A Study on the Association between CCRΔ32 Mutation and HCV Infection in Iranian Patients

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Abstract

Background: Mutations in the coding region of the Chemokine Receptor 5 (CCR5) genes reduce or eliminate CCR5 expression in immune cells and progression of HCV infection. This study aimed to investigate the role of this mutation in HCV infection in Iranian patients in comparison with healthy individuals.

Methods: 100 HCV infected patients and 100 healthy individuals were randomly selected. The CCR5 Δ32 genotypes were determined using specific primers and PCR method.

Results: The agarose gel electrophoresis showed a 189-bp fragment from wild type for both alleles of CCR5 gene. The CCR5- Δ32 allele was not found in any HCV infected and healthy subjects.

Conclusion: The mutation in CCR5 gene was not detected in any of the two groups; therefore, the role of CCR5 gene expression in immune cells and progression of HCV infection needs to be studied in larger samples in our country.

Keywords: CCR5 protein, Hepatitic C, Human, Infection, Mutation

Introduction

Hepatitis C is a complex liver disease and infection and Hepatitis C Virus (HCV) can cause acute and chronic liver disease throughout the world 1-3. Over the decades, the incidence of HCV has increased to 2.8% worldwide which results in more than 185 million infections 4,5. Also, Daw et al reported that HCV has been considered as a global threat in different geographical areas around the world 6. The prevalence of HCV infection is less than 1% of the general population in Iran 7.

HCV is known as a hepatotropic non cytopathic virus which can evade the immune response of the host. The effective immune response to HCV infection requires efficient recruitment and activation of inflammatory cells (monocytes and T lymphocytes) to the infected liver in which chemokines and chemokine receptors are involved and play important roles in the pathogenesis of chronic hepatitis C 8,9. CC-chemokines such as CCL3, CCL4, CCL5 and CCL3L1 bind to Chemokine (C-C motif) Receptor 5 (CCR5), thereby initiating activation and migration of cells. CCR5 gene is located at position 3p21.31 on human chromosome. The coding sequence of this gene is 1056 base pairs, which is translated to a protein with 352 amino acid length 10. This protein is expressed by T helper lymphocytes type 1 (TH1), cytotoxic CD8 (+) T lymphocytes, monocytes, memory T cells (CD45RO), stem cells, dendritic cells, microglia and is known as an important coreceptor for macrophage-tropic virus, including HIV-1, to enter host cells 10,11. The studies showed there are two promoter regions (an upstream promoter region and downstream promoter region), four exons (exon1, exon 2a/2b, exon 3) and two introns in the genomic organization of CCR5 gene 11. A 32 bp deletion in the coding region of this gene, CCR5Δ32, leads to a frame shift in the open reading frame and produces a non-functional CCR5 which is not expressed on the cells surface in homozygous patients (CCR5Δ32/CCR5Δ32) and its expression is decreased in heterozygous individuals (CCR5Δ32/wt) 10,12.

In a study on Caucasian population with homozygous subjects for CCR5Δ32 allele, they were almost completely protected against Human Immunodeficiency Virus (HIV) infection, and heterozygous patients seem to have a delay in the progression of HIV 13. According to previous findings, CCR5Δ32 role in HCV is not the same as HIV for entry into the cell. It is believed CCR5Δ32 homozygosis may increase the risk of HCV infection 14,15. So far, there are no studies about association between CCR5Δ32 mutation and HCV infection in Iranian patients. Therefore, this study aimed to investigate the role of this mutation in HCV infection in Iranian patients in comparison with healthy individuals.
Materials and Methods

Collection of samples

Blood buffy coat samples were collected from 100 HCV patients (age ranges between 15-81 years) and 100 healthy individuals (age range between 8-95 years). None of the participants had received antiviral therapy at the time of the study. All patients were positive for both anti-HCV antibody and serum HCV-RNA.

All specimens were randomly selected from people referred to clinical laboratory of the Iranian Blood Transfusion Organization in Tehran, Iran (IBTO). The protocol of this study was approved by the ethical committee of Blood Transfusion Research Center and the written informed consent was taken from both groups. Blood collection was performed in 10.8 ml K3 EDTA pre-coated glass tubes. After centrifuge of blood samples, buffy coat samples were stored at -80°C for further use.

DNA extraction

Extraction of genomic DNA was performed by the salting out method described previously from blood buffy coat samples. The extracted DNA Optical Density (OD) was obtained at wavelengths of 260 and 280 nm for being quantified by spectrophotometer instrument. Suitable DNA samples (OD260:OD280=1.8-2.0) were stored at -80°C for future use.

