JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 1143-1148

Paraoxonase Q192R Gene Polymorphism and Cardiovascular Risk are Associated with Polycystic Ovarian Syndrome Women

Jayasree Ravindran[1]*, Vijayalakshmi Melanathuru[2] and A. Jamuna Devi[3]

[1]Department of Biotechnology, Rajalakshmi Engineering College, Chennai - 602 105, India.
[2]Department of Biotechnology, Dr. M.G.R. University and Research Centre, Chennai , India.
[3]Department of Biotechnology, Rajalakshmi Engineering College, Chennai 602 105, India.

(Received: 10 February 2015; accepted: 11 March 2015)

The aim of the study was to investigate the lipid profile and its correlation with paraoxonase enzyme in PCOS women and to identify the association of PON1 192 polymorphism with cardiovascular risk. Human paraoxonase 1 (PON1 EC 3.1.8.1) is a HDL-complexed enzyme. It is of clinical importance due to its established associations with inhibition of LDL oxidation. It has anti-oxidative properties too. A decreased paraoxonase activity may contribute to cardiovascular risk. 53 test and 56 control subjects were included in the study. PCOS women exhibited a variety of symptoms whereas the control subjects were chosen to be regularly menstruating women. Blood samples were collected and used for the assay . DNA samples were used for the genetic studies. Polymorphisms were determined by restriction fragment length polymorphism. The results of the study shows a rise in the levels of cholesterol, LDL cholesterol and a decreased HDL in the study subjects when compared to control. A decreased paraoxonase level reported and the genetic variations identified relate the polymorphism in the gene of paraoxonase, with increased risk for cardiovascular diseases. Paraoxonase Q192R Gene Polymorphism are associated with cardiovascular risk.PON1 seems to be related with hyperlipidemia and cardiovascular risk in women with PCOS.The purpose of the study was to assess the impact of paraoxonase level and its genetic variation in PCOS women. The study also investigated if these women harvest a risk for cardiovascular disease.

Key words: Paraoxonase, Polycystic ovary syndrome, Polymorphism, genetic variations, cardiovascular risk.

The polycystic ovary syndrome (PCOS) is one of the most common endocrine metabolic disorders in women[1]. Coronary artery disease (CAD) is a common, complicated and multifactorial disorder and now it has turned out to be a leading cause for morbidity and mortality in women in different parts of the world[2]. Paraoxonase 1 (PON1) a calcium-dependent antioxidant glycoprotein, is produced in the liver and secreted into plasma, where it is associated with high density lipoproteins (HDL). Paraoxonase enzyme (EC 3.1.8.1) is implicated in lipid metabolism and in the prevention of oxidation of LDL. Many genes are involved in the pathogenesis of CVD[3]. PON1 is coded by the HUMPONA gene located on the chromosome 7 (7q 21.3-22.1).

The aim of the investigation was to analyze genetic variation at the PON1-192 and to assess their genetic association with CAD in south Indian women with PCOS. This is a part of genetic analysis to untangle the genetic architecture of paraoxonase gene in heart diseases and its association with PCOS in South Indian women.

* To whom all correspondence should be addressed.
E-mail: jayasree.r@rajalakshmi.edu.in

METHODS

Study design and population

This study was conducted in 53 test subjects with Poly-cystic ovarian syndrome, exhibiting a wide variety of symptoms ranging from amenorrhea, oligomenorrhea, scant flow, dysfunctional bleeding, infertility problems, hair fall and obesity .The subjects were chosen from the out-patients unit at Sri Ramachandra Medical College & Research Institute, Porur, Chennai. PCOS status was confirmed in these subjects by ultrasonography.

Age and ethnicity matched control group consisted of 56 healthy, regularly menstruating women. Informed written consent was obtained from the study subjects. Ethical clearance was obtained following guidelines set down by the institute's ethical committee.

Sample collection

Blood samples were collected after a 12-14 h fast. Blood samples were centrifuged at once, serum was separated and frozen at -70°C until assayed. EDTA samples were collected for the determination of PON1 Genotypes. DNA was stored at –20°C until analyzed.

