Original Article

The Association of PON1 192 Q/R Polymorphism with the Risk of Idiopathic Male Infertility in Northern Iran

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Abstract

Background: Infertility is defined as the inability to achieve pregnancy after 12 months of regular unprotected sexual intercourse. Environmental and genetic factors are involved in male infertility. The polymorphism studies have a crucial role in disease recognition. Paraoxonase (PON) is an oxidant enzyme which is associated with inflammation, oxidative stress and lipid metabolism. The present study aimed to evaluate the relationship between *PON1 192 Q/R* polymorphism and the susceptibility to idiopathic male infertility.

Methods: Samples were collected from 220 patients diagnosed with male infertility and 230 controls genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).

Results: A significant difference in genotype distributions of *PON1 192 Q/R* polymorphism was observed between patients and controls (p=0.001). Our findings revealed that individuals with the variant QR had a significant decreased risk of idiopathic male infertility (OR=0.49, 95%CI=0.33–0.73, p=0.0004). Moreover, analyses showed that R allele may have a protective effect on susceptibility of idiopathic male infertility (OR=0.31, 95%CI=0.21-0.47, p=0.0001).

Conclusion: The data from this study indicates that the *PON1 192 Q/R* polymorphism is associated with decreased risk of idiopathic male infertility. However, more studies should be considered with larger number of patients and control subjects to confirm our results.

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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 about half of the 15% of couples who cannot conceive, the cause is ascribed to male infertility [2]. In spite of advances in clinical diagnostics, 50% of male infertility cases remain unclear which are referred to as idiopathic infertility [3]. No identifiable cause is found in 37-58% of cases of male infertility [4]. Most idiopathic cases are probable to be of genetic origin because many genes have been shown to be involved in human spermatogenesis [5]. Moreover, idiopathic male infertility is assumed to be caused by several factors, including genetic and epigenetic abnormalities, Reactive Oxygen Species (ROS) or endocrine disruption as a result of environmental pollution [6].

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 [7]. Moreover, ROS have a negative effect on sperm parameters [8]. In 40% of infertile men, an increase in the seminal ROS level has been reported [9]. Increased ROS levels can lead to damage with following sperm dysfunction or cell death [8].

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 [10]. The *PON* gene family consists of 3 genes including *PON1*, *PON2*, and *PON3* located on the long arm of chromosome 7. PON proteins localized in the seminiferous tubules and in spermatozoa have been shown to be

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implicated in the pathogenesis of male infertility [11]. Furthermore, PONs can hydrolyze the hydrogen peroxide (H_2O_2) [12]. *PON1* is the first member of the *PON* gene cluster to be discovered. Changes in the size and shape of HDL particles strongly influence the binding affinity and stability of *PON1* and result in a decreased antioxidative capacity [13]. Inactivation of *PON1* reduces the ability of HDL to prevent both the oxidation of LDL and the interaction between macrophages and endothelium [12]. The *PON1* gene has more than seven 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 [14]. 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 [15]. PON has also been shown to play roles in lipid metabolism [13]. The aim of this study was to analyze the *PON1* (Glu/Arg192) gene mutation in infertile men and men without infertility.

