Iraqi Journal of Biotechnology, 2025, Vol. 24, No. 3, 23-29

Investigating the Role of LH, FSH, AMH, and *TGF-B* Gene Expression in Polycystic Ovary Syndrome

[1]Sarah E. Abdul Sahib, [2]Jinan MJ. Al-Saffar

[1,2] Department of Biotechnology, College of Sciences, University of Baghdad

Received: June 6, 2024 / Accepted: July 31, 2024 / Published: November 3, 2025

Abstract: Polycystic ovarian syndrome (PCOS) is one of the most prevalent endocrine disorders in reproductive-age women, with prevalence 10 – 18 % according to diagnostic criteria. Women'slifeare impacted by polycystic ovary syndrome, particularly while trying to conceive. The focus of this research is to establish a connection between polycystic ovary syndrome (PCOS) and the aforementioned variables (LH, FSH, and AMH) and to quantify the impact of transforming growth factor gene expression on PCOS. Eighty women, ranging in age from nineteen to forty-five, were part of the study; thirty of these women had polycystic ovarian syndrome, and thirty of these women had delayed childbearing. The other twenty women were healthy. The hormone levels was analyzed after separating the serum. To determine gene expression, blood samples were collected using a Trizol tube and RT PCR was used for analysis. In conclusion from the results in comparison to control women, those with delayed childbearing had lower levels of TGF-β expression, and the results demonstrated a rise in AMH and LH levels without a change in FSH levels.

Keyword:PCOS, AMH, TGF-β1.

Corresponding author: (Email: jinan.jawad@sc.uobaghdad.edu.iq)

Introduction

Polycystic ovarian syndrome (PCOS) is one of the most endocrine disorders prevalent in female. It is a complex heterogeneous disease; its reason is not clear. PCOS affects 10% of reproductive-age women when using the NIH criteria for diagnosis, and up to 18% of reproductive-age women as per the Rotterdam criteria(1).

Pattern reversal infertility caused by polycystic ovary syndrome accounts for approximately 70% of cases(2)Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism, recurrent anovulation, and polycystic ovaries. Additional symptoms of polycystic ovary syndrome include hirsutism, obesity, insulin resistance, and compensatory hyperinsulinemia(3). Glycemic abnormalities, such as prediabetes, type 2 diabetes mellitus (T2DM), and metabolic syndrome, which raise the risk of cardiovascular disease, are more likely in people with hyperinsulinemia. On the whole, polycystic ovary syndrome is associated with high financial and health care expenses(4)In addition to decreased fibrinolysis, women with polycystic ovary syndrome often have lipid abnormalities, such as low levels of high-density lipoprotein cholesterol and higher levels of triglycerides(5) .When unopposed estrogen is stimulated for an extended period of time, the risk of endometrial cancer increases. A higher

risk of early pregnancy loss is also linked to polycystic ovary syndrome(6).

There is no one diagnostic "gold standard" for polycystic ovary syndrome (PCOS), but rather a collection of symptoms and genetic markers that may or may not be present in any one patient. Because of this, it is exceedingly challenging to establish diagnostic cutoffs for several indicators that may distinguish "normal" women from PCOS patients with high sensitivity and specificity(7).

Blood tests for anti-Mullerian hormone (AMH) could be useful in this area. This dimeric glycoprotein is a member of the transforming growth factor-b family and goes by two names: AMH and MIS(8).

A new way to see how old your ovaries are is by looking at your serum (AMH) levels, which are a measure of ovarian reserve. An autocrine and paracrine regulator of follicular maturation, AMH is produced by the granulosa cells of the preantral and antral follicles. The quantity of antral follicles is correlated with the ageing process and the decrease in serum AMH level(9).

The main cause of polycystic ovary syndrome (PCOS) is heredity, although environmental circumstances and lifestyle choices can play a role10). The ovary is home to several members of the transforming growth factor (TGF-ß) superfamily, such as activins, inhibins, AMH, and BMPs, all of which have been linked to the development of aberrant follicles and hyperandrogenism in polycystic ovarian syndrome (PCOS)(11).

Members of the transforming growth factor-ß family are known to influence follicular and ovarian function. Furthermore, multiple investigations have demonstrated that abnormal regulation of one or more TGF-ß family members may account for some of the difference between PCOS-positive and PCOS-negative women(12).

The current study set out to identify potential correlations between LH, FSH, and AMH hormone levels, as well as TGF-ß gene expression, in women who suffer from polycystic ovaries.