PCR reaction

For detection of CCR5 genotypes, specific primers from the previously published sequences were used (Table 1). The PCR reaction was performed with a total volume of 25 μl, containing 12.5 μl 2× Master Mix (Takara), 0.5 μl in 10 pmol of each forward and reverse primers (CinnaGen) and 5 μl (1-100 ng/μl) extracted genomic DNA. The PCR program was done for 35 cycles on thermocycler (Corbett) as follows: denaturation at 98°C, 10 s; annealing at 56°C, 45 s; extension at 72°C, 45 s. Initial denaturation and final extension were at 98°C for 3 min and at 72°C for 10 min, respectively. Then, 10 μl of the amplified PCR products were electrophoresed on 1.5% agarose gel and stained with DNA green viewer to detect the presence and the proper size of PCR product. The wild type sample was obtained from the previous study as the negative control and a synthesized gene in vector pGEM-B1 containing the CCR5Δ32 gene (Bioneer Corp, Daejeon, South Korea) was used as the positive control. DNA molecular weight marker XIV (100 bp ladder from Roche) was also used to estimate the size of amplified DNA fragments on the gel.

Statistical analysis

Demographic data for the study of subjects were collected by questionnaire. All data were analyzed using the SPSS software for windows (Version 20.0). Chi-square test was used for statistical analysis. P-values<0.05 were considered significant.

Results

The mean age of 200 participants in this research (132 males and 68 females) was 39.5±16 years. There was no significant difference between controls and cases in terms of the mean age (38.3 vs. 40.1, p=0.5). The characteristics of the study subjects in healthy and patient groups are compared in table 2. None of the gender ratio, age, level of education, ALT (Alanine aminotransferase) and AST (Aspartate aminotransferase) levels showed significant difference between two groups. The agarose gel electrophoresis showed 189-bp fragments from wild type for both alleles of CCR5 gene (Figure 1). As shown in table 3, CCR5-Δ32 allele was not found in any of the subjects.

Discussion

HCV is a major cause of acute and chronic hepatitis, cirrhosis and liver cancer worldwide. CCR5 and its ligands by regulating the migration of immune cells to the infected liver play important role in the pathogenesis of hepatitis C. A 32-bp deletion in CCR5 gene reduces or eliminates CCR5 expression in immune cells and progression of HCV infection. Ahlenstiel et al reported that point mutation in the CCR5-Δ32 interrupts the CCR5 signaling pathway and reduces interferon gamma responses in anti-HCV positive haemo-

Table 3. Genotype frequencies of delta 32 CCR5 mutation in HCV infected patients and healthy individuals

<table>
<thead>
<tr>
<th>Subjects sample size</th>
<th>CCR5/CCR5</th>
<th>CCR5Δ32/CCR5Δ32</th>
<th>CCR5/CCR5Δ32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HCV- negative 100 healthy individuals</td>
<td>100 (100%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Anti-HCV- positive 100 patients</td>
<td>100 (100%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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</tbody>
</table>
The CCR5 gene is a chemokine receptor and is involved in the immune response and viral infection. Several studies have been conducted in European countries to investigate the frequency and distribution of the CCR5Δ32 mutation. In northern Europe, 1.1% of the Caucasian population carries the CCR5Δ32 allele, which is a genetic polymorphism located in the promoter region of the CCR5 gene. This mutation has been associated with a decreased risk of chronic HCV infection and AIDS. Studies have also shown that the CCR5Δ32 mutation is more frequent in the Caucasian population compared to other ethnic groups. For example, in Iran, the prevalence of the CCR5Δ32 mutation is reported to be higher in the North West of Iran compared to the rest of the country.

Several studies have been conducted in European countries and showed that the frequency of CCR5Δ32 mutation decreased from northern to southern Europe. The CCR5Δ32 mutation was not detected in any of the two groups; therefore, the mutation in CCR5 gene may be with low frequency in our country and the role of CCR5 gene expression in immune cells and progression of HCV infection needs to be studied in larger samples in our country.

**Conclusion**

Based on our findings, the mutation in CCR5 gene was not detected in any of the two groups; therefore, the mutation in CCR5 gene may be with low frequency in our country and the role of CCR5 gene expression in immune cells and progression of HCV infection needs to be studies in larger samples in our country.

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**References**


