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Polymorphism of the Aromatase Enzyme Gene at the rs700519 Site and Its Relationship with Some Biochemical Variables in Women with Polycystic Ovary Syndrome

[1]R. Zaidan. Mukhlif, and [2]T. M. Jabbar,

[1&2]*College of Science, Department of Biology, University of Tikrit, Iraq.*

tahani.m.jabbar4446@st.tu.edu.iq

Abstract Polycystic Ovary Syndrome (PCOS) is identified as one of the most common hormonal disturbances that can affect females in their reproductive period, having significant implications on fertility observable during the reproductive span and post-menopause (Hanan et al., 2020). This study was designed to decipher the relationship between PCOS and the polymorphism of the aromatase gene at the rs700519 locus along with certain biochemical variables. The research comprised 50 blood samples from women afflicted with PCOS and 25 samples from non-afflicted women, spanning an age range of 16 to 45 years. Additionally, the study involved exploring the polymorphisms of the aromatase gene (CYP19A1) concerning SNPs rs700519 in both afflicted and non-afflicted women. The electrophoretic analysis of the PCR product on a 1% agarose gel for the aromatase gene (CYP19A1) indicated the presence of three genetic patterns (GG, GA, AA). The allele frequency for the aromatase gene SNP CYP19A1 (rs700519) in women with and without PCOS revealed values of 0.49 and 0.54 for the G allele, and 0.51 and 0.46 for the A allele, respectively. The genetic patterns did not exhibit any significant predisposition, with an Odds Ratio (OR) of 0.818 for the mutant A allele at the CYP19A1 rs700519 locus

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Keywords: *SNP, Aromatase gene, Prl, Testo, PCOS.*

1. INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is recognized as a complex and heterogeneous hormonal disorder affecting more than 7% of women in their reproductive phase (1). Its global prevalence is estimated to be around 5-10%, depending on the diagnostic criteria utilized (2). PCOS remains one of the common ailments among women, posing a significant challenge to healthcare providers. Patients often navigate from one doctor to another in an attempt to understand their condition, usually undergoing lengthy periods before obtaining a definitive diagnosis, often without arriving at a substantial solution. The syndrome conglomerates several medical specialties aiming to enhance the healthcare of afflicted women, integrating fields such as obstetrics and gynecology, pediatrics, endocrinology, internal medicine, genetics, psychology, and laboratory medicine, all striving to understand PCOS comprehensively and diagnose its multifaceted aspects. The economic burden of PCOS was previously estimated to be around 3.7 billion USD in 2020, considering only the initial diagnosis costs and reproductive endocrine disorders, excluding the costs associated with pregnancy-related or long-term diseases.(3)

Numerous studies have pointed to a link between PCOS and a malfunction in the gene responsible for insulin function. Despite the varied causes of this disease, it is vital to address it effectively. PCOS stands as a primary cause of infertility among women, yet it does not imply that affected individuals cannot conceive. It can be managed through the use of medications specifically designed to treat this disorder, such as anti-androgen drugs. In some cases, surgical intervention, like ovarian drilling, may be necessitated to ameliorate the condition (4). The principal cause of this syndrome remains unclear to date, but there is evidence suggesting the involvement of genetic factors or a dysfunction in the pituitary gland affecting ovarian response to pituitary hormones.

A theory suggests that a decrease in dopamine secretion in the brain's upper center might play a role in the onset of the syndrome. Some studies also indicate that an increase in Luteinizing Hormone (LH) could lead to a decrease in estrogen secretion, causing irregularity in the response of ovarian follicles.(5)

Women with PCOS often exhibit symptoms such as menstrual irregularities like oligomenorrhea or amenorrhea, acne, hirsutism, obesity, insulin resistance, elevated levels of

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Insulin-like Growth Factor-1 (IGF-1), infertility, lactation, presence of ovarian cysts, acanthosis nigricans (manifested as dark patches appearing on the neck and armpits), depression, occasional perspiration, frontal baldness, and hair loss.(6,7)

