



The association of *PON1* 192 Q/R polymorphism and the risk of female infertility

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Abstract

Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months of regular unprotected sexual intercourse. Both environmental and genetic factors are involved in female infertility. Paraoxonase (PON) is an oxidant enzyme which plays an important role in various diseases and is associated with inflammation, oxidative stress and lipid metabolism. The present study was aimed to evaluate the *PON1* 192 Q/R gene polymorphism in female infertility. Samples were obtained from 150 patients diagnosed with female infertility and 150 controls subjects and genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). The *PON* genotype frequencies amongst the 70 cases were C/C=40%, C/T=52.8% and T/T=7.2%; the C and T allele frequencies were 66% and 34%, respectively. The *PON* genotype frequencies amongst the 73 controls were C/C=45.20%, C/T=50.70% and T/T=4.1%; the C and T allele frequencies were 70% and 30%, respectively. We observed a significant difference in the genotype distributions of *PON1* 192 Q/R polymorphism between patients and controls ($P=0.03$). Our findings revealed that individuals with the variant QR had a significant decrease risk of female infertility (OR= 0.55, 95% CI= 0.33 – 0.91, $P=0.019$). The data from this study indicates that the *PON1* 192 Q/R polymorphism may be associated with decreased risk of female infertility. Although more studies should be considered with larger number of patients and control subjects to confirm our results.

Key words: Female infertility, gene polymorphism, *PON*.

Introduction

Infertility is a disorder of the reproductive system defined by the failure to gain a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (1). In spite of advances in clinical diagnostics 50% of infertility cases remains unclear which are referred to as idiopathic infertility (2). Most idiopathic cases are probable to be of genetic origin because many genes have been shown to be involved in human metabolic abnormalities (3). Moreover, infertility is assumed to be caused by several factors, including genetic and epigenetic abnormalities, reactive oxygen species or endocrine disruption as a result of environmental pollution (4).

Reactive oxygen species (ROS) are generated as a by-product in mitochondria of normal mammalian cells. Low levels of ROS have physiological functions including activation and regulation of signal transduction pathways, modulation of activities of redox-sensitive transcription factors, and regulation of mitochondrial enzyme activities, but at high levels ROS are toxic to the cell (5). Increased ROS levels can lead to damage with following oocyte dysfunction or cell death (6). Paraoxonase (PON) is a HDL-associated enzyme and a family of Ca^{2+} depended hydrolase that inhibits low-density lipoprotein oxidation. It has antioxidant function and protects cells from oxidative stress (7).

The PON gene family consists of 3 genes including *PON1*, *PON2*, and *PON3* that located on the long arm of chromosome 7 (8). *PON1* is the first member of the PON gene cluster to be discovered. Changes in the size and shape of HDL particles strongly influences the binding affinity and stability of *PON1* and results in a decreased antioxidative capacity (9). Inactivation of

PON1 reduces the ability of HDL to prevent both the oxidation of LDL and the interaction between macrophages and endothelium (10).

The *PON1* gene has two polymorphisms in the coding region and five in promoter region. *PON1* gene substitution of glutamine (Q) by arginine (R) at position 192 and leucine (L) by methionine (M) at position 55 of coding region has been shown (11). *PON1* and *PON3* are predominantly expressed in the liver and secreted into blood. *PON2* is more widely expressed in a number of tissues including the brain, liver, kidney, and testis but not detectable in the blood (12). Oxidative stress has been associated with several adverse health effects including atherosclerosis, pre-eclampsia, endometriosis, polycystic ovary syndrome (PCOS) and female infertility (13). The aim of this study was to analyze the *PON1* (Glu/Arg192) gene mutation in infertile women and women without infertility.

Materials and methods

In the present study 300 subjects including 150 women with infertility and 150 healthy women as the control group were assessed. Controls and patients were selected from same population living in the Guilan province, north of Iran, including unrelated subjects that were recruited between 2013 and 2014. Patients were referred to the in vitro fertilization unit of Alzahra hospital, Rasht, Guilan, Iran for analysis. In this study we tried to select the women in the 28-40 age range because strong evidence suggests that the couples who are trying to get pregnant, become less fertile as they get older. Patients had at least two years of infertility history. Women with unexplained infertility were enrolled in this study. Infertile patients of known cause, such as hormo-

nal, structural, immunological, and coagulation abnormalities were excluded. The couple not conceiving after at least 1 year of unprotected intercourse; the women being younger than 40; confirmation of an ovulatory cycle by midluteal serum progesterone level; symptoms suggestive of endometriosis; and a normal spermogram of the partner according to the World Health Organization criteria (sperm concentration at least 20 million/mL and 50% progressive motile spermatozoa within 1h of ejaculation). Couples who presented with other gynecological pathologies or coexisting causes of infertility besides endometriosis were excluded. Women with additional obvious causes of infertility such as any abnormality in anatomy, tubal factor, ovulatory dysfunction, and polycystic ovarian syndrome were also excluded from the study.

