Introduction

Chronic hepatitis C is caused by the hepatitis C virus (HCV) infection. This virus is a RNA virus and belongs to Flaviviridae family that can develop a wide range of clinical complications including hepatocellular carcinoma, liver failure and liver cirrhosis [1]. Hepatitis C is one of the main causes of mortality in the world and HCV has infected 170 million people in the world and 15% of its population is symptomatic [2, 3]. This viral infection leads to pro inflammatory cytokines production. The cellular immune responses in hepatitis C have an important role in liver damages and viral functions, although the exact mechanisms of such functionality are not well known yet [4, 5].

Cytokines play a crucial role in the body against infectious agents. They generally activate the immune system as a defense factor and also prevent viral replication in the body [6]. The variation of polymorphisms in cytokine genes could be an effective factor in the control of hepatitis C virus infection; therefore, in this study the association between interleukin 20 (IL 20) polymorphism and HCV infection was investigated. IL 20 is produced by different cells in liver including hepatocytes, sinusoidal endothelial cells, liver kupffer cells and lymphocytes. IL 20 belongs to the interleukin 10 (IL 10) family and has a chief regulatory part in HCV infection like IL10 [7-9].

The level of IL 20 production is associated with autoimmune diseases such as autoimmune hepatitis and it seems that its production level has an effect on the incidence of hepatocellular carcinoma and lesions related to immune response such as liver failure and liver cirrhosis [10, 11]. IL 20 coding sequence is a 195 kilo base region which is located on the long arm of chromosome 1 [12].

Genetic variations such as nucleotide polymorphisms in cytokine genes can raise or lower the severity of the inflammatory infections [13]. Changing the alleles can be considered as an effective factor to prevent diseases from progression to liver failure and cirrhosis. Several polymorphisms have been identified in the sequence of human IL 20 gene. In this study, IL 20 polymorphism (rs1518108) at position 3978 (in a region near to the gene...
was investigated. The aim of this study was to assess the association between nucleotide polymorphism at position 3978 of IL 20 gene and chronic HCV infection in patients and healthy control subjects who were referred to gastroenterology ward of Tehran Taleghani Hospital.

Materials and Methods

This research was conducted as a case-control study. Patients’ and healthy controls’ samples were collected from the gastroenterology ward of Tehran Taleghani Hospital between years 2010 and 2011, then according to the clinical criteria, ELISA tests were performed on samples. ELISA test positive results for patients’ samples and negative results for the healthy control group samples were confirmed and the results were evaluated. The patient group consisted of 105 patients and the control group consisted of 135 healthy people. The demographic data and clinical history of patients were gathered based on a questionnaire completed by the assistance of a trained general practitioner. Peripheral blood samples were taken and informed consent was obtained from all participants as well.

The study protocols and written informed consent were reviewed and approved by the ethics committee at the Research Center for Gastroenterology and Liver Diseases, Shaheed Beheshti University of Medical Sciences (Code 522). Genomic DNA was extracted from blood samples using standard phenol-chloroform method [14]. The subjected gene sequence was retrieved from GenBank and examined for primer design, and a primer pair was designed for the gene using Gene Runner software subsequently. Finally, sequences of the primers were subjected to BLAST online search engine (NCBI). Nucleotide sequences of primers used in this study are as follow: IL20 primer pairs Forward: 5’GCCCAGACAGGTGTATGAGC3’ Reverse: 5’GAGTTATCAAAAGTTAAAGTCATTG 3’. The PCR-RFLP method was used to investigate the presence of variation at polymorphism site of IL20 gene. The enzymatic digested products are illustrated in figure 1. Genotypic frequencies of CC, TT, CT in patient group were 36.2, 15.2, 48.6 and in the healthy group were 52.6, 13.3, 34.1% respectively. The results of this study highlighted a significant difference in genotype frequencies between patients and control group \((p=0.053)\). The results of enzymatic digestion of Apal1 restriction site are as follow: length of CC genotype (homozygote C) undigested product was 386 bp, and CT genotype (heterozygote) digested products were 386, 223, 163 bp and TT genotype (homozygote T) digested product were 223 and 163 bp. The enzymatic digested products are illustrated in figure 1.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients N (%)</th>
<th>Healthy controls N (%)</th>
<th>OR (CI 95%)</th>
<th>p-Value</th>
<th>OR (CI 95%)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>38 (36.2)</td>
<td>71 (52.6)</td>
<td>Reference 1</td>
<td>-</td>
<td>Reference 1</td>
<td>-</td>
</tr>
<tr>
<td>CT</td>
<td>51 (48.6)</td>
<td>46 (34.1)</td>
<td>2.072 (1.183-3.628)</td>
<td>0.011</td>
<td>2.283 (1.210-4.306)</td>
<td>0.011</td>
</tr>
<tr>
<td>TT</td>
<td>16 (15.2)</td>
<td>18 (13.3)</td>
<td>1.66 (0.761-3.624)</td>
<td>0.203</td>
<td>1.835 (0.789-4.267)</td>
<td>0.158</td>
</tr>
<tr>
<td>C</td>
<td>127 (60.5)</td>
<td>188 (69.6)</td>
<td>-</td>
<td>-</td>
<td>Reference 1</td>
<td>-</td>
</tr>
<tr>
<td>T</td>
<td>83 (39.5)</td>
<td>82 (30.4)</td>
<td>1.498 (1.025-2.189)</td>
<td>0.035</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a: adjusted for age and gender, b: unadjusted for age and gender