Materials and Methods

In the present study, 450 subjects including 220 men with idiopathic male infertility and 230 healthy men as the control group were assessed. Data on patient characteristics at the study entry for each subject were collected from the infertility clinic of Alzahra Educational and Remedial Hospital (Rasht, Iran). All patients underwent at least two semen analyses and those with a history of orchitis, obstruction of the vas deferens, hypogonadotroic hypogonadism, varicoceles, systemic diseases and sperm antibodies were excluded. Patients for at least two years had an infertility history with their spouses with confirmed normal gynecological assessment. Semen analysis results, age, smoking status, sperm motility and family history data were evaluated. A spermogram was made according to World Health Organizations (WHO) guidelines [16]. Also, the healthy married male volunteers who had at least one child without assisted reproductive technologies were recruited as control group. Peripheral bloods (2 *ml*) were collected in the EDTA-coated tubes (Venoject, Belgium), which was used for DNA extraction. This project has been approved by the ethical committee of University of Guilan and informed consent was obtained from all subjects and has been performed according 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 pajoohan, 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' TC CTTCTGCCACCACTCGAAC3'). Each DNA sample was stored in TE buffer (5 *mμ* Tris–HCl, 0.1 *mμ* EDTA, pH=8.5) at -20 *℃* until analysis. Amplification of the *PON1* 192 Q/R polymorphism was accomplished with the use of polymerase chain reaction (PCR). The PCR was performed in 20 *μl*. The PCR conditions for the *PON1* were as follows: 95*°C* for 5 *min*, 34 cycles at 95*°C* for 30 *s*, annealing at 60*°C* for 40 *s*. 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 idiopathic male infertility, odds ratios with 95% confidence intervals (95% CI) were evaluated by logistic regression. A value of $p<0.05$ was considered statistically significant. Also, analyses for cases and controls were performed by age (two groups: ≤30 years and >30 years), smoking status, family history of infertility and semen parameters. The homozygosity with the more frequent allele among controls was set as the reference group. A value of $p<0.05$ was considered statistically significant.

Results

The current study included a total of 220 patients with idiopathic male infertility and 230 disease-free control subjects. The mean age of study subjects was (36.2±2.1 years) and for controls (34.5±2.3 years), that was not significantly different between infertile patients and controls ($p>0.05$). 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. The prevalence of genotype frequencies for QQ, QR and RR were 40.8, 54.7 and 4.3% in controls, and 57.2, 38.7, 4.5% in infertile subjects, respectively. Statistical analysis showed that there was significant difference between two groups ($p=0.004$).

The results indicated that the subgroup with QR genotypes was associated with decreased risk of idiopathic male infertility (OR=0.49, 95%CI=0.33-0.73, p= 0.0004). Moreover, analyses showed that R allele may have a protective effect on susceptibility of idiopathic male infertility (OR=0.31, 95%CI=0.21-0.47, p=0.0001). All information about allele and genotype frequencies and associated ORs (95%CI) for infertile cases and controls are summarized in table 2.

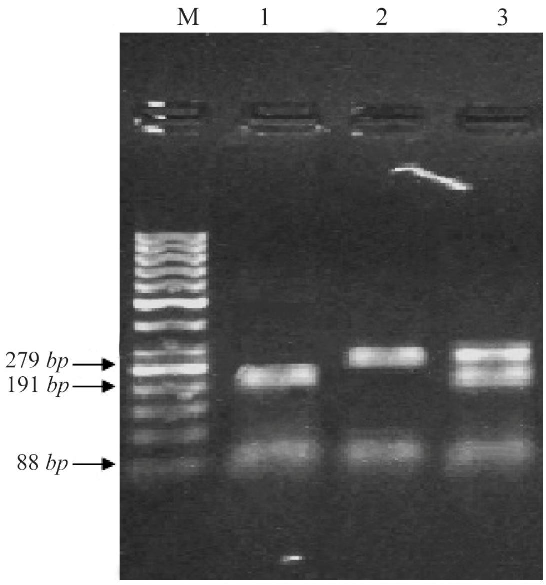

Figure 1. Detection of *PON1* gene polymorphism by PCR-RFLP using *ALWI* restriction enzyme. Lane 1, fragments presenting the RR genotype for the mutant homozygous patient; lane 2, fragments indicating the QQ genotype for the wild type homozygous patient; lane 3, fragments showing genotype for heterozygous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

Table l. Characteristics of idiopathic male infertility patients and controls enrolled in the study