Chemical studies

Lipid parameters like triglycerides (TG), total cholesterol and HDL were assayed. Total cholesterol was estimated by Parekh and Jung method[4] and triglyceride by Rice method[5]. HDL was calculated by Eisenberg et al method[6]. The LDL level was calculated using the Friedewald equation. (Table 1) PON1 activity was assessed by the rate of enzymatic hydrolysis of paraoxon (Sigma chemical Co) to *p*-nitrophenol. The amount of *p*-nitrophenol generated was monitored with a continuously recording spectrophotometer by the increase in absorbance at 405 nm and 25°C [7].

Genotyping study

Human Genomic DNA was isolated from the blood using Modified Miller's Protocol [8]. PCR was used to amplify two loci of the PON1 gene from 100-ng genomic DNA in a total reaction volume of 20 µL, as described by Humbert *et al* [9]. 5' TGA AAG ACT TAA ACT GCC AGT C 3'a forward primer and 5' CCT GAG AAT CTG AGT AAA TCC ACT 3'a reverse primer was used. Genotyping was performed by RFLP (Restriction Fragment Length Polymorphism) analysis. PCR amplicons were digested with *DpnII* enzyme that recognizes 5' GATC 3' site and produce two fragments (175bp + 63bp). In normal, *DpnII* will recognize the single site and produce two fragments. In heterozygous mutant type, *DpnII* produces three fragments (238bp, 175bp and 63bp). Gel electrophoresis was carried out in 0.7% agarose gel.

Statistical analysis

Student *t* test was used to compare means for continuous variables. The Hardy-Weinberg equilibrium was assessed by using chi-square test. The odds ratio with 95% confidence intervals was calculated using standard epidemiological and association methods and significance was assessed by the chi-square test. All *P* values are two tailed; $P < 0.05$ was considered statistically significant. To examine the synergistic effect of PON1 (-192) gene polymorphisms and the risk of CAD, we conducted the multivariate analysis using the SPSS software (version 11.5). Allele frequencies were calculated as the number of occurrences of the test allele in the population divided by the total number of alleles. The carriage rate was calculated as the number of individuals carrying at least one copy of the test allele divided by the total number of individuals. Bonferroni correction was used to correct the level of significance. The sample size calculations revealed that the present sample had enough power (>80%) to detect an odds ratio of around 1.5 or higher at alpha level of 5%. This was based on distribution of allele frequencies in previous studies. The haplotype frequency estimation was computed by using Arlequin version 3.1 software.

RESULTS AND DISCUSSION

Lipid profile was studied in the study subjects and control groups and the results are shown in Table 1. According to the National Educational Program guidelines, prevalence of abnormal lipid level is either, border line normal or high approaching 70% in PCOS[10]. The current investigation is also in accordance with the above guidelines. This study shows a rise in LDL cholesterol that may be a causative risk factor for CV disorder[11]. The variations in lipoprotein

profile especially that of HDL and LDL causes a pathological status which may lead to tissue damage, proliferations and inflammations eventually leading to CV abnormalities[12]. The study has revealed a significant borderline risk in cholesterol level, which indicates the primary alterations in lipid metabolism in subjects with PCOS. The study is in accordance with a previous study the Third report of National Cholesterol Education Program Expert Panel, 2002[13]. A significant fall in HDL-Cholesterol in PCOS women clearly suggests an early risk for CVD. As HDL removes cholesterol from tissues, the antiatherogenic role of HDL is low in these subjects [14]. The decreased HDL level reported from the current study states that there is a low scavenging mechanism of HDL lipoprotein in PCOS women leading them to the risk of heart diseases. The present investigation is in

Table 1. Lipid profile in test and control subjects

Parameters	Control	Test
Cholesterol	134.11 ± 31.09	171.79 ± 48.83***
Triglycerides	132.64 ± 60.84	166.09 ± 10.71***
HDL	40.83 ± 7.14	32.14 ± 12.71***
LDL	80.79 ± 24.26	106.72 ± 9.84***

Student *t* test was used to compare means. *P< 0.05 was considered statistically significant.

