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"Polymorphism of the Aromatase Gene at the rs2414096 Locus and Its Association with Enzyme Concentration in Women with Polycystic Ovary Syndrome in Salah al-Din"

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Abstract

Polycystic Ovary Syndrome (PCOS) is characterized as a complex multifactorial endocrine disorder affecting approximately 15-20% of all women of reproductive age. The underlying cause is believed to be influenced by several factors including genetic variations, geographical locations, and environmental conditions (including physical and chemical impacts). This study was designed to explore the relationship between PCOS and the aromatase enzyme, and its relation to enzyme concentration in women affected with PCOS. This study was conducted on 50 blood samples from women diagnosed with PCOS and 25 blood samples from women not affected by the syndrome, with ages ranging from 16 to 45 years old.

The study also included the detection of polymorphisms in the aromatase gene (CYP19A1) for SNPs (rs2414096) in women affected by PCOS and unaffected women (control group). The results of the electrophoretic migration of the Polymerase Chain Reaction (PCR) product on 1% agarose gel for the aromatase gene (CYP19A1) revealed three genetic patterns (GG, GA, AA), where variations in the allele frequencies for the SNP of the aromatase gene (rs2414096) CYP19A1 were observed. The frequency of the G allele in affected women was 0.7 and 0.74 in unaffected women. Conversely, the frequency of the A allele in affected women was 0.3 and 0.26 in the control group. The genetic patterns did not record any significant predominance in the locus, with the Odds Ratio (OR) value for the mutant A allele in the CYP19A1 (rs2414096) gene being 0.8198. Since the OR value is equal to one, the mutant allele does not constitute a risk factor for the disease, and no significant differences were observed between the dominance types of the genetic patterns for the aromatase gene.

Keywords: SNP, Aromatase Enzyme, Polycystic Ovary Syndrome.

Introduction

Polycystic Ovarian Syndrome (PCOS) is recognized as a common and complex endocrine disorder, affecting a proportion ranging from 5 to 20% of women of reproductive age. This disorder bears both short and long-term implications, impacting various bodily systems and leading to reproductive and metabolic complications among women. Affected individuals experience varying degrees of endocrine disruptions and psychological disorders, also facing heightened risks and complications associated with pregnancy, including gestational diabetes and preterm birth.

PCOS accounts for over 75% of infertility cases, primarily due to abnormalities in the ovulation process. This syndrome is a multifactorial condition, with its onset attributed to both genetic and environmental factors, manifesting in a variety of symptoms. Women with PCOS typically exhibit characteristics such as polycystic ovarian morphology (PCOM), irregular menstrual cycles, and elevated levels of androgens (hyperandrogenism), which assist in the diagnosis of the syndrome.

Diagnosis of this syndrome can be based on the Rotterdam criteria or the guidelines established by the National Institutes of Health, encompassing symptoms such as increased risk of infertility, diabetes mellitus, hypertension, and obesity. In addition to sterility, women with PCOS are at a higher likelihood of encountering gynecological health issues, such as abnormal uterine bleeding and menopause cessation. These complications potentially escalate the risk of developing uterine or endometrial cancer in the future. Although existing studies presume a significant influence of genetic and environmental factors in the onset of this syndrome, the exact cause remains largely unidentified and uncertain.

One of the critical genes involved in the biosynthesis pathway of steroid hormones, which has a prominent place in molecular genetic studies, is the aromatase gene, also known as CYP19A1. This gene plays a vital role in female fertility, being responsible for the genetic expression of the aromatase enzyme that facilitates the conversion of C19 androgens, including testosterone and androstenedione, to estrogens such as estradiol and estrone.

The CYP19A1 gene, which encodes for P450aromtase located on the long arm of chromosome 15 at locus q21.2, spans across ten exons extending over 91 kilobases. Variations in the CYP19A1 gene can influence steroid composition in the adrenal glands and ovaries, altering hormone levels and consequently impacting hormone-related phenotypic patterns. Aromatase, a crucial enzyme in estrogen synthesis, is expressed in various biological tissues including the ovaries, testes, adipose tissue, bones, placenta, and brain. In females, aromatase activity is more pronounced in the ovaries, maintaining a hormonal equilibrium between estrogen and androgens in both sexes.

Among the techniques utilized to detect mutations is the Polymerase Chain Reaction (PCR), a biotechnological method employed to amplify and replicate DNA in the original sample. The current study employs a specialized

tetra amplification refractory mutation-polymerase chain reaction system, or Tetra ARM-PCR.