Genetics play a significant role in the causality of the syndrome, with the genetic nature of the patients reverting to a dominant type gene, accompanying the emergence of baldness in women affected by the syndrome. The genetic cause remains undiscovered to date. Moreover, obesity and changes in body composition are correlated with insulin resistance, which is one of the critical accompanying symptoms of the syndrome (8). Genetics has played a vital role in the occurrence of the syndrome, and studies have shown that identical twins are more susceptible to developing PCOS compared to non-identical twins or non-twin siblings. Genetic studies indicate that PCOS has a dominant autosomal inheritance.(9)

Aromatase enzyme (CYP19A1) stands as one of the critical genes responsible for the biosynthetic pathway of steroid hormones, constituting a complex enzyme (cytochrome P450arom) (10). It is known as a steroidogenic enzyme belonging to the cytochrome P450 complex family, playing a vital role in the conversion of androgens to estrogens. A deficiency in aromatase disrupts this pathway, halting the conversion process, affecting ovarian function, and increasing androgen levels due to the failure to convert C19 androgens to C18 estrogens. The CYP19A1 gene harbors single nucleotide polymorphisms (SNP), notably rs700519 (C/T), located in the exon region (11). The CYP19A1 gene spans over 123 kilobases, with the regulatory region covering about 93 kilobases and the coding region approximately 30 kilobases (Serdar et al., 2003). The current study employs techniques such as Tetra ARM – PCR.(12) . To investigate the relationship between Single Nucleotide Polymorphisms (SNP) at the rs700519 locus in the aromatase gene CYP19A1 and its correlation with certain biochemical variables in the incidence of Polycystic Ovary Syndrome (PCOS).

2. MATERIALS AND METHODS

For this study, a total of 75 samples were collected from individuals aged between 16 and 45 years, from November 1, 2022, to March 1, 2023. The samples were divided into two groups: 50 samples from patients and 25 samples from healthy individuals. The samples were collected from gynecological consultations at Tikrit Teaching Hospital and outpatient clinics through necessary analyses and ultrasound examinations to confirm whether or not they were affected by the syndrome. The prolactin and testosterone hormones were measured, where 5 ml of venous blood was drawn from each woman, whether affected by PCOS or not, during days 2-6 of their menstrual cycle. These samples were divided into two sections for analysis and study:

Section One:

2 ml of venous blood was placed in a tube containing EDTA as an anticoagulant. The sample was then stored in an ultra-low temperature unit for later use in DNA extraction and molecular studies.

Section Two:

3 ml of the blood was placed in test tubes containing gel (Gel tube). Subsequently, the samples were separated using a centrifuge at a speed of 3500 rpm for 15 minutes to obtain blood serum. The serum was transferred to Eppendorf test tubes and stored at a temperature of -20°C. All sample-related information was recorded and documented, ready for use in biochemical variable tests in the current study.

The concentration of the male hormone, testosterone, was estimated using the instructions provided with the ready analytical kit manufactured by Monobind Inc., USA, specialized in ELISA technique. The absorbance was read at a wavelength of 450 nm using a specific ELISA reader, 15 minutes after adding the solution. Moreover, a ready analytical kit prepared by Monobind Inc., USA, containing all the necessary steps to measure PRL hormone concentration, was also utilized. The absorbance was measured using a wavelength reader specific to the ELISA technique at 450 nm, and the reading was taken 30 minutes after adding the reaction stop solution.

3. MOLECULAR STUDY

This study encompassed the following:

1. DNA Extraction: DNA was extracted from blood samples of each woman, whether affected by PCOS or not, using a kit prepared by GENEAID for the purpose of extracting genomic DNA.

2. Detection of Aromatase Gene (CYP19A1) Polymorphisms: The polymorphisms at the rs700519 site were detected using the Tetra-ARMS PCR technique. The four primers were designed according to the guidelines referred to by Anderson et al. (13) for this study. A PCR-Premix kit was used to detect the polymorphisms of the aromatase gene (CYP19A1) at the rs700519 site, which was prepared by the Korean company Macrogene.