A total of 150 blood samples from women with at least one child and no history of infertility or miscarriage were used as controls. Peripheral bloods (2 ml) were obtained in the EDTA-coated tubes (Venoject, Belgium), which were used for DNA extraction. This project has been approved by the local licensing committee of the University of Guilan and informed consent was obtained from all subjects and has been performed according to the Helsinki Declaration of 1975, as revised in 1983.

Genotyping

Genomic DNA was extracted from whole-blood samples using a DNA Extractor Gpp Solution Kit (Gen pajooan, Iran) according to the manufacturer's instructions. The region of *PON1* including the (192 Q/R) SNP site was amplified using primers:

(F: 5' CACGAAGGCTCCATCCCAC3' and R: 5' TCCTTCTGCCACCACTCGAAC3'). Amplification of the *PON1* 192 Q/R polymorphism was accomplished by polymerase chain reaction (PCR). The primers were designed by means of Oligo7 software (version 7.54, USA). The PCR were performed in 20 μ L. The PCR conditions for the *PON1* were as follow: 95°C for 5 min, 34 cycles at 95°C for 30 second, annealing at 60°C for 40 second. Polymerase chain reaction products were subsequently digested with restriction enzyme *ALWI*. Enzyme digestion products were separated on 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

Statistical analysis

Statistical analyses were performed using MedCalc (version 12.1, Mariakerke, Belgium). Analysis of difference in allele and genotype frequencies between cases and controls were compared by the χ^2 test. To estimate the association between the *PON1* 192 Q/R variant and the risk of female infertility, Odds ratios with 95% confidence intervals (95% CI) were evaluated by logistic regression. A value of $P < 0.05$ was considered statistically significant.

Results

The current study included a total of 150 patients with infertility (mean age: 35.1 ± 2.1) and 150 disease-free control subjects (mean age, 34.5 ± 2.3). Control contributors were chosen to have a *PON1* genotypic distribution similar to that of the patients. Genotyping of 192 Q/R was done by PCR-RFLP method (Figure 1). The main characteristics of the patients are presented in table 1. Analysis suggested that age, smoking status and family history of infertility were not significantly different between cases and controls.

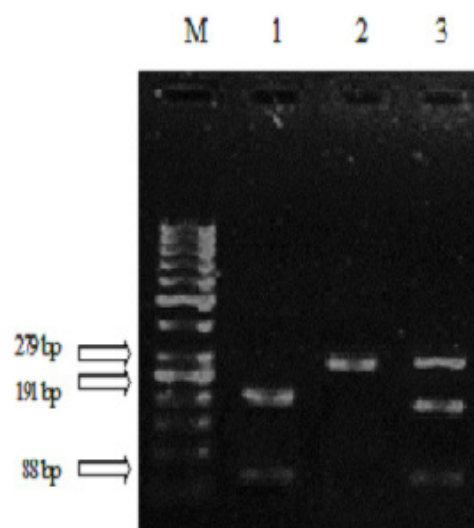


Figure 1. Detection of *PON1* gene polymorphism by PCR-RFLP using *ALWI* restriction enzyme. Lane 1, fragments presenting the RR genotype for the mutant homozygous patient; lane 2, fragments indicating the QQ genotype for the wild type homozygous patient; Lane 3, fragments showing genotype for heterozygous patients; M= 50 bp DNA marker.

Table 1. Characteristics of female infertility in patients and controls enrolled in the study.

| Variable | Controls (n = 150) | Cases (n = 150) | P value |
|-------------------------------|--------------------|-----------------|---------|
| | No. (%) | No. (%) | |
| Age | | | 0.385 |
| ≤ 30 | 14 (9.3) | 9 (6.0) | |
| > 30 | 136 (90.6) | 141 (94.0) | |
| Smoking status | | | 0.035 |
| Never | 90 (60.0) | 75 (50.0) | |
| Former | 19 (12.6) | 36 (24.0) | |
| Current | 41 (27.4) | 39 (26.0) | |
| Family history of infertility | | | 0.174 |
| No | 120 (80.0) | 109 (72.6) | |
| Yes | 30 (20.0) | 41 (27.4) | |

Table 2. Allele and genotype frequencies of *PON1* 192 Q/R polymorphism among infertile cases and controls.

