Study of Paraoxonase -1 Gene Polymorphism in a Healthy Population of Khorramabad, Iran

Kobra Chehari¹, Farideh Sepahvand², Sirous Ghobadi¹, Ahmad Ismaili³* and Ezat Rafiei Alavy⁴

Abstract

Human serum paraoxonase (HuPON1; EC 3.1.8.1), a calcium-dependent esterase, is synthesized in the liver and widely distributed in tissues including liver, kidney, intestine, and serum, where it is associated exclusively with high-density lipoprotein. Human paraoxonase-1 plays an important role in prevention of atherosclerosis and also protection against organophosphate-induced neurotoxicity. Paraoxonase-1 shows 2 common polymorphisms: Q/R at position 192 and M/L at position 55. In this study, paraoxonase-1 192 and 55 polymorphisms were investigated in 64 healthy Iranian individuals. Genomic DNA was isolated from whole blood by the Bartlett method, and paraoxonase-1 genotypes were determined by polymerase chain reaction amplification followed by restriction isotyping and gel electrophoresis. The chi-square test was used to evaluate the Hardy-Weinberg equilibrium. The genotype frequencies for paraoxonase 1-Q192R were approximately 47% (QQ), 41% (QR) and 12% (RR) and for paraoxonase-1 M55L, 44% (LL), 44% (ML) and 12% (MM). Thus, the frequency of alleles R, L, Q, and M were 0.33, 0.66, 0.67, and 0.34 respectively. In conclusion, the frequencies of paraoxonase-1 192 and 55 polymorphisms in this group of Iranian population were different from those seen in other Asian populations from Japan and China but similar to European (Caucasians).

Keywords: Atherosclerosis, Haplotype, Paraoxonase, Polymorphism, Polymerase Chain Reaction.

Introduction

Human paraoxonase-1 (PON1) belongs to the family of serum paraoxonases consisting of PON1, PON2, and PON3. The 3 human PON genes are located adjacent to each other at bands q21-q22 on chromosome7 [1]. Paraoxonase 1 (PON1) is a calcium-dependent esterase composed of 354 amino acids (45 kDa), which is synthesized in the liver and secreted into the plasma where it is associated with high-density lipoproteins (HDL) [2-4]. The concentration of PON1 in human plasma is ~50 mg/L, but can vary by as much as 13-fold from one individual to another [5]. PON1 hydrolyzes many active metabolites of organophosphorus insecticides, including paraoxon (a catabolite of the insecticide parathion), diazoxon and chlorpyrifosoxon, detoxifies various neurotoxic agents like sarin and soman and hydrolyses the aliphatic lactones such as γ-butyrolactone and homocysteine-methiolactone [6-8].

Increased enzyme activity has been associated with higher HDL levels in some populations, but not in all studies [9, 10]. The PON 192 and 55 polymorphisms have been associated with risk of ischemic stroke in a small study of younger adults and older patients respectively [11, 12]. PON1 is assumed to be involved in the lipid metabolism and to be a protective factor against atherosclerosis. It prevents the formation of oxidized LDL, inactivates LDL-derived oxidized phospholipids once they are formed and prevents oxidation of HDL phospholipids [6]. Genetic factors include polymorphisms in the coding and promoter regions of the PON1 gene that might influence the PON1 expression and its catalytic activity [13-16]. Two common polymorphisms in the coding region of the PON1 gene have been identified, Q192R glutamine to arginine substitution at position 192 and L55M leucine to methionine substitution at position 55[17]. Q192R polymorphism affects PON1 activity towards paraoxon, diazoxon, soman and sarin and is associated with coronary artery disease, familial hypercholesterolemia, type 2 diabetes and Parkinson’s disease [18]. L55M polymorphism can affect the PON1 mRNA and protein levels and its activity.

Since there are not enough data for the Iranian population, this study was under taken to investigate the distribution of the PON1-192 and -55 polymorphisms in healthy individuals of Khorramabad in west of Iran.

Materials and Methods

Subjects

The study included 64 healthy unrelated individuals (28 male and 36 female subjects), aged 55 years [35–75]. Healthy volunteers in the central laboratory of Khorramabad were screened for the study. The protocol was approved by Razi University Ethical Committee, and
informed consent was obtained from all subjects. Blood samples were drawn into tubes with sodium citrate 3.8% to analyze PON1 genotypes. The samples were then stored at -70°C until the next measurements.

