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Unlocking the Genetic Link: PON1 -108C/T Polymorphism and Cardiovascular Risk in Women Battling Polycystic Ovarian Syndrome

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ABSTRACT

An important enzyme linked to high-density lipoprotein (HDL) is called *paraoxonase* 1 (PON1); it is well-known for its antioxidative qualities, which are vital in preventing oxidation of both HDL and low-density lipoprotein (LDL). In order to investigate potential connections between PON1 genotyping and its activity and a number of medical disorders, such as diabetes, cancer, heart disease, and fertility problems, more recent studies have focused on these topics. Genetic variations within the PON1 gene can disrupt metabolic processes, leading to imbalances between pro-oxidants and antioxidants, thereby potentially contributing to increased cardiovascular risk. This study involves 56 control and 53 polycystic ovary syndrome (PCOS) affected women, PON1 -108 C/T polymorphism frequencies were found using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. Through evaluating enzyme activity and polymorphism, potential associations between these two with cardiovascular risk. These findings revealed that both PCOS subjects and controls carrying the -108 TT genotype demonstrated decreased paraoxonase activity compared to those harboring -108 C alleles. Additionally, the research demonstrated a noteworthy correlation between the PON1 -108 C/T polymorphism and women with PCOS, suggesting its possible importance as a cardiovascular risk factor in this particular population. These results underscore the critical importance of comprehending genetic variations in PON1 and their implications for cardiovascular health, particularly in the context of PCOS. Further investigation into the interplay between PON1 polymorphisms, enzyme activity, and cardiovascular risk may offer valuable insights into preventive and therapeutic strategies tailored to address the unique needs of individuals, especially women, affected by PCOS.

INTRODUCTION

One of the leading causes of mortality and morbidity worldwide for both men and women is cardiovascular disease (CVD). High blood pressure, smoking, being overweight or obese, having diabetes mellitus, and having high cholesterol are traditional risk factors that can explain the epidemic of CVD in both men and women[1]. The erroneous assumption that women are "secured" from coronary artery disease often leads to an underestimation of the prevalence of heart disease in women.[2].The increased risk of CVD is probably the reason for metabolic disturbance associated with Polycystic Ovarian Syndrome (PCOS). Dyslipidaemia, diabetes and obesity significantly influence CVD in women with PCOS[3-6]. Evidence shows that more extensive coronary artery disease and myocardial infarction are likely observed in women with PCOS[7-9]. Infertility symptoms, hormone imbalances, and oxidative stress are the hallmarks of PCOS, a complex disorder affecting premenopausal women. Women with PCOS may be more susceptible to cardiovascular disease (CVD) due to a higher level of oxidative stress.

Studies and experiments have demonstrated that the calcium-dependent esterase *Paraoxonase* 1 is specifically anchored to HDL-C and is known to be an enzyme that fights free radicals because it hydrolyzes the lipid peroxides in oxidized lipoprotein.[10]. PON1 gene was also identified as a cluster of enzymes having organophosphates as substrates. It prevented cellular damage from the toxic agents, which uncovered the genetic polymorphisms[11,12]. PON1 gene polymorphism affects PON1 expression and action, which in turn affects women's susceptibility to PCOS.[13]. A functional single nucleotide polymorphisms (SNPs) were observed while screening PON1 gene C (-108)T located in promotor and coding sequence of the paraoxonase gene respectively in the long arm of human chromosome 7[14,15]. This study aims to understand the enzyme activity and upstream polymorphism to prove the association of -108C/T polymorphism and CVD risk.

SUBJECTS:

Study samples comprise 109 individuals, 53 cases with PCOS recruited from the unit of Gynecology and Obstetrics at Sri Ramachandra Medical College and Research Institute, Porur, Chennai. An ultrasonographic examination was used to confirm the PCOS diagnosis. (Fig.1(b)). The signs of insufficient flow, dysfunctional bleeding, infertility, and obesity leads to PCOS diagnosis. Controls were 56 cases with normal ovaries and regularly menstruating women of a similar age group (Fig.1(a)).