| | controls | | patients | | |
|-----------|------------|------------|--------------------|----------------|----------------|
| | (n = 150) | | (n = 150) | | |
| | n (%) | n (%) | OR (95% CI) | P ^a | P ^b |
| Alleles | | | | | |
| Q | 307(47.6) | 196 (46.0) | 1.00 (reference) | 0.045 | - |
| R | 116 (36.3) | 104 (30.6) | 1.40 (1.02- 1.93) | | 0.037 |
| Genotypes | | | | | |
| QQ | 41 (27.3) | 58 (38.6) | 1.00 (reference) | 0.031 | - |
| QR | 102 (68.0) | 80 (53.4) | 0.55 (0.33 – 0.91) | | 0.019 |
| RR | 7 (4.7) | 12 (8.0) | 1.21 (0.43 – 3.34) | | 0.710 |

^a allele and genotype frequencies in cases and controls were compared using χ^2 test.

^b significance level for allele and genotype frequencies in cases and controls.

The prevalence of genotype frequencies for QQ, QR and RR were 27.3%, 68.0% and 4.7% in controls, and 38.6%, 53.4% and 8.0% in infertile subjects, respectively. Statistical analysis showed that there is significant difference between two groups ($P= 0.03$). The results indicated that the subgroups with QR genotypes were associated with decreased risk of female infertility (OR= 0.55, 95% CI= 0.33 – 0.91, $P= 0.019$). Moreover, significant association were found in allele frequencies ($P= 0.04$). All information about allele and genotype frequencies and associated ORs (95%CI) for infertile cases and controls are summarized in table 2.

Discussion

In this case-controls study we evaluated the role of *PON1* 192 Q/R polymorphism in 150 infertile patients and 150 controls. Our results suggest that there is a significant association in genotype distribution between cases and controls ($P= 0.03$). The individuals with QR genotypes were associated with decreased risk of female infertility (OR= 0.55, 95% CI= 0.33 – 0.91, $P= 0.019$).

In spite of enormous progress in the finding out of reproductive biology, the underlining mechanism of female infertility remains unclear in about 50% of cases that are referred to idiopathic infertility (14). Paraoxonase is a high density lipoprotein (HDL)-associated enzyme that prevents low-density lipoprotein (LDL) oxidative modification (15). *PON1* is a calcium-dependended esterase that circulates in plasma associated with high density lipoprotein (HDL) and contributes to the protective effect of this lipoprotein on LDL oxidation (16). The involvement of antioxidants in the regulation of oxidative stress and inflammation and their impact on female fertility have been well documented (17). Strong evidence implicated oxidative stress in the pathogenesis of infertility causing diseases in women (18). The reasons for a reduction-oxidation imbalance in the woman may vary from the chronic inflammatory diseases such as obesity or diabetes to a deficient diet, smoking, or alcohol abuse (19). Several studies have presented increased susceptibility of low density lipoprotein (LDL) to oxidation and higher levels of oxidized LDL in smokers (20). It has been demonstrated that *PON1* genetic variations

have a crucial role to increasing the risk in many kind of disease. Bhattacharyya and colleagues reported that the *PON* gene may contribute to genetic susceptibility of cardiovascular risk (21). Moreover, Aydin and colleagues found that *PON1* 192 Q/R polymorphism was significant associated with stroke severity (22). Erlich and colleagues revealed that *PON1* 192 Q/R polymorphisms are associated with increase Alzheimer risk (23). Furthermore, Jackson et al demonstrated that oxidative stress might play a role in the development and progression of endometriosis (13). Also, accomplished studies have investigated the relations between inter-individual variations with female infertility. It has been shown that there is no association between 1562C/T of *MMP9* and female infertility (24). It was shown that low activity of *PON1* can be a risk factor for male infertility probably due to a decrease in antioxidant activity of *PON1* and increase in lipid peroxidation (25) Tavailani et al., 2014). It was also suggested that genetic variations in antioxidant genes may contribute to oxidative sperm DNA damage and male infertility (26).

According to the role of *PON* in oxidative stress and also the effect of genetic variations of *PON* which might be effective on female germ cells and fertility, in present study we investigated the association between 192 Q/R *PON1* SNP and susceptibility to female infertility. To our knowledge, the current research is the first genetic variation study on the association between this polymorphic region of *PON1* with female infertility risk. In this study we observed a significant difference in the genotype distributions of *PON1* 192 Q/R polymorphism between patients and controls and we showed that individuals with the variant QR had a significant decrease risk of female infertility.

In conclusion, our results indicated that the *PON1* 192 Q/R polymorphism is associated with decreased risk of female infertility. Further studies with larger numbers of patients and controls are needed to confirm our results.

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