Subjects and Methods

Subjects

Eighty women, ranging in age from 19 to 45, who were not yet menopausal participated in the present study. From November 2022 through March 2023, these instances were chosen from the medical records of Kamal Al Samarrai Hospital in Baghdad, Iraq. There are three subsets within the female population:

1. First group:30women with polycystic ovary syndrome (PCOS).
2. Second group:30women with Delayed childbirth.
3. Third group:(control group) 20 healthywomen.

Gynecologist have used ultrasound waves to confirm that women with polycystic ovary syndrome (PCOS) have the condition. The research, which took place at the University of Baghdad's Department of Biotechnology at the College of Science, had ethical clearance from the University of Baghdad (CSEC/0524/0042).

Collecting Blood Sample:

Blood sampling About 5 ml of venous blood was withdrawn by a medical syringe on the follicular phase (2-3) day of the menstrual cycle of each subject (patients and controls). The blood sample was divided into (4.5ml) in gel tubes after a while, they were placed in a centrifuge at 3000 rpm for 10 minutes to separate the serum to be frozen for use, when necessary, for the hormones test (LH (Biomerieux,

FRANCE), FSH (Biomerieux, FRANCE), and AMH (Ansh Labs, USA)) and (0.5 ml) put in tube containing TRIzol, Hand mixed and then frozen, for TGF-ß gene expression (Promega, USA).

Quantitative real-time PCR analysis

The Mic qPCR Cycler (Bio Molecular System, Australia) was used to measure expression levels, and SYBR Green PCR master mix (Promega, USA) was used for real-time qPCR analysis. The manufacturer's instructions (Promega) were followed to conduct all qPCRs in a final volume of 10 µL, all additions are shown in table (1). The amplification thermal cycling conditions included an initial denaturation cycle at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 20 s and annealing at 65°C for 20 s. The final cycle was extension at 72°C for 20 s, and the process began with one cycle at 37 °C for 15 minutes to activate the reverse transcriptase enzyme, which converts messenger RNA to complementary DNA.ß-Globin was used as housekeeping gene tonormalize mRNA levels, relative mRNA expression was evaluated using the $^{2-}\Delta\Delta CT$ technique according to Livak method(13). This study's qPCR primer was created by NCBI prime3, Primer name and sequences are shown in table (2).

Table (1): Component of q PCR utilized.

Master mix components	Stock	Unit	Final	Unit	Volume
					1 Sample
qPCR Master Mix	2	X	1	X	5
RT mix	50	x	1	x	0.25
$MgCl_2$					0.25
Forward primer	10	µM	0.5	µM	0.5
Reverse primer	10	µM	0.5	µM	0.5
Nuclease Free Water					2.5
RNA		ng/µl		ng/µl	1
Total volume					10
Aliquot per single rxn	9µl of Master mix per tube and add 1µl of Template				

Table (2): Sequences of The Primers.

Primer Name	Seq.	Annealing Temp. (Cº)
β-Globin-F	ACACAACTGTGTTCACTAGC	65
β-Globin-R	CAACTTCATCCACGTTCACC	
TGF-β1_exp-F	TACCTGAACCCGTGTTGCTCTC	
TGF-β1_exp-R	GTTGCTGAGGTATCGCCAGGAA	

Statistical analysis

Mean ± Standard Division (SD), ANOVA table (Duncan test), was calculating for parametric data. Median, 25%-75% percentile, Kruskal-Wallis, was calculating for non-parametric data. by using Statistical program: IBM SPSS V27.0.

Results and Discussion

The Median age (25% - 75% percentile) of the PCOS group was 26.0 (22.0-29.25) years and 30.0 (25.75-36.0) years in women with Delayed childbirth compared to27.0 (21.75-33.25) years in the control group as shown in Table 3. The results of BMI showed that the mean ± SEin the PCOS group was significantly higher ($p < 0.05$) than that inthe women with Delayed childbirthand incontrol group (30.28 ± 0.82 kg/m^2 and 26.94 ± 1.31 kg/m^2*vs.* 26.19 ± 1.08kg/m^2,

respectively)as shown in Table 3.These results agreed with study, also found BMI elevate in PCOS women compared to control(14). This increasein BMI due to obesity is closely associated with PCOS, which affects 6–12% of women of reproductive age(15)Compared to controls with the same body mass index (BMI), women with polycystic ovary syndrome (PCOS) are more likely to have excess fat in the middle of their bodies, which may raise their risk of obesity(16).

With a body mass index (BMI) of 30 kg/m2 or higher, women are considered obese and more likely to suffer from polycystic ovary syndrome (PCOS), according to the present BMI results. On a global scale, between 38% and 88% of PCOS-afflicted women are overweight or obese. Obesity in women with polycystic ovary syndrome (PCOS) is caused by hyperandrogenism, which influences fat accumulation in adipose tissue(17)

Table (3): Baseline characteristics of study groups.