Table 2. Level of Paraoxonase in test and control subjects

Paraoxonase	Control	Test	F value
Activity in (nmol/mt)	207.91 ± 7.47	174.50 ±19.56**	37.26

Student *t* test was used to compare means. *P< 0.05 was considered statistically significant

Table 3. Genotype and allele frequencies of Q192R Polymorphism of PON1 gene

Subjects		Genotype			Allele frequency		X^2
	N	QQ	QR	RR	Q	R	
Control	56	51	6	0	0.947	0.053	0.176
PCOS	53	49	4	0	0.962	0.038	0.082

X^2 - Hardy Weignberg Equilibrium

Table 4. Percent and gene frequencies of Q192R Polymorphism of PON1gene

Genotypes	Control (%)	Case (%)	P value*
Homozygote reference (QQ)	89.5%	92.5%	0.8327
Heterozygote (QR)	10.5%	7.5%	-
Homozygote variant (RR)	0.0%	0.0%	-
MAF*	5.3%	3.8%	0.8367

MAF * - Minor Allele Frequency

Table 5. Odd ratio of Q192R Polymorphism of PON1 gene

Case vs Control	ODDs Ratio	P value**	95 % CI	
QR vs QQ	0.69	0.83273	0.185	2.609
RR + QR vs QQ	0.69	0.83273	0.185	2.609
R vs Q	0.63	0.83671	0.173	2.296

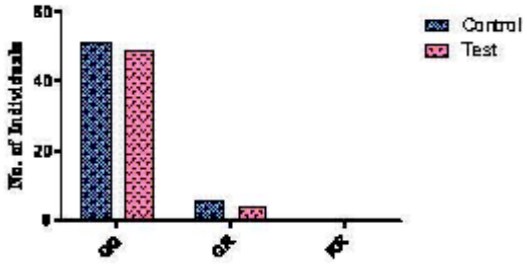

Fig. 1. PCR Reaction of Q192R Polymorphism

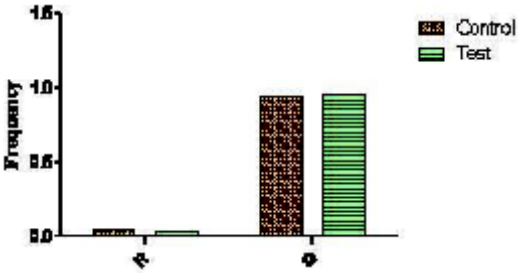

Fig. 2. RFLP Reaction of Q192R Polymorphism

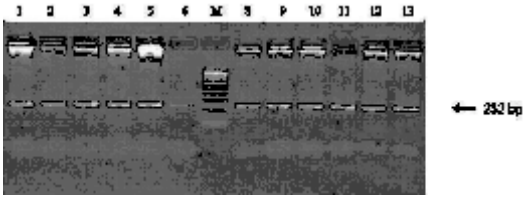

Fig. 3. Restriction Digestion Pattern of Q192R Polymorphism

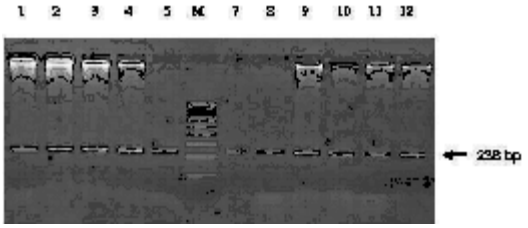

Fig. 4. Genotype Frequency of Q192R Polymorphism

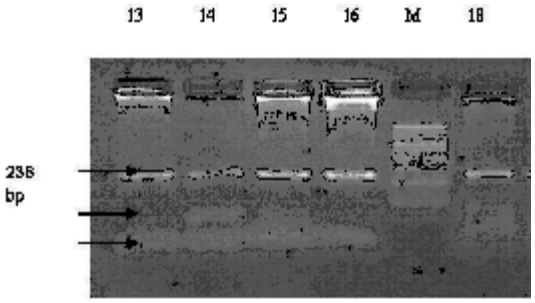

Fig. 5. Allele Frequency of Q192R Polymorphism

accordance with a study which has concluded a decrease in PON1 activity might contribute to an increased probability for the development of cardio vascular disorder in PCOS women[15]. The paraoxonase level and its genetic background contribute to an increased oxidative stress[16].