- rs700519: G wild/A mutant
- Annealing Temperature: 59°C
- Product Sizes:
- G allele: 272 bp
- A allele: 326 bp

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- Two outer primers: 546 bp

This methodological approach ensures a comprehensive and detailed analysis, facilitating a deeper understanding of the complex interactions and potential genetic underpinnings of Polycystic Ovary Syndrome

Position	Primer Name	Primer Sequence
1	IF19	TCTCTTCTGTGGAAATCCTTCG
2	IR19	CCATAGAAGTTCTGATAGCAGAAAAAATAT
3	OF19	TTACACAGAAGAGTCACAATCTTAGCA
4	OR19	TAACTCTGGCACCTTAACATGAAGT

Upon electrophoresis of the PCR reaction output for the purpose of detecting the polymorphism of the aromatase gene CYP19A1 on a 1% agarose gel for a period of 45 minutes, and upon imaging with a UV Transilluminator, the site rs700519 revealed that the wild allele (G) appears at the band of 272 bp, while the mutant allele (A) manifests at the 326 bp band.

4. RESULTS

The study demonstrated the percentage distribution of phenotypic patterns among women afflicted with PCOS, as delineated in Table 1. The prolactin hormone levels were measured across patient and control samples as depicted in Figure 1, while Figure 2 illustrates the concentration of the testosterone hormone between the samples of individuals with the syndrome and the healthy controls .

The molecular analysis of the aromatase gene in SNP rs700519 reveals the genetic patterns resulting from the electrophoretic migration using the T-ARMS PCR technique on a 1% agarose gel for the aromatase gene at the rs700519 site, along with a volumetric guide (DNA ladder 100bp Marker), as displayed in Figure 3 .

Table 2 elucidates the allelic frequency in patient and control samples, alongside the OR, CI, \(X^2\), and p-Value metrics. Furthermore, Table 3 indicates the types of dominances in patients and controls for the site rs700519 in the aromatase gene, accompanied by \(X^2\), OR, CI, and p-Value statistics.

Table (1): *Percentage Distribution of Phenotypic Patterns in Women with PCOS*

Yes (%)	No (%)
Menstrual Regularity: 7 (14%)	43 (86%)
Hirsutism: 32 (64%)	18 (36%)
Appearance of Baldness: 34 (68%)	16 (32%)
Acne: 42 (84%)	8 (16%)

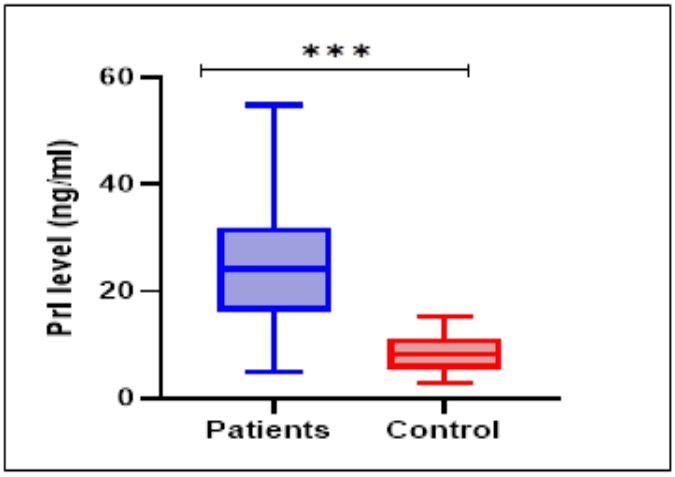

Figure (1) *illustrates the concentrations between the patient and healthy samples for prolactin hormone.*

