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Association of paraoxonase-1 108 C >T gene polymorphism with polycystic ovarian syndrome

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ABSTRACT: Polycystic ovarian syndrome (PCOS) is increasingly recognized as a significant health concern among women of reproductive age, exerting its influence on the reproductive system and overall female physiology. Paraoxonase-1 (PON1) gene polymorphism, -108 C >T in the promoter region, have been identified as factors that influence both the stability of the enzyme and its active site. This, in turn, contributes to increase oxidative stress, a recognized risk factor associated with PCOS. This study aimed to investigate the connection between paraoxonase-1-108 C >T gen polymorphisms with PCOS in Iraqi women in a case-control study included 40 women with PCOS and 40 women with normal cycles and no symptoms of hyperandrogenism, and no history of PCOS. Whole blood was used to extract the DNA, and using specified sets of primers, the gene fragments corresponding to the -108 C >T were amplified using a conventional polymerase reaction (PCR). Direct sequencing was used to carry out the genotyping and the results showed that the age, body mass index (BMI), and comorbidities of the two groups were similar. The prevalence of homozygous genotype (TT) of the -108 C >T was greater in PCOS women (27.5%) compared to healthy women (10%), with a significant difference (OR= 5.04, 95%CI=1.11-22.97, p=0.037). The prevalence of the mutant allele (T) was found to be greater in women with PCOS (56.25% versus 141.25%) compared to healthy women. This difference was nearly statistically significant (OR=1.85, 95%CI= 0.98–3.43, p= 0.059). These results confirmed the significance of -108 C >T as a risk factor for PCOS in Iraqi women.

KEYWORDS: PCOS; Paraoxonase-1; PON1 -108 C>T gene polymorphisms; PON1 (rs705397) gene polymorphisms.

1. INTRODUCTION

Polycystic ovarian syndrome is a prevalent reproductive endocrine illness that affects 3–10% of women in their reproductive years. Its hallmarks include polycystic ovarian morphology (PCOM), hyperandrogenism, ovarian dysfunction, and oligo-ovulation [1]. The Rotterdam criteria need two of the following at minimum: excess androgens, irregular or missing menstrual cycles, for indications of polycystic ovaries [2]. In order to comprehend PCOS's genetic susceptibility, researchers have focused on genes linked to steroidogenic and metabolic pathways [3].

However, PCOS is the result of a complex interplay of various genetic factors. There are currently no well recognized genetic indicators for PCOS susceptibility, despite studies. Paraoxonase 1 (PON1) is an antioxidant enzyme with many functions [4]. PON1, which is mostly produced in the liver and binds to high-density lipoprotein HDL cholesterol that is in the blood, is essential for maintaining cardiovascular health. Its roles involve blocking the oxidation of low-density lipoprotein (LDL) in arterial walls, shielding cell membranes from free radical damage, assisting macrophages in removing cholesterol, taking part in the breakdown of homocysteine thiolactone, and decreasing the risk of cardiovascular disease [5]. The well-studied -108 C >T genetic variation in the PON1 gene's promoter region affects the stability and active site of the enzyme, affecting its levels and catalytic efficiency, respectively [6]. Obese women with PCOS been shown to have lower PON1 activity [7,8]. Changes in PON1 activity may be observed in PCOS patients due to the correlation shown in women with PCOS, obesity, and oxidative stress. It is true that earlier research on PCOS-afflicted women found lower PON1 activity, which was negatively correlated with

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hyperandrogenemia [9,10]. Numerous investigations have examined any potential link between these genetic variations and PCOS and its associated symptoms [11,12].

This study aimed to Genotyping of PON1 (108C >T) Polymorphisms in PCOS and compared with controls.

2. RESULTS

2.1. Demographic Characteristic

Table 1 displays the study population's demographic characteristics. The mean age of women with PCOS was (29.7±15.49) years, which was very close to that of Healthy women (30.18±5.25 years) with no significant difference. Although, PCOS women demonstrated higher Body Mass Index than controls (29.34±6.06 kg/m2 vs. 28.22±6.78 kg/m2), the difference was not significant. There were very few cases of Diabetes type 2 and hypertension in the two groups with no significant differences.