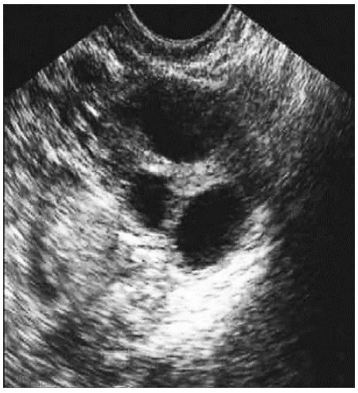


Figure 1(a): The ultrasonographical examination of normal ovary

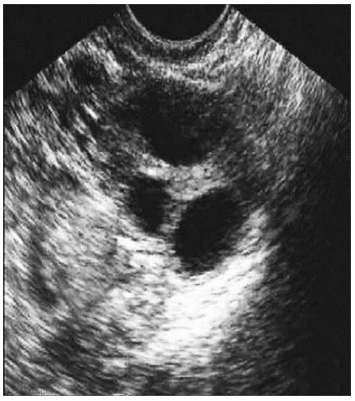


Figure 1(b): The ultrasonographical examination of PCOS ovary

2.MATERIALS AND METHODS:

5ml of blood were taken from both the control and experimental groups, and stored at -20°C to facilitate additional analysis. The serum was separated and used for biochemical studies. For the genetic study, DNA was isolated from the blood. All the chemicals used were of analytical grade. The plastic containers and glassware's used for the experiment were purchased from reputed vendors like qualigens, SRI, Star Labs, Tarsons, etc.

2.1 Determination of lipid profile

The blood sample collected was used to determine the lipid pofile. Cholesterol level was estimated by Parekh and Jung method (1970). Estimation of triglycerides was done by the method of Rice (1973). HDL- cholesterol was determined by precipitation of apolipoprotein with heparin manganese chloride reagent by Fisenberg method (1984). LDL- cholesterol was determined by Wilson and Springer method (1973).

2.2 Estimation of Paraoxonase

Paraoxanase activity was measured using a spectrophotometric assay using paraoxon (O, O-diethyl-O-P-nitrophenyl-phosphate). One nanomol of 4-nitrophenol generated per minute, under the specified test conditions, is the definition of one unit of paraoxonase activity.

2.3 Genotypic analysis

Genomic DNA was extracted using the Modified Miller's Protocol for genomic research. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used to conduct the genomic analysis of C(-108)T. The primers were created in order to examine the C(-108)T genotype (Table.1).

PCR was initiated with a denaturation step at 94°C for 5 minutes, followed by 30 cycles comprising denaturation at 94°C for 1 minute, annealing at 51.1°C for 30 seconds, and extension at 72°C for 1 minute. Subsequently, a final extension was performed at 72°C for 6-8 minutes after the completion of the cycles.

For two hours, the PCR amplicons were incubated at 37◦C with the specific restriction enzyme BsrBI. After being digested, the PCR amplicons were put through gel electrophoresis and data analysis.

3. RESULTS:

The study revealed an altered lipid profile and a dyslipidaemia status among the subjects. In this study, there has been a notable rise in triglycerides. (Table, 2). Additionally, a slight decline in HDL levels and a notable rise in LDL levels were noted. Triglycerides may accumulate because of elevated lipogenesis, reduced clearance, or decreased fatty acid oxidation. The over-accumulation of triglycerides can lead to excessive metabolites such as diacylglycerols, fatty acids, and ceramides. Lipid accumulation in non-adipose tissues has detrimental effects and raises the possibility of cardiovascular disease (CVD). According to the present study, paraoxonase enzyme levels have decreased, which could help PCOS patients develop AHD (Table 3.3).

Primer Name	Primer Sequence	Primer Length	GC%	Tm	Product Size
PC1	5' AGC TAG CTG CCG ACC CGG CGG GGA GGA 3'	28 mer	75%	>75◦C	240 bp
PC2	5' GGC TGC AGC CCT CAC CAC	23 mer	69.6%	69.6◦C	240 bp

Table 1: Sequence of primer used in amplifying the promotor of PON1 gene polymorphism

S. No	Parameters	Control	Test	F Value	P Value
1	Cholesterol	174.8022 ± 10.8701	212.4630 ±18.8243	13.248	0.0000**
2	Triglycerides	108.3022 ± 22.3991	177.1304 ± 16.8900	4.723	0.032*
3	HDL	57.4543 ± 5.0144	38.5637 ± 7.02422	2.604	0.110
4	LDL	106.7261± 9.8432	131.8696 ± 22.3991	3.655	0.059

Table 2: Dyslipidemic status of subjects.