**DNA Extraction**

Genomic DNA was isolated from whole blood by Bartlet method. This method is, fast, inexpensive and accurate [19]. In order to quality assurance, DNA samples were tested for DNA size and integrity, using agarose gel electrophoresis.

**Polymerase chain reaction (PCR)**

PON1 genotypes were determined by PCR amplification and restriction isotyping [20-21]. Primers used for PCR analyses are illustrated in Table 1. The PCR was carried out in small reaction tubes (25 μl volume), containing 200 M dNTPs, 0.5 M of each primer, 1.5 mM MgCl₂, 100 ng genomic DNA and 1U Taq DNA polymerase (Cinnagen, Iran). Prior to the first cycle, PCR reaction was heated to a temperature of 95 °C for 5 min (initialization step). Then the reaction mixture was exposed to 25 cycles at 94°C for 45s (denaturation step), 60°C for 45s (annealing step), and 72°C for 1 min (elongation step), followed by a final extension time of 5 min. The PCR products were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining.

**Table 1.** PCR primers used for PON1 polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism PON1.55</th>
<th>F Primer</th>
<th>R Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorphism PON1.192</td>
<td>5’-GAAGAGTGTAGTATAGCCCCAG-3’</td>
<td>5’-TTTAATCCAGCTAATGAAAGCC-3’</td>
</tr>
<tr>
<td>Polymorphism PON1.192</td>
<td>5’-TTTAATCCAGCTAATGAAAGCC-3’</td>
<td>5’-TTTAATCCAGCTAATGAAAGCC-3’</td>
</tr>
</tbody>
</table>

**Restriction fragment length polymorphism (RFLP)**

The PON1-55 (170-bp) PCR product (10 μl) was digested with 5U of NlaIII restriction endonuclease (Fermentas) in the presence of 0.1 mg/ml bovine serum albumin at 37°C (overnight). The digested products were separated by electrophoresis on a 2% agarose gel at 70 V, and visualized by UV after ethidium bromide staining. Digestion resulted in 126 and 44 bp fragments for the PON1-55 MM polymorphism and a nondigested 170 bp fragment for the PON1-55 LL polymorphism, and 170, 126, and 44 bp fragments for the PON1-55 ML polymorphism [20]. The PON1-192 (99 bp) PCR product (10 μl) was digested with 8 U of BspPI (Fermentas) overnight at 55°C. Digestion with BspPI resulted in 66 and 33 bp fragments for the PON1-192 RR polymorphism and a nondigested 99 bp fragment for the PON1-192 QQ polymorphism and 99, 66, and 33 bp fragments for the PON1-192 QR polymorphism [21]. It should be noted that PCR reaction and cycling were the same for both PON1-192 and PON1-55 polymorphisms.

**Statistical analyses**

The chi-square test was used to evaluate the Hardy-Weinberg equilibrium. Allele frequencies were estimated by the gene counting methods. Differences at p<0.05 were considered statistically significant.

**Results**

Figure 1 illustrates the results of the visualization of the Genomic DNA on a 1% agarose gel electrophoresis. The lengths of the PCR products for PON1-192 and PON1-55 were 99 and 170 bp, respectively. Figure 2 and 3 illustrate the results of the PCR amplifications and digestion for PON1-192. Also Figure 4 and 5 show the results of the PCR amplifications and digestion for PON1-55.

**Figure 1.** The visualization of the Genomic DNA on a 1% agarose gel electrophoresis.

**Figure 2.** The visualization of PON1-192 (99 bp) PCR product on a 2% agarose gel electrophoresis. The first lane from right refers to DNA size Marker Φ X174 DNA.

**Figure 3.** Determination of PON1-192 gene polymorphism by PCR-RFLP procedure using BspPI restriction enzyme.
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Figure 4. The visualization of PON1-55 (170 bp) PCR product on a 2% agarose gel electrophoresis. The first lane from left refers to DNA size Marker Φ X174 DNA.

Figure 5. Determination of PON1-55 gene polymorphism by PCR-RFLP procedure using NlaIII restriction enzyme. The first lane from left refers to DNA size Marker Φ X174 DNA. The length of digestion products and genotypes are reported respectively on the right side and upside.

Genotype and allele frequencies of PON1 Polymorphisms
As shown in Table 2, the most common PON1-192 and PON1-55 genotypes were QQ (46.9%), LM and LL (43.75%), respectively. Thus the most frequent allele were Q (67%) and L (66%). The 192 RR and 55 MM genotypes were the rarest genotypes found in the population (12.5%). No significant deviation of PON1-192 and 55 genotype frequencies was established using Hardy-Weinberg equilibrium calculations. Neither the QR nor the RR genotypes occurred in the MM homozygotes, suggesting that these haplotypes may not exit or may be rare in this population (Table 3).

