Full Length Research Article

Association between Paraoxonase-1 L55M Gene Polymorphism and Polycystic Ovarian Syndrome

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder affecting women of reproductive age. Oxidative stress may contribute to its pathogenesis. Paraoxonase-1 (PON1) is an antioxidant enzyme associated with high- density lipoprotein, and its genetic polymorphisms may alter enzyme activity. This study aimed to investigate the association between PON1 L55M polymorphism (rs854560) and PCOS in Iraqi women.

Methods: This case-control study included 80 women aged 20–35 years who were in good general health and matched for age and body mass index (BMI). Genomic DNA was extracted using a commercial kit. The PON1 L55M polymorphism was amplified by polymerase chain reaction and genotyped by direct sequencing. Statistical analysis was performed using SPSS version 25.

Results: No significant differences were observed between patients and controls regarding age or BMI. Genotype frequencies of rs854560 showed no significant differences between the two groups. Likewise, allele distribution was not significantly associated with PCOS ($P > .05$).

Conclusions: The PON1 L55M polymorphism was not associated with susceptibility to PCOS in this sample of Iraqi women. Further studies with larger sample sizes are recommended.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is estimated to affect 3% to 10% of women of reproductive age and represents one of the most prevalent reproductive endocrine disorders. It is characterized by signs and symptoms such as hyperandrogenism and ovarian dysfunction, including irregular ovulation and the development of polycystic ovarian morphology (PCOM) [1].

According to the Rotterdam criteria [2], at least two of the following must be present for PCOS to be diagnosed: polycystic ovaries, irregular or absent menstrual cycles (oligo/amenorrhea), and clinical or biochemical signs of hyperandrogenism. Because of the complex genetic basis of PCOS, researchers are investigating genes involved in steroidogenic and metabolic pathways to better understand the condition's genetic predisposition. The interplay between multiple genes and environmental factors further adds to the syndrome's complexity [3].

There are currently no well-recognized genetic indicators to identify PCOS susceptibility. The Paraoxonase-1 gene, which is located on chromosome 7, codes for the versatile calcium-dependent antioxidant enzyme called paraoxonase-1 (PON1). The majority of this enzyme's synthesis occurs in the liver, and it passes into the bloodstream, where it binds to HDL cholesterol [4]. PON1 is important for maintaining cardiovascular health. Some of its roles include preventing low-density lipoprotein (LDL) in artery walls from oxidizing, protecting cell membranes from oxidative stress, helping macrophages remove cholesterol, taking part in the breakdown of homocysteine thiolactone, and ultimately lowering the risk of cardiovascular disease [5].

The L55M mutation situated in exon 3 of the *PON1* gene has been recognized as a pivotal factor that influences the enzyme's active site and its overall stability. These variations exhibit distinct effects on the levels and catalytic efficiency of PON1, respectively [6]. Importantly, diminished PON1 activity has also been detected in individuals with obesity, and this has been associated with an earlier onset of cardiometabolic abnormalities [7,8]. Given the intimate connection between obesity and oxidative stress, a common occurrence in women with PCOS, it is reasonable to consider that changes in PON1 activity may also manifest in individuals with PCOS. Previous studies have indeed shown reduced PON1 activity in women diagnosed with PCOS, and this decrease was found to be inversely correlated with hyperandrogenemia [9,10]. Furthermore, specific research teams have investigated the potential link between these genetic variations and PCOS, along with its related attributes [11,12]. This study aimed to investigate the connection between paraoxonase-1 SNP rs854560 polymorphism with PCOS in Iraqi women.

METHODS

Participants

Two separate groups were created from the research populations.

Group 1: 40 women with PCOS were included.

Group 2: 40 healthy women (matched by age and BMI to group 1), have a regular menstrual cycle, show no symptoms of hyperandrogenism, and have no history of PCOS.

From May 1 to October 15, 2022, all of the women in this study were admitted to AL-Yarmouk Hospital in Baghdad, Iraq.