Table 1. The population under study's demographic characteristics

Variables	PCOS (40)	Healthy (40)	*p*-value
Age, years			
Mean ±SD	29.7±15.49	30.18±5.25	0.487
Range	19-41	20-41	
Body Mass Index, kg/m²			
Mean ±SD	29.34±6.06	28.22±6.78	0.152
Range	19.7-44.9	20.3-57.26	
Comorbidities			
Diabetes	2 (5%)	3 (7.5%)	1.0
Hypertension	3 (7.5%)	1 (2.5%)	0.615

The -108 C >T (rs705397)

The paraoxonase-1 gene fragment corresponding to the -108 C >T polymorphism was amplified using a particular pair of primers, and its predicted length was 118 bp. Gel electrophoresis of PCR products is shown in Figure 1. Three genotypes (CC, CT, and CT) appeared in the sequencing results for -108 C >T shown in Figure 2.

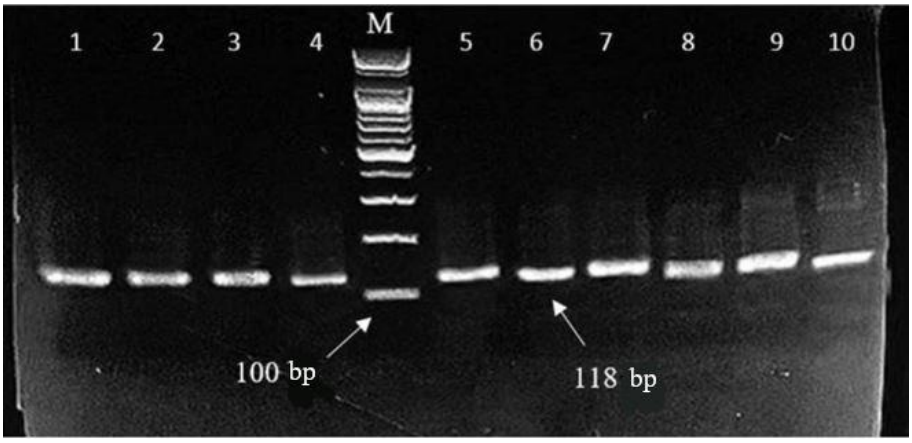

Figure 1. The -108 C> T gene's gel electrophoresis, which was amplified using a particular pair of primers by conventional PCR. Ethidium bromide was used to stain the PCR products, which were then visible under UV light in Lanes 1–10: -108 C >T, M: molecular marker molecular marker.

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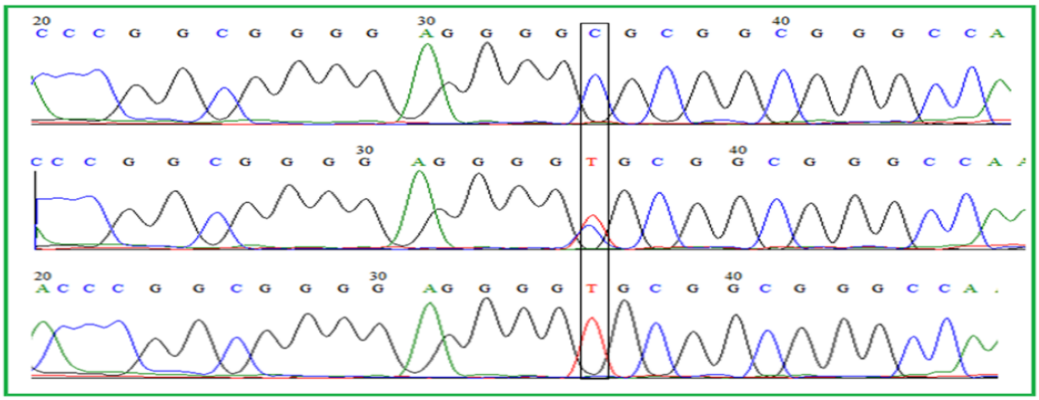

Figure 2. Examination of the forward strand's -108 C >T sequence. The polymorphism sites are represented by the bases in the frames. The homozygous wild type genotype (CC) is represented by the C in the upper frame, the heterozygous genotype (CT) is shown by the T in the middle frame, and the mutant genotype (TT) is shown by the T in the lower frame.