Variable	Controls (n=230) No. (%)	Cases (n=220) No. (%)	p
Age (mean±SD)	34.5±2.3	36.2±2.1	0.365
≤30	44 (19.3)	34 (15.4)	
>30	186 (80.8)	186 (84.5)	
Smoking status			0.181
Never	114 (49.5)	90 (41.0)	
Former	42 (18.2)	48 (21.8)	
Current	74 (32.2)	82 (37.2)	
Family history of infertility			0.121
No	171 (74.3)	148 (67.2)	
Yes	59 (25.6)	72 (32.8)	
Semen parameters (mean±SEM)			
Concentration ($\times 10^6$ /*ml*)	100±2.4	80.7±4.21	<0.001
Motility (%)	63.5±1.21	35.7±0.63	<0.001
Volume (*ml*)	2.9±0.05	2.85±0.06	<0.001

Table 2. Allele and genotype frequencies of PON1 192 Q/R polymorphism among cases and controls and the associations with risk of idiopathic male infertility

	Controls (n=230)	infertile cases (n=220)			
	n (%)	n (%)	OR (95% CI)	p[a]	p[b]
Alleles (192 Q/R)					
Q	460(68.0)	336 (76.0)	1.00 (reference)	0.0001	-
R	146 (32.0)	34 (24.0)	0.31 (0.21-0.47)	0.0001	
Genotypes (192 Q/R)					
QQ	94 (40.86)	126 (57.27)	1.00 (reference)	0.001	-
QR	126 (54.78)	84 (38.18)	0.49 (0.33-0.73)	0.0004	
RR	10 (4.34)	10 (4.54)	0.74 (0.29-1.86)	0.530	

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.

Discussion

In this case-control study, the role of *PON1* 192 Q/R polymorphism in 220 infertile patients and 230 controls was evaluated. Our results suggest that there is a significant association in genotype distribution between cases and controls (p= 0.001). The individuals with QR genotypes were associated with decreased risk of idiopathic male infertility (OR=0.49, 95%CI=0.33-0.73, p=0.0004). It has been shown that R allele may have a protective effect on susceptibility of idiopathic male infertility (OR=0.31, 95%CI=0.21-0.47, p=0.0001).

In spite of enormous progress in discovering the reproductive biology, the underlying mechanism of male infertility remains unclear in about 50% of cases referred to as idiopathic male infertile patients [17]. Paraxonase is a High Density Lipoprotein (HDL)-associated enzyme that prevents Low-Density Lipoprotein (LDL) oxidative modification [18]. In humans, *PON1* gene is expressed basically in the liver and kidney [19]. PON1 is a calcium-depended esterase that circulates in plasma associated with HDL and contributes to the protective effect of this lipoprotein on LDL oxidation [20]. Several studies have presented increased susceptibility of LDL to oxidation [21]. It has been demonstrated that *PON1* genetic variations have a crucial role in increasing the risk in many kinds of diseases. Bhattacharyya *et al* reported that the *PON* gene may contribute to genetic susceptibility of cardiovascular risk [22]. Moreover, Aydin *et al* found that *PON1* 192 Q/R polymorphism was significantly associated with stroke severity [23]. Recently, Erlich *et al* revealed that *PON1* 192 Q/R polymorphisms are associated with increased Alzheimer risk [24].

According to the role of PON in oxidative stress and also the effect of genetic variations of PON which might be effective in male germ line cells and fertility, in the present study, the association between 192 Q/R *PON1* SNP and susceptibility to idiopathic male infertility was investigated. In Iranian population, the PON1 gene polymorphism was associated with systemic lupus erythematosus [14], High LDL/HDL ratios [25] and psoriasis [26]. The finding of the current study is inconsistent with those of Marsillach who found that the *PON1* 192 Q/R polymorphism was not associated with male infertility risk [27]. However, our results indicate that *PON1* 192 Q/R polymorphism had a significant decreased risk of idiopathic male infertility and the protective effect of QR was more predominant among other subgroups (OR=0.49, 95%CI=0.33-0.73, p=0.0004). The contradictory results of these reports may be due to the differences in sample sizes, gene pool and the impact of other genetic and environmental factors.

Conclusion

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

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