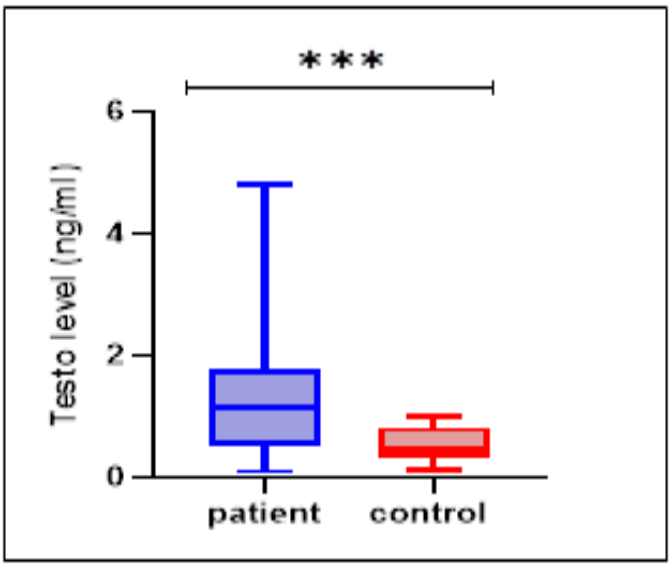

Figure (2) *demonstrates the concentrations between the patient and healthy samples for testosterone hormone."*

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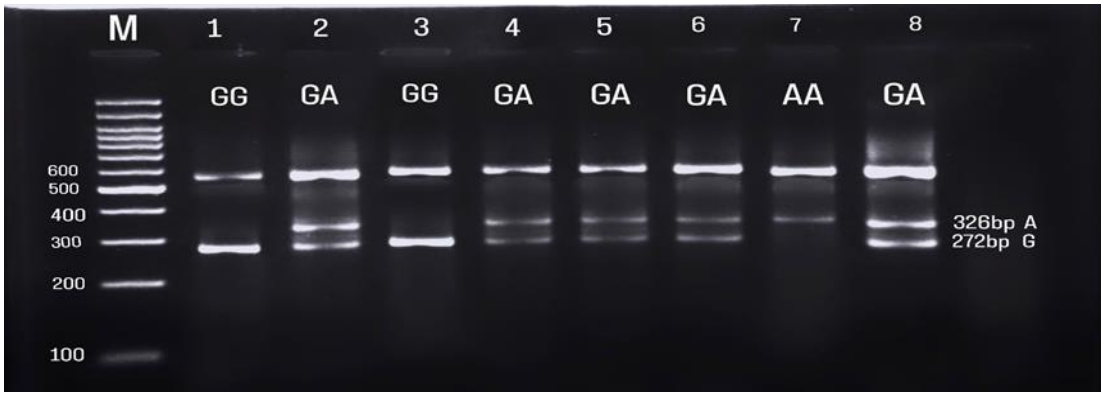

Figure (3) *displays the genetic patterns resulting from electrophoretic migration using T-ARMS PCR technique on 1% agarose gel for the aromatase gene at the site (rs700519), along with a size guide (DNA ladder 100bp Marker)."*

Table (2) *illustrates the allelic frequency in the samples from both the patients and the healthy individuals, along with the values of OR, CI, χ^2, and p-value."*

Alleles (rs700519)	Study participants							
	patient NO.	**Allele frequency**	**Healthy control no.**	**Allele frequency in Healthy control**	**OR**	**95% CI**	**χ2**	**p-value**
G (reference)	**49**	**0.49**	**27**	**0.54**	**1(ref.)**			
A	**51**	**0.51**	**23**	**0.46**	**0.818**	**0.4159 - 1.584**	**0.33**	**0.59**
Total	**100**		**50**					

Table (3) *illustrates the types of dominances in patients and healthy individuals for the site rs2414096 in the aromatase gene, along with the values of χ^2, OR, CI, and p-Value."*

Rs700519	patients	control	X^2	OR (95% CI)	p-Valu
Codominant					
GG	7	4		1 (ref.)	
GA	35	19	0.005	0.95 (0.2421-3.197)	0.94
AA	8	2	0.68	0.43 (0.06980 - 2.9)	0.4
Dominant					
GG	7	4		1(ref.)	
GA + AA	43	21	0.05	0.8547 (0.2249 -2.847)	0.81
Recessive					
GG+GA	42	23		1(ref.)	
AA	8	2	0.92	0.4565(0.09224 -2.226)	0.33
Over dominant					
GG + AA	15	6		1(ref.)	
GA	35	19	0.29	1.357 (0.4798 - 4.253)	0.58