The homozygous genotype (TT) was more frequent in patients (27.5%) than controls (10%) with a significant difference (OR= 5.04, 95%CI=1.11-22.97, p=0.037). According to Table (2), there was a nearly statistically significant difference in the frequency of the mutant allele (T) between the patients and healthy women (56.25% versus 141.25%) at the allelic level (OR=1.85, 95%CI= 0.98–3.43, p= 0.059).

Table 2. Genotypes and alleles of PON 1 -108 C**>**T in women with PCOS and Healthy

Rs705397	PCOS (n=40)	Healthy (n=40)	*P*-value	OR(95%CI)
Genotypes				
CC	6 (15%)	11 (27.5%)	0.105	1.0
CT	23 (57.5%)	25 (62.5%)	0.093	3.0 (0.83-10.17)
TT	11 (27.5%)	4 (10%)	0.037	5.04 (1.11-22.97)
HWE	0.278	0.067		
Dominant model				
CC+CT	29 (72.5%)	36 (90%)	0.053	1.0
TT	11 (27.5%)	4 (10%)		3.14 (0.98-11.85)
Recessive model				
CC	6 (15%)	11 (27.5%)	0.177	1.0
TT+CT	34 (85%)	29 (72.5%)		2.15 (0.71-6.53)
Alleles				
C	35 (43.75%)	47 (58.75%)	0.059	1.0
T	45 (56.25%)	33 (41.25%)		1.83 (0.98-3.43)

3. DISCUSSION

Age, BMI, and comorbidities were not significantly differing in the patients and the healthy group in the current study. The purpose of this careful matching was to produce a cohort in which the characteristics of the PCOS patients were well matched with the controls. It's important to note, though, that certain research have found notable BMI variations between PCOS patients and healthy groups. BMI is essential to comprehending PCOS because of the known link between the syndrome and obesity [13, 14]. The BMIs of many PCOS-affected women are often greater than those of non-affected females. Excess weight and obesity can worsen the symptoms of PCOS and increase the chance of developing related diseases such infertility [13]. Insulin resistance, which is frequently brought on by high body fat levels and interferes with the production of hormones, is a significant role in PCOS and exacerbates its characteristic symptoms [14]. Furthermore, adipokines which is a family of hormones and chemicals produced by fat cells in adipose tissue that can accelerate the development of PCOS and further upset the balance of hormones [15]. This study concentrated on the -108 C >T polymorphism (rs705397) in the promoter region of the PON1 gene, which may affect the gene's levels of expression and, in turn, its enzymatic activity. PON1 is important because it plays a role in lipid metabolism and antioxidant defense in PCOS, which is characterized by hormonal abnormalities and metabolic problems [16]. The homozygous genotype (TT) was found to be much more common in patients (27.5%) than in healthy women (10%) as indicated by Odds ratio (OR)

results that measure of association between an exposure and an outcome (OR = 5.04, 95%CI=1.11-22.97, p=0.037) which indicate that the individuals with TT genotype have an incidance of PCOS by about 5 times than those with CC genotype. The frequency of the mutant allele (T) in patients and healthy women was almost significantly different at the allelic level (OR=1.85, 95%CI= 0.98- 3.43, p= 0.059) (56.25% versus 41.25%).