S. No	Paraoxonase	Control	Test	F Value	P Value
1	Activity in nmol/ml	207.9152 ±7.4727	174.5065 ±19.5609	37.265	0.0

Table3: *Paraoxanase* activity

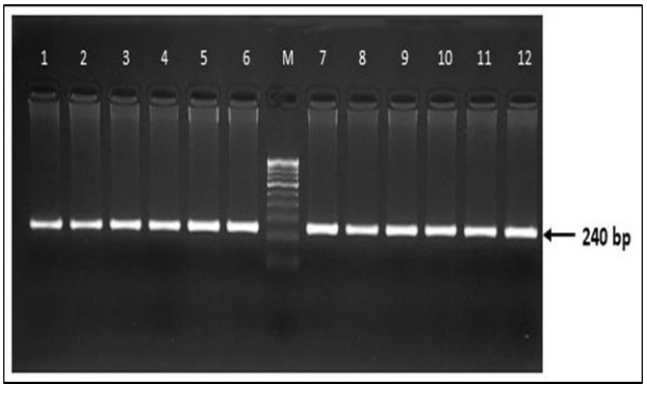


Figure 2: PCR Reaction of -108C/T PolymorphISM

Figure 3: RFLP Reaction for -108C/T Polymorphism

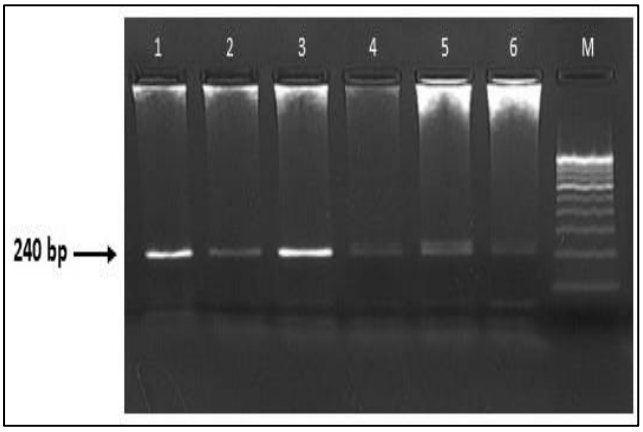
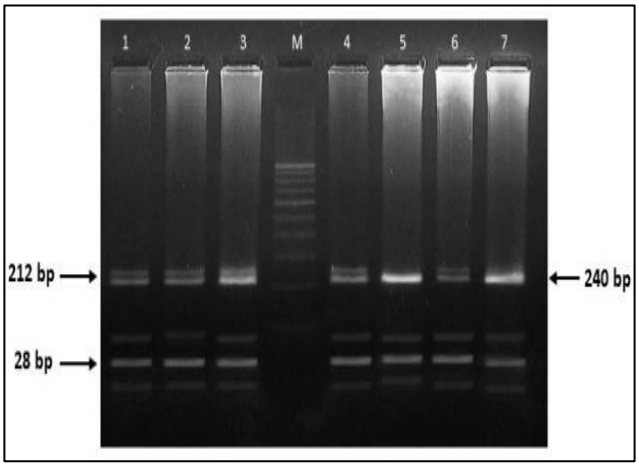