Preparation and DNA extraction

Genomic DNA was extracted using a commercially available extraction kit according to the manufacturer's instructions. DNA purity and concentration were assessed prior to PCR amplification. Primers targeting the *PON1* SNP (rs854560) are listed in Table 1. PCR amplification was performed under the following cycling conditions: initial denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 40 seconds, annealing at 61°C for 35 seconds, and extension at 72°C for 40 seconds, with a final extension step at 72°C for 15 minutes.

Preparation PCR mixture

PCR cycling was performed for SNP rs854560 with PCR Express (Thermal Cycler, BioRad, USA), the reaction component shown in (Table 2), and the temperature program shown in Table 1.

Inclusion Criteria

According to the Rotterdam criteria, women diagnosed with PCOS were included in this study.

Exclusion Criteria

Patients with ovarian failure, Cushing's syndrome, late- onset congenital adrenal hyperplasia, androgen-secreting malignancies (ovarian and adrenal), as well as pregnant women and those using medications that affect endocrine parameters, were excluded from the study.

Statistical Analysis

Continuous variables were presented as Mean ± standard deviation (SD) and assessed using Student's t-test. To explore the relationship between genotypes and alleles with PCOS, logistic regression analysis was employed, facilitating the computation of the odds ratio (OR) along with its associated 95% confidence interval (CI). The adherence of the polymorphism to Hardy-Weinberg equilibrium (HWE) was evaluated using the Chi- square test. Significance was established at a threshold of *P* .05. All statistical analyses were carried out utilizing SPSS, specifically version 25.

RESULTS

The mean age of PCOS-afflicted women was 29.7±5.1years, which was very similar to that of controls (30.18±5.25 years) and did not differ significantly, according to Table 4. The two groups did not significantly differ in the number of instances of hypertension and type 2 diabetes.

The *PON1* SNP rs854560 Polymorphism

The paraoxonase-1 gene fragment matching to the SNP rs854560 polymorphism, which should be 249 bp long, was amplified using a particular pair of primers. Figure 1 shows the gel electrophoresis of PCR products.

Agarose gel electrophoresis of PCR- amplified products for the PON1 gene polymorphism rs854560 is shown. Lanes 1–6 represent samples from patients with polycystic ovary syndrome (PCOS), while lanes 7– 12 correspond to healthy control subjects. Lane M indicates the molecular weight marker (DNA ladder). The expected amplification products are observed at 249 bp and 100 bp, confirming successful PCR amplification of the target fragments. The banding pattern is consistent across samples, allowing comparison of genotype distribution between PCOS patients and controls.

Genotype frequencies were comparable between PCOS patients and controls with no statistically significant differences observed in either genotype or allele distributions. Although the Odds Ratios (ORs) in Table 5 have confidence intervals (CIs) that cross 1.0, indicating non-significance, the wide ranges (e.g., 0.43– 7.59) suggest substantial variability. This limitation should be acknowledged.

This table presents the distribution of PON1 rs854560 genotypes (TT, AT, and AA) and alleles (T and A) in PCOS patients and control subjects. It also includes the results of Hardy– Weinberg equilibrium (HWE) analysis, as well as dominant and recessive genetic models. Odds ratios (OR) with 95% confidence intervals (CI) and corresponding p- values are reported. No significant associations were observed between PON1 rs854560 polymorphism and PCOS risk under genotype, allele, or genetic model comparisons (*P* > .05).