These results are consistent with earlier studies conducted on Spanish women, which found that PCOS patients were substantially more likely to have the homozygous -108 T/T genotype, which was correlated with lower PON1 expression [17]. PON1 may directly enhance susceptibility to insulin resistance, according to data from Leviev I. et al. (2001) [18]. Liu et al. (2016) conducted a meta-analysis which consistently suggested that the variation T allele of PON-1 (-108 C >T) was associated with a higher risk of PCOS, this finding affected various models of allelic distribution, including T vs. C, homozygous (TT vs. CC), and recessive (TT vs. TC+CC). However, the dominant model, TT + TC vs. CC, did not show any association [19]. The expression, protein levels, and the lactonase activity of PON-1 were discovered to be strongly impacted by the -108 C >T variants of PON-1 [20]. The development of PCOS may be impacted by insufficient PON-1 lactonase activity, according to the study, given the function of this enzyme in lowering oxidative damage and managing inflammation. The findings suggest that determining the PON-1 -108 C >T polymorphism may be a viable method for predicting the risk of PCOS [21]. This is in contrast to the findings of Ferk et al. (2014), who discovered no proof that the -108 C >T PON1 polymorphism and PCOS are related. Similarly, a recent study conducted in the Chengdu region on Chinese PCOS patients found no link between the -108 C >T PON1 polymorphism and PCOS [22]. The difference between these results may be due to place of residence, ethnic group and a sample size.

4. CONCLUSION

Collectively, the results of this study revealed that Iraqi women's PCOS may be related to the PON1-108 C >T polymorphism. The genotype (TT) of the SNP -108C>T in PON1 gene is considered as risk factor for PCOS. Women carrying the (TT) genotype of -108C>T polymorphism in PON1 gene should avoid the modifiable risk factors such as contraceptive.

5. MATERIALS AND METHODS

The research samples were split into two groups: First Group (PCOS): This cohort included 40 women who met the Rotterdam criteria (2003) for PCOS [13].

Group 2 (Healthy): Comprising 40 women who were matched to the patients in Group 1 in terms of age and body mass index (BMI). The healthy group did not exhibit hyperandrogenism, had regular menstrual cycles, or a history of PCOS.

Patients with androgen-secreting malignancies (ovarian and adrenal), late-onset congenital hyperplasia, ovarian failure, Cushing's disease, hyperprolactinemia, and those using drugs that alter endocrine parameters were excluded from both groups. The study did not include pregnant women.

5.1. DNA extraction

DNA was extracted from whole blood using a commercially ready kit for DNA extraction (Geneaid Biotech, Taiwan) according to manufacturer's instructions. The (A260/A280) ratio was used to determine the DNA's purity. A high quality DNA sample is indicated by an A260/A280 ratio in the range of 1.8 to 2.0, which is a widely accepted value.

5.2. Gene amplification and genotyping

The gene fragments corresponding the -108 C >T were amplified using specific sets of primers shown in Table 3. PCR primers were obtained from previous studies with PCR amplicon length for -108 C > T of 118 base pair [22].

Table 3. The sequences of the primers and Product size for -108 C >T (rs705397)

	Primer Sequence 5'→3'	Product size (bp)	Annealing
Forward	5'-GAC CGC AAG CCA CGC CTT CTG TGC ACC-3'	118	63
Reverse	5'-TAT ATT TAA TTG CAG CCG CAG CCC TGC TGG GGC AGC GCC GAT TGG CCC GCC GC-3'	118	

A 4 µl of DNA, 1 µl of each of the four oligonucleotides, 25 µl of mastermix, and 17 µl of deRNAase water made up the 50 µl PCR reaction (Pioneer, Korea). The reaction conditions were carried out using the temperature program in a PCR device listed in Table 4.

Table 4. The program of amplification for-108 C >T (rs705397)

Stage	Steps	Temp	Time	No. of cycle
One	Initial denaturation	95	10 min	1
	Denaturation	94	45 sec	
Two	Annealing	63	45 sec	35
	Extension	72	1 min	
Three	Final extension	72	7 min	1

Statistical Analysis

After continuous variables were provided as mean standard deviation (SD), the student's t-test was utilized to investigate them [24]. The association between alleles and genotypes and PCOS was examined using logistic regression. This test was used to calculate the odds ratio (OR) and related 95% confidence interval (CI). The chi-square test was used to quantify polymorphism's deviation from Hardy-Weinberg equilibrium (HWE). The significance threshold of $P < 0.05$was selected. The statistical software utilized for all computations was SPSS version 25.

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