Groups	Age	Probability	BMI (kg/m^2)	Probability
PCOS	26.0(22.0-29.25)	($p < 0.05$)	30.28 ± 0.82	($p < 0.05$)
Delayed Childbirth	30.0(25.75-36.0)		26.94 ± 1.31	
Control	27.0(21.75-33.25)		26.19 ± 1.08	

BMI: Body mass index.
Data conducted by Median (25% - 75% percentile).

According to Table 4, the PCOS group had a substantially higher level of luteinizing hormone (LH) than the delayed childbearing women and control groups ($p < 0.05$). These results compatible with previous study on 100 Iraqi women, that found significant increase in the level of LHin PCOS women(18). Table 4 shows that there was no statistically significant difference ($p > 0.05$) in the follicle stimulating hormone (FSH) values between the three groups. These results were compatible with study carried out on Iraqi women (19),also found not significant difference in FSH level. Even though the exact cause of polycystic ovarian syndrome is still a mystery, numerous studies have linked the condition to hormones including follicle-stimulating hormone (FSH) and luteinizing hormone (LH), leading researchers to believe that it is inherited. Ovulatory disruption results from an overabundance of gonadotropin hormone due to an increase in LH secretion and a reduction in FSH production(20).For a long time, it was believed that anovulation in PCOS women was caused by insufficient FSH relative to LH(21)

Table (4): LH and FSH level among the studied groups

Groups	LH (mIU/ml)	Probability	FSH (mIU/ml)	Probability
PCOS	7.04 ± 0.49 ^{A}	($p < 0.05$)	4.86 ± 0.34 ^{A}	($p > 0.05$)
Delayed Childbirth	4.90 ± 0.67 ^{B}		5.03 ± 0.76 ^{A}	
Control	4.37 ± 1.18 ^{B}		4.61 ± 0.49 ^{A}	

LH: Luteinizing hormone, FSH: Follicle stimulating hormone.
The data conducted by Mean ± (SD).
Duncan test: The same letter refers to no statistical difference ($p > 0.05$).

As showed in Table 5, there was significant increased level ($p < 0.05$) in the level of AMH hormone in PCOS group compared to the delayed

childbirth women and control groups.These results were compatible with the results of Amadi *et al.*(22), who referred that PCOS is associated with elevated levels of the anti-Müllerian hormone (AMH), which could be used as a diagnostic tool in the future.

Table (5): The median of AMH among study groups.

Groups	AMH (ng/ml)	Probability
PCOS	4.95 (2.40-7.40) ^{A}	($p < 0.05$)
Delayed Childbirth	1.95 (0.90-3.49) ^{B}	
Control	3.25 (2.80-3.60) ^{B}	

AMH: Anti-Mullerian Hormone.
The data conducted by Median (25% - 75% percentile)
Duncan Test: The same letter refers to no statistical difference ($p > 0.05$).

TGF-β 1 gene expression

The results of the transforming growth factor β 1 (TGF-β1) are shown in Table (6). There was increase in TGF-β gene expression in women with PCOS (6.44 ± 2.67), and less in delayed childbirth women (2.23 ± 0.44) compared with healthy women (1.16 ± 0.27), these increased not statistically significant ($p > 0.05$). The mean of housekeeping gene β. Globin and TGF-β1 gene explained in table (7).

Table (6): The gene expression ofTGF-β1 among study groups

Groups	TGF-β1	Probability
PCOS	6.44 ± 2.67^{A}	($p > 0.05$)
Delayed Childbirth	2.23 ± 0.44^{A}	
Control	1.16 ± 0.27^{A}	

TGF-β1: Transforming growth factor-β1.
The data conducted by The Mean ± (SD).
Duncan test: The same letter refers to no statistical difference ($p > 0.05$).

Table (7): Folding expression level of TGF-β 1 gene among study groups

Group	Mean of β. Globin	Mean of TGF-β1	Mean of ΔCT	Mean of ΔΔCT	Mean of Folding
PCOS	18.14	28.87	10.73	-1.36	6.43
Delayed Childbirth	16.72	28.01	11.28	-0.81	2.22
Control	15.48	27.58	12.10	-5.07	1.15

Figure (1) showed the amplification of transforming growth factor – β1 (TGF-β1) in samples by real-time polymerase chain reaction, in addition to their melting curve showed in figure (2).