The present study demonstrates that a decreased paraoxonase level serves as a marker for cardiovascular disease in PCOS subjects (Table 2). The decrease in the level of HDL and paraoxonase enzyme leads to an accumulation of oxidized LDL, which induces a state of lipotoxicity[17]. The present investigation supports the fact that subjects with PCOS have all possibilities of harvesting the risk associated with cardiovascular disease. Correlation of various researches in lipid profile and paraoxonase level has concluded that PON1 associated with HDL exerts a protective effect against oxidative damage of the system. Our results agree with conclusions that reduced serum PON1 activity may contribute to the increased susceptibility for the development of CVD in PCOS women[18]. A decreased activity of the enzyme enhances the risk for cardiovascular disease[10].

Studies with respect to the PON1 Q192R polymorphism indicate diminished PON1 concentrations (Fig.1) and an increased prevalence of the RR allele indicating a risk for CHD[13].

In the current study of Q192R, (rs662) polymorphism (glutamine to arginine conversion at the 192nd position) was screened for, in the PON1 gene in the PCOS population .The results of the Q192R polymorphism is shown in the Table 3. The RFLP reaction for the same polymorphism is represented in the

Fig. 2. The restriction digestion pattern results are depicted in the Fig. 3. The QQ genotype was found in equal numbers in tests and the control (Fig. 4). The Q allele was found to be much higher in PCOS group and control (Fig. 5). However, the QR genotype was found only in very few control and tests. The Minor Allele Frequency (MAF) was 3.8% in PCOS which is lower, compared to 5.3% among control (Table 4 & 5). The frequency distribution results suggest that the R allele could act as a protective factor.

The Q192R (rs 662) variation in PON1 gene is an important determinant of serum

paraoxonase activity and contribute for the risk of cardiovascular disease in the subjects. The study is in accordance with a genetic study stating that the lower paraoxonase activity and the compositional changes in HDL and LDL could contribute for cardiovascular risk[10]. It is clear that PON1-192 RR genotype and *R allele are significantly associated with CAD risk among south Indian women .

From the present study, we have established a co-relation between polycystic ovarian syndrome, paraoxonase enzyme level, its polymorphism and the possibilities for the risk of cardio-vascular disease. We observed a reduction in the levels of HDL and PON-1 enzyme and an elevated LDL level. DNA analysis of blood samples revealed polymorphism in the 192nd position of the paraoxonase 1 gene that reduces the enzymatic action and its ability to prevent the oxidation of LDL in serum. This results in dyslipidemia and the elevated cholesterol when accumulated leads to atherosclerosis. The polymorphism study determines that a low PON1 activity contributes for higher risk of cardiovascular disease in the subjects with PCOS.

CONCLUSIONS

Paraoxonase Q192R Gene Polymorphism are associated with cardiovascular risk. PON1 seems to be related with hyperlipidemia and cardiovascular risk in women with PCOS. PCOS women harvest a risk for cardiovascular disorders. Hormonal imbalance in PCOS women can lead to various metabolic disorders. A frequent lipid profiling is advised for the PCOS subjects to rule out the risk of cardiovascular disorder. A research in a larger study population is required to infer the level of cardiovascular risk in PCOS women. A dietary control of lipid is recommended for PCOS women.

ACKNOWLEDGMENTS

The authors are thankful to Dr. M. Dhanalakshmi, Department of Obstetrics and Gynecology, Sri Ramachandra Medical College and Research Center, Chennai for providing the necessary samples to carry out the investigation.

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