PCR performance accuracy was also controlled and confirmed by positive and negative controls. PCR products were then subjected to apal1 restriction enzyme digestion. This enzyme cuts the interleukin 20 at position 223 if the nucleotide changes at that position. RFLP products were run in 2% agarose gel and stained with ethidium bromide. Finally, 10% of whole samples were genotyped using sequencing technique (direct sequencing method) to confirm PCR-RFLP results. In this study, the statistical analysis was performed using SPSS-16 software. Logistic regression was performed to analyze the association between genotypes and a chi-square test was used to evaluate Hardy-Weinberg equilibrium. The risk of chronic hepatitis C was calculated with 95% confidence interval.

Results

In this study, general and clinical information of patients and healthy individuals such as, age gender and liver damages are shown in table 1. The mean age of both the patient and the healthy control groups are close. The prevalence of hepatitis C diseases was higher in men. The adjusted and unadjusted data for age and gender are reported in table 2. Statistical analysis showed that no association between genotypes of studied polymorphism and liver damage could be spotted.

Table 1. General and clinical information of studied population

<table>
<thead>
<tr>
<th>General information</th>
<th>Patients</th>
<th>Healthy controls</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (Years)</td>
<td>47.92</td>
<td>46.50</td>
<td>0.455</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>73</td>
<td>0.001</td>
</tr>
<tr>
<td>Male</td>
<td>85</td>
<td>62</td>
<td>0.001</td>
</tr>
<tr>
<td>HCV</td>
<td>105</td>
<td>-</td>
<td>0.362</td>
</tr>
</tbody>
</table>

Table 2. Adjusted and unadjusted data for age and gender

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients N (%)</th>
<th>Healthy controls N (%)</th>
<th>OR* (CI 95%)</th>
<th>p-Value</th>
<th>OR* (CI 95%)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>38 (36.2)</td>
<td>71 (52.6)</td>
<td>Reference 1</td>
<td>-</td>
<td>Reference 1</td>
<td>-</td>
</tr>
<tr>
<td>CT</td>
<td>51 (48.6)</td>
<td>46 (34.1)</td>
<td>2.072 (1.183-3.628)</td>
<td>0.011</td>
<td>2.283 (1.210-4.306)</td>
<td>0.011</td>
</tr>
<tr>
<td>TT</td>
<td>16 (15.2)</td>
<td>18 (13.3)</td>
<td>1.66 (0.761-3.624)</td>
<td>0.203</td>
<td>1.835 (0.789-4.267)</td>
<td>0.158</td>
</tr>
<tr>
<td>C</td>
<td>127 (60.5)</td>
<td>188 (69.6)</td>
<td>-</td>
<td>-</td>
<td>Reference 1</td>
<td>-</td>
</tr>
<tr>
<td>T</td>
<td>83 (39.5)</td>
<td>82 (30.4)</td>
<td>1.498 (1.025-2.189)</td>
<td>0.035</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a: adjusted for age and gender, b: unadjusted for age and gender
observed in the present study. A meaningful difference in genotypes prevalence was also with hepatitis C. This reflects the high prevalence of IL 20 rs1518108 polymorphism in the studied patients. A represen-ted higher percentage of T minor allele patients with healthy controls than patients (64% in controls vs. 48% in patients) [13, 25]. In the present study, heterozygote CT genotype was more than TT homozygote in rs1518108 polymorphism. Similar to this study, Truelove et al., have reached to the same results on hepatitis B patients which heterozy-gote CT genotype was reported higher than homozygote TT in rs1518108 polymorphism [13, 16]. Another key aspect in studying patients with chronic hepatitis infection is frequency of cirrhotic patients among them. Hamada et al., studied the association between IL 10 level variations and liver cirrhosis; they concluded that high production of IL 10 can reduce disease progression to cirrhosis phase [17] but there hasn’t been any report of association between IL 20 and cirrhosis process yet. No significant difference was found between patients with chronic disease and people with cirrhosis in the present study. Further surveys on treatment response at different stages of the disease and IL 20 polymorphisms are suggested. A significant association between genotypes of healthy controls and hepatitis C at rs1518108 polymorphism of IL 20 was observed that represents higher risk of chronic hepatitis C infection for rs1518108 polymorphism carriers of IL 20. According to the results obtained in this study, allele T frequency of this polymorphism in the patient group was relatively higher than healthy control group which represents higher frequency of the rs1518108 IL 20 polymorphism in the studied population. No significant difference in the genotype of this polymorphism at the studied site has been found between chronic patients and people with cirrhosis.

**Acknowledgements**

The present project was supported by the Research Center for Gastroenterology and Liver Diseases (RCGLD), Shahid Beheshti University of Medical sciences, Tehran, Iran. The authors would like to thank all the members of RCGLD laboratory, especially Mahsa Khanyaghma, Hanieh Mirtalebi, Azita Hasani and Parvaneh Mohammadi for their critical help and technical support during the project. We also gratefully acknowledge Mr. Mohsen Mahlouji for language editing of the manuscript.
Authors’ Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

References


Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Shahid Beheshti University of Medical Sciences.