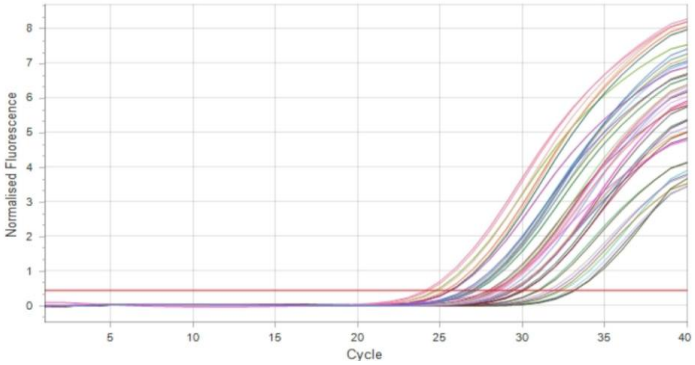

Figure (1): Plots of TGF-β1 Amplification by RT-qPCR

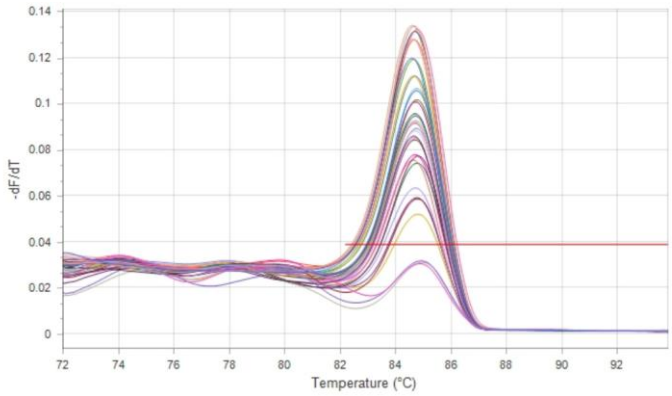

Figure (2): Melt curve of TGF- β1gene

The current results of TGF-β1gene expression were compatible with the study of Gao *et al*.(23) and study of Shen and Wang(24). They also found TGF-β1 gene expression elevated in PCOS patient.More and more data points to the TGF-β-signaling pathway as an essential component in PCOS aetiology. Recent years have seen a proliferation of theories suggesting that TGF-β dysregulation, leading to an increase in collagen formation and deposition, is responsible for the elevated stromal tissues seen in polycystic ovary syndrome (PCOS) ovaries. The fibrosis of numerous organs and tissues is indeed linked to TGF-β dysregulation(25,26). TGF-β1 play a pivotal role in follicular growth regulation and mammals' development, so dysregulation in expression of these gene affects on PCOS patient, may lead to cardiovascular and metabolic abnormalities (24). TGF-β1 signaling is dynamic during fetal ovary development and may be important in aetiology of PCOS, and could provide a link between genetic basis and fetal predisposition to PCOS .

Conclusion

LH and AMH are significantly higher in polycystic ovary syndrome (PCOS) patients than healthy women, and lowest results in women with delayed childbirth. While FSH were no significant change in these three groups. The gene expression of TGF-β 1 was not significant in three groups, but elevate in PCOS patient, low in delayed childbirth and the least expression in control women.

References

1. Maqbool, M., Gani, I., & Geer, M. I. (2019). Polycystic ovarian syndrome: A multifaceted disease. International Journal of Pharmaceutical Sciences and Research, 10(3). https://doi.org/10.13040/IJPSR.0975-8232.10
2. Cunha, A., & Póvoa, A. M. (2021). Infertility management in women with polycystic ovary syndrome: A review. Porto Biomedical Journal, 6(1), e116.
3. Al-Juaifari, B. A. J., & Al-Jumaili, E. F. (2020). Correlation of body mass index and some hormones (estradiol, luteinizing hormone, follicle-stimulating hormone) with polycystic ovary syndrome among young females (20–35 years). Biomedical and Pharmacology Journal, 13(1), 193–198.
4. Al-Shattawi, S. S. M., & Al-Jumili, E. F. (2019). The effect of hypercytokinemia in the pathogenesis of polycystic ovary syndrome in Iraqi women. Indian Journal of Public Health Research & Development, 10(2), 768. https://doi.org/10.5958/0976-5506.2019.00387.5
5. Al-Shattawi, S. S. M., Al-Jumili, E. F., & Al-Azzam, M. A. (2018). The relationship between obesity and polycystic ovary syndrome in a sample of Iraqi infertile women. Iraqi Journal of Biotechnology, 17.
6. Yang, A. M., Xu, X., Han, Y., Wei, J. J., Hao, G. M., Cui, N., et al. (2021). Risk factors for different types of pregnancy