5. DISCUSSION

Polycystic ovary syndrome (PCOS) is recognized as a multifactorial disease affecting a significant number of women globally. Those afflicted encounter various social and health issues stemming from stress. Consequently, diverse aspects of this syndrome have been studied to attain a conclusive result. In 1972, women diagnosed with infertility had small, glossy ovaries, and genetic inhibitors played a role in the polycystic ovary syndrome .(14)

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Recent studies illustrate that genetics play a pivotal role in the onset of the syndrome. However, the modes of inheritance remain elusive. Research suggests that this disorder may be the outcome of a complex interaction between multiple genes and familial factors, which culminate in the manifestation of the syndrome's phenotypic pattern (15). Female sex hormones are central to the regulation and control of the female reproductive system's functions. These hormones significantly influence the development of secondary sexual characteristics that emerge during puberty and sexual maturation. The diagnosis of polycystic ovary syndrome can be facilitated through the assessment of prolactin and testosterone hormone levels, and evaluating the increase or decrease in luteinizing hormone (LH) and follicle-stimulating hormone (FSH), followed by comparing these ratios with the standard levels .(16)

The current study's findings reveal that 86% of the women in the PCOS group experience irregular menstrual cycles, with 64% exhibiting hirsutism, approximately 68% showing signs of baldness at the front of the head, and about 84% suffering from acne, as delineated in Table 1. The study also indicated a significant rise in prolactin levels in the serum of women afflicted with PCOS, with the lowest concentration recorded at 5.090 and the highest at 54.80. The average and standard deviation were noted to be 25.07 ± 11.35 (ng/ml), contrasting with the control group which recorded a range of 8.385 ± 3.671 (ng/ml). A highly significant difference was found between the patient and control samples with a p-value ≤ 0.0001, as depicted in Figure 1.

Additionally, the study demonstrated a significant elevation in the levels of testosterone hormone in the serum of women afflicted with PCOS, with the patient samples registering the lowest concentration at 0.1000 and the highest at 4.820. The average and standard deviation were 1.273 ± 0.9691 (ng/ml), while the control samples noted a range of 0.5332 ± 0.2829 (ng/ml). A highly significant difference was observed between the patient and control samples with a p-value ≤ 0.004, as illustrated in Figure 2. This study aligns with many others conducted over past years, affirming an increase in testosterone levels. This elevation, resultant from the excessive secretion of androgens, leads to the onset of symptoms accompanying PCOS, such as hirsutism, irregular menstrual cycles, and baldness, coupled with a hormonal imbalance in ovarian secretion .(17)

Upon analyzing the genetic patterns of the aromatase gene at the site rs2414096 for the study sample of women with PCOS, the observed count for allele G in patients was 49 with an allelic frequency of 0.49, while for allele A, it was 51 with a frequency of 0.51. In control samples, allele G appeared 27 times with a frequency of 0.54, and allele A was observed 23 times with a frequency of 0.46, as illustrated in Table 2. The data depicted in Tables 4 and 5 indicate that the odds ratio (OR) for women carrying allele G is 1, whereas for those carrying the mutant allele A, the OR was 0.818. Since the odds ratio was equal to one and the confidence interval (CI) was not less than 95%, allele A does not constitute a risk factor for the disease. The statistical analysis results, when applying the Chi-square (χ^2) test to the afflicted women, showed a calculated χ^2 value of 0.33, with no significant difference (P=0.59). Regarding the types of dominances, no significant difference was observed in the genetic patterns of the aromatase gene, as shown in Table 3.

6. CONCLUSIONS

1 .A statistically significant disparity is evident in the concentrations of prolactin and testosterone hormones. Based on the conclusions reached in this study, we highlight the intricate relationships between these variables and their impacts on polycystic ovary syndrome (PCOS).

2 .An increase was observed in the frequency of GA and GG genetic patterns in the SNP (rs700519) associated with the aromatase enzyme gene. Despite this increase in the frequency of genetic patterns, no significant difference was detected between the genetic patterns and types of dominance.

3. The results attained indicate that irregular menstrual cycles were the most prevalent symptom accompanying polycystic ovary syndrome. This trend underscores the importance of focusing on providing medical care and early diagnosis for PCOS patients experiencing this symptom, with the aim of enhancing their quality of life and preventing potential health issues.

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