Table 2. Genotype and Allele Frequency of PON1 Polymorphisms

<table>
<thead>
<tr>
<th>Polymorphic Site</th>
<th>Frequency</th>
<th>PON1-192 Genotypes</th>
<th>L55M Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QQ: 46.9%</td>
<td>MM: 12.5%</td>
<td>QQ: 12.5%</td>
</tr>
<tr>
<td>Genotype</td>
<td>QR: 40.6%</td>
<td>ML: 43.75%</td>
<td>QR: 20.3%</td>
</tr>
<tr>
<td></td>
<td>RR: 12.5%</td>
<td>LL: 43.75%</td>
<td>RR: 10.9%</td>
</tr>
<tr>
<td>Allele</td>
<td>Q: 0.67</td>
<td>M: 0.34%</td>
<td>QQ: 12.5%</td>
</tr>
<tr>
<td></td>
<td>R: 0.33</td>
<td>L: 0.66%</td>
<td>MM: 0%</td>
</tr>
</tbody>
</table>

Table 3. Genotype combination for PON1-192 and 55 Polymorphisms

<table>
<thead>
<tr>
<th>PON1-55 Genotypes</th>
<th>PON1-192 Genotypes</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL</td>
<td>12.5%</td>
<td></td>
</tr>
<tr>
<td>LM</td>
<td>21.9%</td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>12.5%</td>
<td></td>
</tr>
</tbody>
</table>

Discussion
In this study findings are in agreement with previously reported data for Iranian population, which showed predominance of Q192 and L55 alleles over R192 and M55 alleles (Table 4) [22]. Asian population shows predominance of R192 over Q192 allele and a very low frequency of M55 allele [23-24]. The observed variation in Q192 allele frequency is similar to previously reported data showing that European populations have Q192 allele frequencies between 0.67 and 0.74 [25-27]. In present investigation, the PON1 genotype frequencies found were comparable to those reported in European (Caucasian) populations (Table 4). The frequency of the R allele in European populations is approximately 0.3 and the L allele 0.6 suggesting that Iranian subjects may display higher genetic affinities to Caucasian than Asian populations [25-27]. In a study, R variant was significantly correlated with the highest triglyceride (TG) concentrations, indicating an association between 192 R variant and TG levels in an Iranian population [22].

Table 4. Allele frequencies of polymorphisms in different ethnic groups

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>Q192R</th>
<th>L55M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asian</td>
<td>Q</td>
<td>R</td>
</tr>
<tr>
<td>Iranian</td>
<td>0.67</td>
<td>0.33</td>
</tr>
<tr>
<td>Chinese</td>
<td>0.35</td>
<td>0.65</td>
</tr>
<tr>
<td>Japanese</td>
<td>0.40</td>
<td>0.60</td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Italy</td>
<td>0.65</td>
<td>0.35</td>
</tr>
<tr>
<td>Spain</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Finland</td>
<td>0.69</td>
<td>0.31</td>
</tr>
</tbody>
</table>

The RR genotypes also displayed higher levels of total cholesterol and LDL compared to QQ and QR genotypes [22]. Thus, it would appear that subjects possessing the R allele may be at higher risk of cardiovascular disease due to their lipid profile. Based on our observations, an Iranian population with an R frequency of 0.33 may be more resistant to some organophosphatetoxicity and coronary heart disease (CHD) compared to Chinese, with an R frequency of 0.65.

PON1 Q192R is the most studied allelic variant in the PON1 gene. The Q192R variant determines PON1’s substrate specific activity, in particular toward organophosphates. The R allele exhibits several-fold higher activity toward paraoxon, whereas the arylesterase activity and PON1 antigen concentration are similar in
both isozymes. Recently, it has been shown that PON1 position 192 is involved in HDL binding. The PON1-192Q binds HDL with a 3-fold lower affinity than the R isozyme and consequently exhibits significantly reduced stability, lipolactonase activity, and macrophage cholesterol efflux [28].

Conclusion

It is noteworthy that the knowledge of PON1 polymorphism distribution can be useful in epidemiological studies related not only to atherosclerosis but also to toxicology, or pharmacogenetics. In conclusion, the frequencies of paraoxonase-1 192 and 55 polymorphisms in this group of Iranian population were different from those seen in other Asian populations from Japan and China but similar to European (Caucasians).

References