Figures

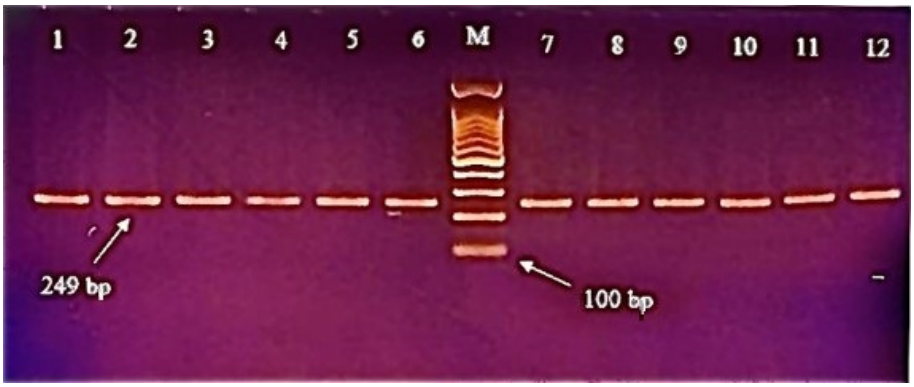

Figure 1: Agarose gel electrophoresis of PCR-amplified products for the PON1 gene polymorphism rs854560 is shown. Lanes 1–6 represent samples from patients with polycystic ovary syndrome (PCOS), while lanes 7–12 correspond to healthy control subjects. Lane M indicates the molecular weight marker (DNA ladder). The expected amplification products are observed at 249 bp and 100 bp, confirming successful PCR amplification of the target fragments. The banding pattern is consistent across samples, allowing comparison of genotype distribution between PCOS patients and controls.

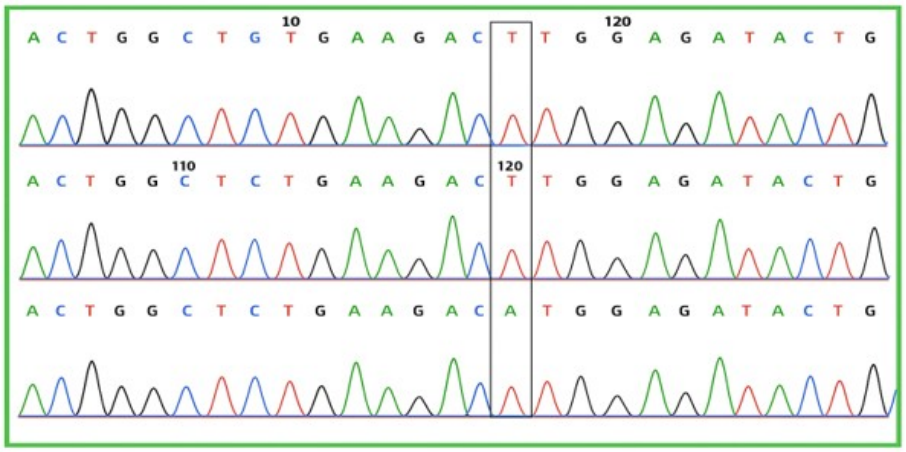

Figure 2: Sequence analysis of the PON1 SNP rs854560 on the forward strand. The chromatograms show the polymorphic sites: Top panel: Homozygous mutant genotype (TT), Middle panel: Heterozygous genotype (TA), Bottom panel: Homozygous wild-type genotype (AA). Peaks correspond to the polymorphism sites.

Tables

Parameter	Details
Forward primer	5′ TGAATTATTCTGAACCTATTAAAGAAGA 3′
Reverse primer	5′ AAGACTTAAACTGCCAGTCCTAGA 3′
Product size	249 bp
Total reaction volume	25 µL
Initial denaturation	94°C, 4 min
Denaturation	94°C, 40 sec
Annealing	61°C, 35 sec
Extension	72°C, 40 sec
Final extension	72°C, 15 min
Number of cycles	35

Table 1: PCR primers, reaction mixture components, and thermal cycling conditions used for amplification of PON1 rs854560 polymorphism.

Variables	Patients (n=40)	Controls (n=40)	*P*-value
Age, years			.487
Mean ±SD	29.7±5.1	30.18±5.25	
Range	19-41	20-41	
Body Mass Index, kg/m²			.152
Mean ±SD	29.34±6.06	28.22±6.78	
Range	19.7-44.9	20.3-57.26	
Comorbidities			
Diabetes	2(5%)	2(5%)	1.0
Hypertension	3(7.5%)	1(2.5%)	.615

A *P*-value of <0.05 was considered significant.

Table 2: Demographic and clinical characteristics of women with polycystic ovary syndrome (PCOS) and healthy controls. This table summarizes the demographic and clinical characteristics of the study participants, including age, body mass index (BMI), and comorbidities (diabetes mellitus and hypertension) in both PCOS patients and control subjects. Continuous variables are presented as mean ± standard deviation (SD) and range, while categorical variables are presented as number and percentage. Statistical analysis showed no significant differences between the two groups in age, BMI, or comorbidities (P > .05), indicating that the study groups were well matched.