losses: Analysis of 15,210 pregnancies after embryo transfer. Frontiers in Endocrinology, 12.
7. Dewailly, D. (2016). Diagnostic criteria for PCOS: Is there a need for a rethink? Best Practice & Research Clinical Obstetrics & Gynaecology, 37, 5–11.
8. Bhattacharya, K., Saha, I., Sen, D., Bose, C., Chaudhuri, G. R., Dutta, S., et al. (2022). Role of anti-Müllerian hormone in polycystic ovary syndrome. East Fertility Society Journal, 27, 32. https://doi.org/10.1186/s43043-022-00123-5
9. Shrikhande, L., Shrikhande, B., & Shrikhande, A. (2020). AMH and its clinical implications. Journal of Obstetrics and Gynecology of India, 70(5), 337–341.
10. Asghari, R., Shokri-Asl, V., Rezaei, H., Tavallaie, M., Khafaei, M., Abdolmaleki, A., et al. (2021). Alteration of TGFB1, GDF9, and BMPR2 gene expression in preantral follicles of an estradiol valerate-induced polycystic ovary mouse model. Clinical and Experimental Reproductive Medicine.
11. Bhati, M., Prabhu, Y. D., Renu, K., Vellingiri, B., Thiagarajan, P., Panda, A., et al. (2020). Role of TGF-β signaling in PCOS-associated focal segmental glomerulosclerosis. Clinica Chimica Acta, 510, 244–251.
12. Kristensen, S. G., Kumar, A., Kalra, B., Pors, S. E., Bøtkjær, J. A., Mamsen, L. S., et al. (2019). Quantitative differences in TGF-β family members measured in small antral follicle fluids from women with or without PCO. Journal of Clinical Endocrinology & Metabolism, 104(12), 6371–6384.
13. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods, 25(4), 402–408.
14. Bhattacharya, K., Sengupta, P., Dutta, S., Chaudhuri, P., Das Mukhopadhyay, L., & Syamal, A. K. (2021). Waist-to-height ratio and BMI as predictive markers for insulin resistance in women with PCOS in Kolkata, India. Endocrine, 72(1), 86–95.
15. Neubronner, S. A., Indran, I. R., Chan, Y. H., Win, A., Thu, P., & Yong, E. L. (2021). Effect of body mass index on phenotypic features of polycystic ovary syndrome in Singapore women. BMC Women's Health, 21. https://doi.org/10.1186/s12905-021-01277-6
16. Barber, T. M., & Franks, S. (2021). Obesity and polycystic ovary syndrome. Clinical Endocrinology, 95(4), 531–541.
17. Khan, S. Y. (2023). [Article information incomplete]. Punjab University Journal of Zoology, 38(2), 171–179.
18. Association of glutathione-S-transferase omega-2 (GSTO2) gene polymorphism with incidence of polycystic ovary syndrome in Iraqi women. (n.d.). Iraqi Journal of Biotechnology. https://jige.uobaghdad.edu.iq/index.php/IJB/article/view/539
19. Effect of HNF1A gene polymorphism on risk of polycystic ovary syndrome in Iraqi women. (n.d.). Iraqi Journal of Biotechnology. https://jige.uobaghdad.edu.iq/index.php/IJB/article/view/484
20. Saadia, Z. (2020). Follicle-stimulating hormone (LH:FSH) ratio in polycystic ovary syndrome: Obese versus non-obese women.
21. Shah, A., Dodson, W. C., Kris-Etherton, P. M., Kunselman, A. R., Stetter, C. M., Gnatuk, C. L., et al. (2021). Effects of oral contraception and lifestyle modification on incretins and TGF-β superfamily hormones in PCOS. Journal of Clinical Endocrinology & Metabolism, 106(1), 108–119.
22. Amadi, C. F., Okolonkwo, B. N., George-Oparati, M. I., & Odiabara, K. K. (2023). Understanding the relationship between polycystic ovarian syndrome and dyslipidemia. Asian Journal of Biochemistry, Genetics and Molecular Biology, 15(3), 1–11.
23. Gao, M., Liu, X., Gu, H., Xu, H., Zhong, W., Wei, X., et al. (2024). Association between single nucleotide polymorphisms, TGF-β1 promoter methylation, and polycystic ovary syndrome. BMC Pregnancy and Childbirth, 24(1).
24. Shen, H., & Wang, Y. (2019). Activation of TGF-β1/Smad3 signaling pathway inhibits ovarian follicle development in polycystic ovary syndrome by promoting granulosa cell apoptosis. Journal of Cellular Physiology, 234(7), 11976–11985.
25. Raja-Khan, N., Urbanek, M., Rodgers, R. J., & Legro, R. S. (2013). The role of TGF-β in polycystic ovary syndrome. Reproductive Sciences, 21(1), 20–31. https://doi.org/10.1177/1933719113485294