Figure 4: Restriction Digestion Pattern of -108C/T Polymorphism



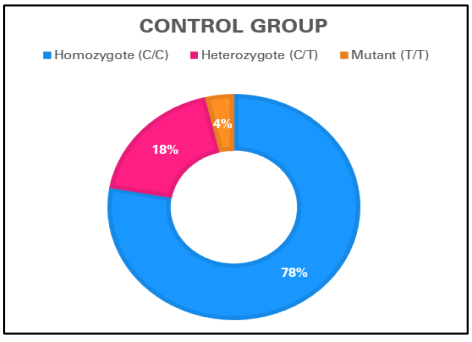


Figure 5(a): Represents the Control group

Figure 5(b): Represents the PCOS group

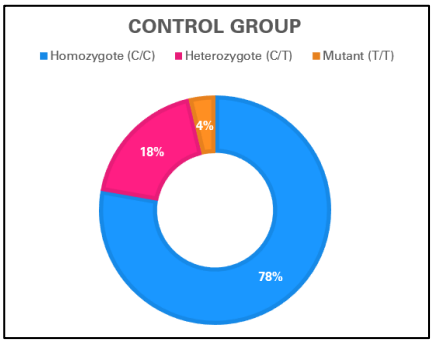


Figure 5(b): Represents the PCOS group

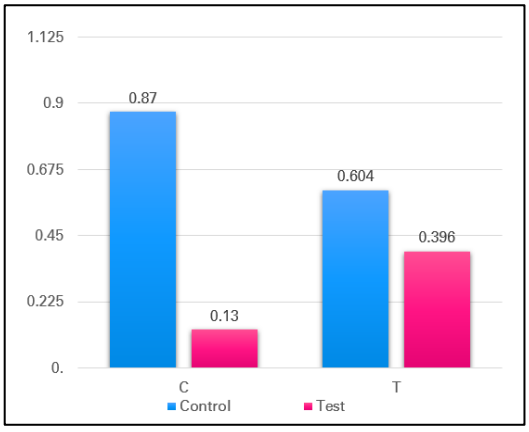


Figure 6: Allelic frequency of C-108T polymorphism

The number of natural alleles in the control group is shown in Figure 6, with the majority of them belonging to the homozygous group (CC). In contrast, the heterozygous type (C/T) and the mutant (T/T) both significantly contribute to the enzymatic behavior in Figure 5(a).

The T allele was higher in PCOS compared to control. Compared to the control group, PCOS patients had higher genotypes of CT and TT. The control group had a higher CC genotype than the subjects. The Minor Allele Frequency (MAF) was 39.6% in PCOS subjects, which is much higher, compared to 13% among control (Fig.(5b)). Thus, the above polymorphism results show that the TT genotype might contribute to the risk at the functional level of the enzyme, contributing to CV risks in PCOS women.

4. DISCUSSION:

Oxidative stress is associated with a number of conditions, including aging and chronic diseases related to aging, such as insulin resistance, atherosclerosis, and ischemia reperfusion injury. Apart from the well-known risk factors like diabetes, high blood pressure, centrally obese status, and dyslipidemia, a recent study indicates that women with PCOS may be more susceptible to cardiovascular disease due to higher levels of oxidative stress and reduced antioxidant capacity.[16,17]. These patients need to take anti-oxidant supplements, like vitamin C and E, and maintain a healthy diet in order to build up an adequate antioxidant defense system and minimize the harmful effects of excessive oxidative stress. The etiology of insulin resistance and cardiovascular disease is complicated by oxidative stress[18,19]. Aside from affecting insulin action, oxidative stress causes chronic inflammation, which contributes to type 2 diabetes, atherosclerosis, and heart disease. Chronic inflammation,

moreover, is at the root of PCOS pathophysiology. Women with PCOS have reduced serum PON1 activity, which increases their risk of atherosclerosis. [20,21]. In our investigation, the activity of PON1 in the blood was decreased in the PCOS group. Liver PON1 mRNA expression is influenced by genetic and environmental factors, while proinflammatory mediators and androgens decrease liver PON1 expression. The PON1 gene, predominantly expressed in the liver, encodes serum paraoxonase. Additionally, some research indicates that in women with PCOS, insulin resistance, PON1, and dyslipidemia increase the risk of cardiovascular disease. [22], As a result, utilizing medicines that modify these factors can help PCOS patients avoid long-term health consequences.

5. CONCLUSION:

In this study, the enzyme activity of *Paraoxonase 1* (PON1) and the polymorphism of its respective gene were interpreted. The significant difference in the *Paraoxonase 1* (PON1) enzyme may result in the risk factors of CV disease in PCOS women. Hence, a correlation exists between PON1 −108C/T polymorphism, enzyme activity, and risk factors due to the abnormal profile in PCOS subjects. Therefore, there is a possible chance of CV diseases in these subjects.

6.ABBREVIATIONS:

PCOS – Poly-Cystic Ovary Syndrome, PON1 – *Paraoxonase 1*, HDL – High-Density Lipoprotein, LDL – Low-Density Lipoprotein, CVD – Cardiovascular Disease, PCR-RFLP – Polymerase Chain Reaction-Restriction Fragment Length Polymorphism.

7.GENETIC NOMENCLATURE AND PRIMER INFORMATION:

The gene PON1: *Paraoxonase 1* is a single nucleotide variant of nucleotide change −108C/T polymorphism (NM_000446.6(PON1):c.-108C>T). The rsID of −108C/T polymorphism is **rs705379**. The gene sequence accession number is NM_000446. The primer sequences used in this research is included in the table 1 along with the location of primers.

COMPETING INTERESTS:

No conflicts of interests

DATA AVAILABILITY STATEMENT:

The sequence is not reported in our any of the previous studies. So, the data availability statement is not applicable.

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