Rs854560	PCOS (n=40)	Controls (n=40)	*P*-value	OR (95%CI)
Genotypes				
TT	15(37.5%)	18(45%)	0.707	1.0
AT	19(47.5%)	16(40%)	0.423	1.8(0.43-7.59)
AA	6(15%)	6(15%)	0.628	1.42(0.34-5.88)
HWE	0.996	0.871		
Dominant				
model	34(85%)	22(55%)	0.502	1.0
AA+AT	6(15%)	18(45%)		1.59(0.41-6.12)
AA				
Recessive model	15(37.5%)	18(45%)	0.496	1.0
TT	25(62.5%)	22(55%)		1.36(0.56-3.33)
AT+AA				
Alleles				
T	49(61.25%)	52(65%)	0.410	1.0
A	31(38.75%)	28(35%)		1.31(0.69-2.51)

A *P*-value of <.05 was considered significant. OR the odds ratio and CI its corresponding 95 % confidence interval.

Table 3: Genotype, allele, and genetic model distributions of PON1 rs854560 (L55M) polymorphism in women with polycystic ovary syndrome (PCOS) and healthy controls.

DISCUSSION

Age and comorbidities did not significantly differ between the patients and control group in this study.

The SNP rs854560 polymorphism is a genetic variation in the *PON1* gene that results in the substitution of leucine (L) with methionine (M) at position 55 of the protein [14]. It is located in the exon region of the *PON1* gene, which may influence its enzymatic activity.

In this study, the distribution of these genotypes among both PCOS patients and the control group exhibited a consistent pattern, without any significant differences observed. Likewise, the distribution of alleles between the two groups showed no statistically significant difference. These findings are consistent with those reported by Nalkiran et al., (2019) who also found no significant differences in the L55M genotype between PCOS patients and the control group [15].

Furthermore, the outcomes of this study are in line with several other investigations that failed to demonstrate a significant association between the L55M polymorphism and the risk of developing PCOS [16,17].

In Egyptian women with PCOS, a significant prevalence of MM genotypes was observed, which was found to significantly increase the risk of developing PCOS [18].

A study conducted by Motovali-Bashi et al., in 2015 aimed to investigate the impact of the *PON1* L55M polymorphism on PON1 activity; their findings indicated that PON1 activity was indeed influenced by the SNP rs854560 polymorphism. In both the patient and control groups, individuals with the LL genotype displayed the highest PONase activity, while those with the MM genotype exhibited the lowest activity. Interestingly, all three genotypes (LL, LM, MM) in the patient group showed lower PONase activity compared to the control group. Notably, the MM genotype was found to be more prevalent among infertile females as compared to the control group. It is essential to highlight that PON1 activity can vary significantly among individuals and is subject to influence from various environmental factors [19].

On the other hand, a study published in 2003 reported no consistent effect of the L55M polymorphism on arylesterase activity of PON1 [20]. There are a number of reasons for this disparity in research, but the most crucial ones are sample size, ethnic differences, and selection methods.

The present study suggests that the PON1 L55M polymorphism is not significantly associated with PCOS susceptibility in Iraqi women. Further large-scale studies are recommended.

CONFLICT OF INTEREST

The authors of this study confirm that they do not have any conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Hiba Hayder Kadhum: Conceptualization, laboratory investigations, data collection, and writing–original draft preparation.

Raid J. M.: Supervision, project administration, and critical review of the manuscript.

Qasim Sharhan: Supervision, validation, and final editing of the manuscript.

Fadia J.: Clinical consultation and patient examination

AI Use Declaration

The authors used artificial intelligence (AI)- assisted tools during the preparation of the manuscript for language enhancement, grammar correction, sentence restructuring, and improvement of readability. The AI tools were also used to assist in paraphrasing certain text passages to improve clarity and reduce textual similarity. However, AI was not used for data collection, data analysis, interpretation of results, generation of scientific findings, or drawing conclusions.

All scientific content, data, analyses, results, and conclusions presented in the manuscript were developed, verified, and approved by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the work.

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