The objective of this study is twofold:

1. To investigate the relationship between the Single Nucleotide Polymorphism (SNP) and rs2414096 in the CYP19A1 aromatase enzyme gene and its association with the onset of PCOS.
2. To elucidate the relationship between the polymorphism of the gene and the level of aromatase enzyme.

Materials and Methods

In this study, a total of 75 samples were collected from individuals within the age range of 16-45 years during the period from November 1, 2022, to March 1, 2023. These samples were divided into two groups, gathered from patients and healthy individuals consulting gynecology clinics at Tikrit Teaching Hospital and outpatient clinics. The diagnosis or non-diagnosis of PCOS was confirmed through necessary analyses and sonography, during which the Aromatase enzyme levels were measured.

Preparation of Serum

5 ml of venous blood was drawn from each woman, whether affected by PCOS or not, between days 2-6 of the menstrual cycle. These samples were further divided for analysis and study into two sections:

Section One: 2 ml of venous blood was placed in a tube containing the anticoagulant EDTA. This sample was then stored in an ultra-cooling unit for later DNA extraction and molecular studies.

Section Two: 3 ml of blood was deposited into test tubes containing silicon (Gel tube). Following this, the samples were separated using a centrifuge at a speed of 3500 rotations per minute for 15 minutes to obtain the blood serum. The serum was then transferred to Eppendorf test tubes and stored at a temperature of -20°C. All related sample information was recorded and documented in preparation for biochemical variable examinations in the current study. The concentration of the Aromatase enzyme (CYP19A1) was measured using a kit produced by Sunlong Company, following the attached instructions. A Sandwich ELISA system was utilized, with absorbance (OD) read at a wavelength of 450nm using a Microtiter plate reader. The assay should be conducted within 15 minutes after adding the reaction termination solution. The efficacy of the Aromatase enzyme in the samples was calculated.

Molecular Study

This segment of the study encompassed the following:

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

Aromatase Gene Polymorphism Detection: The polymorphism of the aromatase gene (CYP19A1) at the site rs2414096 was detected using the Tetra-ARMS PCR technique. Four primers were designed according to the guidance

provided by Anderson et al. (10) specific to this study. A PCR-Premix kit was used to detect the polymorphism of the aromatase gene (CYP19A1) at the site rs2414096, supplied by the Korean company Macrogene.

For the rs2414096 marker, the details are as follows:

- G wild / A mutant
- Annealing Temperature: 59°C
- Product Size:
  - For A allele: 208 bp
  - For G allele: 282 bp
  - For two outer primers: 432 bp

Through the above methods and materials, the study aimed to explore the relationship between the polymorphisms of the specified SNP and its relation to the onset of PCOS, as well as its correlation with Aromatase enzyme levels.

No.	Primer Name	Primer Sequence
1	IF96	TCTTTTGTTACCCTCAAAAAAGACTACA
2	IR96	GAGATTTAGCTTAAGAGCCTTTTCTTACAC
3	OF96	TTTCTAATACAAGTCAATTGGTGCATT
4	OR96	GTTCTTTACAATTCTGCCATCCTCT

During the electrophoresis of the PCR product for the purpose of detecting the polymorphisms of the aromatase gene (CYP19A1) on a 1% agarose gel for a duration of 45 minutes, and upon visualization using a UV Transilluminator, the locus rs2414096 indicated that the wild allele (G) appears at the band of 282 base pairs (bp), whereas the mutant allele (A) manifests at the band of 208 base pairs (bp).

Results

The current study highlights the age difference among women affected by Polycystic Ovary Syndrome (PCOS), ranging between 16-40 years as depicted in Table 1. The aromatase enzyme level was measured in both the patient and control samples, with the concentrations delineated in Figure 1. Moreover, the molecular analysis of the aromatase gene in SNP rs2414096 illustrates the resultant genetic patterns from the electrophoresis conducted using the T-ARMS PCR technique on a 1% agarose gel for the aromatase gene at locus rs2414096, accompanied by a volumetric guide (DNA ladder 100bp Marker) as presented in Figure 2.

Table 2 elucidates the allele frequency in the patient and control samples, along with the OR, CI, χ^2, and p-Value metrics. Furthermore, Table 3 demonstrates the dominance types in both patients and controls for the rs2414096 locus in the aromatase gene, complemented by χ^2, OR, CI, and p-Value statistics.

