BMP-15* showed no significant distinction between the control and patients with PCOS (40). Lower level of *BMP-15* expression are associated with subfertility, damage to ovulation and even with ovarian failure in women(40).

3.3 Correlation between *BMP-15* with hormonal profile

The result showed in table (3) there were no correlation between *BMP-15* gene expression and hormonal profile of (E2, PRL, FSH and LH).

Table 3 Correlation between *BMP-15* gene expression and hormonal profile

Hormones	Correlation coefficient-r with fold of <i>BMP-15</i> gene	P-value
Estradiol	0.09 NS	0.509
Prolactin	0.02 NS	0.894
Testosterone	-0.06 NS	0.700
LH	-0.02 NS	0.930
FSH	-0.02 NS	0.870
NS: Non-Significant.		

4. CONCLUSION

This study approved there are a high level of the hormone (LH, PRL, T and FSH) and low levels of the E2 hormone in women with PCOS, also showed there were overexpression in gene *BMP-15* in patient with PCOS, but not correlation to reproductive hormones levels.

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