Table 1: Percentage Distribution of Age Groups Affected by Polycystic Ovary Syndrome (PCOS)

Percentile	Count	Age Group
44%	22	15 - 24
38%	19	25 - 34
18%	9	35 - 44

Figure 1: Illustration of Aromatase Enzyme Concentrations Between Patient and Control Samples

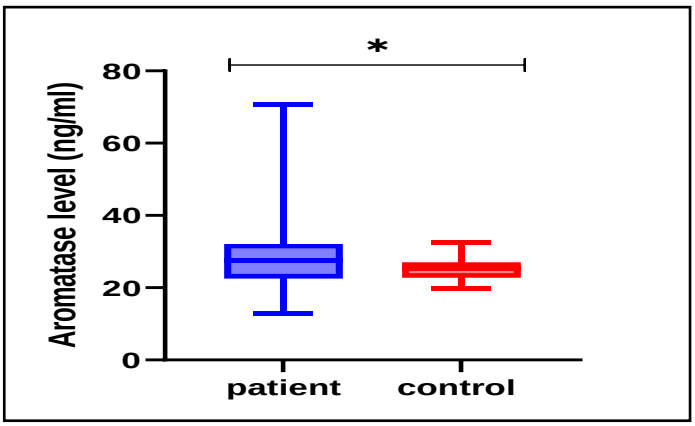

Figure 2: Genetic Patterns Resulting from Electrophoresis Using the T-ARMS PCR Technique on a 1% Agarose Gel for the Aromatase Gene at the rs2414096 Locus, Accompanied by a Volumetric Guide (DNA Ladder 100bp Marker)

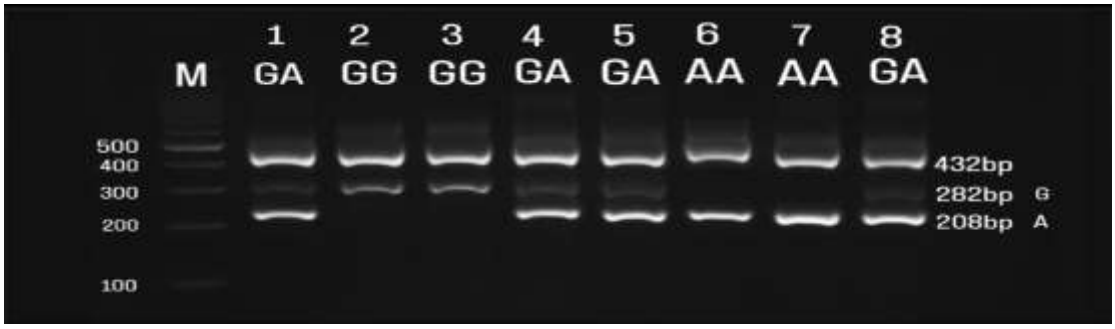

Table 1: Illustrates the Allele Frequency in Patient and Control Samples, Along with OR, CI, χ^2, and p-Value Values

Alleles (rs2414096)	Study participants							
	patient NO.	Allele frequency	Healthy control no.	Allele frequency in Healthy control	OR	95% CI	χ2	p-value
G (reference)	70	0.7	37	0.74	1			
A	30	0.3	13	0.26	0.8198	0.5450-0.9739	0.26	0.609
Total	100		50					

rs2414096	patients	control	X^2	OR (95% CI)	p-Value
Codominant					
GG	**27**	**16**	**1 (ref.)**		
GA	**16**	**5**	**1.149**	**0.5273 (0.1811- 1.645**	**0.28**
AA	**7**	**4**	**0.002**	**0.9643 (0.2811 - 4.009**	**0.958**
Dominant					
GG	**27**	**16**	**1(ref.)**		
GA + AA	**23**	**9**	**0.68**	**0.6603(0.2517 - 1.757)**	**0.409**
Recessive					
GG+GA	**43**	**19**	**1(ref.)**		
AA	**7**	**4**	**0.14**	**1.293 (0.3855 -4.991)**	**0.706**
Over dominant					
GG + AA	**24**	**20**	**1(ref.)**		
GA	**16**	**5**	**2.814**	**0.3750 (0.1315 -1.130)**	**0.093**

Table 2: Illustrates the Types of Dominance in Patients and Controls for the rs2414096 Locus in the Aromatase Gene, Along with χ^2, OR, CI, and p-Value Values

DISCUSSION

It is important to acknowledge that Polycystic Ovary Syndrome (PCOS) is a syndrome, not a disease, reflecting a variety of potential causes and presenting with variable clinical symptoms. Recent studies indicate that genetics play a major role in the occurrence of the syndrome, but the modes of inheritance remain elusive. Research suggests that this disorder might be the result of complex interactions between multiple genes and familial factors, culminating in the phenotypic manifestation of the syndrome (11). The exact mechanism behind PCOS is still unclear, although genetics significantly contributes to its onset. Autosomal dominant inheritance is believed to be a contributing factor in PCOS, hence women with one or more family members affected by the syndrome (like a mother or a sister) are more prone to developing it (12). Women have a 50% chance of developing PCOS if their mothers and sisters are affected (13). The exact pathway through which PCOS occurs remains unclear to date (14).

The current study highlights the age difference among women affected by PCOS, ranging from 16 to 40 years, as depicted in Table 1. A significant increase in the incidence rate was observed among women aged 15-24 years (44%) and 25-34 years (38%), with the rate decreasing to 18% in the 35-44 age group. Women's age can influence the diagnosis of PCOS, as they were diagnosed according to the 2003 Rotterdam criteria. Depending on the age group, they might exhibit varying phenotypic patterns. A study comprising 453 women with PCOS showed that younger women exhibited higher incidence rates and clinical symptoms like acne and hirsutism, with a decrease in cholesterol and triglyceride levels compared to older women (15).

The concentration of aromatase enzyme was measured, and the study results of 50 patient samples showed a range between the minimum

concentration of 12.85 and the maximum of 70.65. The mean and standard deviation (SD) ranged between (30.38±13.25) ng/ml. In the control group, the results from 25 samples ranged from a minimum concentration of 19.87 to a maximum of 32.53, with the mean and SD ranging between (25.07±2.932) ng/ml. A significant difference was found between patient and control samples (P≤0.01), as illustrated in Figure 1. This study concurs with reference 16, which indicated an increase in the aromatase concentration at a probability level of (p≤ 0.05) in patients with PCOS who have an elevated body mass compared to those with normal weights. Conversely, decreased aromatase activity contributes to an increase in testosterone hormone, which plays a role in hyperandrogenism, another characteristic feature of PCOS. Increased luteinizing hormone secretion stimulates the theca granulosa cells to synthesize excessive testosterone, whereas a decrease in aromatase concentration causes a disruption in aromatase activity, thereby limiting its function. Consequently, the granulosa cells are unable to convert androgens to estrogens, resulting in insufficient estrogen amounts for egg maturation, leading to chronic anovulation. Conversely, increased aromatase levels result in an increase in egg sizes and numbers, causing ovarian cysts. This study did not examine androgens and estrogens to verify the results (17).

Upon analyzing the genetic patterns of the aromatase gene at locus rs2414096 for the study sample of individuals with PCOS, the observed number for allele G in patients was 70 with an allelic frequency of 0.7, while allele A was observed 30 times with a frequency of 0.3. In the control samples, allele G appeared 37 times with a frequency of 0.74, and allele A appeared 13 times with a frequency of 0.26, as shown in Table 1.

Table 1 demonstrated that the odds ratio (OR) for women carrying allele G was 1, whereas for those carrying the mutant allele A, the OR was 0.8198. An odds ratio greater than one with a confidence interval (CI) not less than 95% indicates that since the OR equals one, allele A does not constitute a risk factor for the disease. The statistical analysis results indicate that upon applying the Chi-square (χ^2) test to the affected women, the calculated χ^2 value was 0.26, and there was no significant difference (p=0.609). Regarding the types of dominance, no significant difference was observed in the genetic patterns of the aromatase gene as depicted in Table 2.

Conclusions

1. There is a statistically significant difference in the concentration of aromatase enzyme activity. Based on the conclusions reached by these results, it sheds light on the complex relationships between these variables and their impacts on Polycystic Ovary Syndrome (PCOS).
2. An increase in the frequency of the GA and GG genetic patterns in the SNP(rs2414096) related to the aromatase enzyme gene was observed. Despite this increase in the frequency of genetic patterns, no significant difference was detected between the enzyme ratio, genetic patterns, and types of dominance.

3. The results obtained indicate that the age groups ranging between 16-24 years are more susceptible to developing PCOS. This disparity can be attributed to the heightened biosynthetic activity and elevated sexual activity during this age period. Additionally, it seems that the psychological conditions encountered by women during this stage of life play a significant role in increasing the probability of developing PCOS.

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