Original Article

Association between interleukin-10 gene promoter polymorphisms and susceptibility to liver cirrhosis

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Abstract: We conducted a case-control study to investigate the association between three common SNPs in IL-10 gene (rs1800896, rs1800871 and rs1800872) and the development of liver cirrhosis in a Chinese population. Between January 2013 and December 2014, a total of 318 patients with liver cirrhosis and 318 health control subjects were enrolled into our study. The IL-10 rs1800896, rs1800871 and rs1800872 polymorphisms were analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). By multivariate logistic regression analysis, we found that individuals with the AA genotype and GA+AA genotype of IL-10 rs1800896 were more likely to have an increased risk of liver cirrhosis when compared with the GG genotype, and the ORs (95% CI) for the AA genotype and GA+AA genotype were 2.04 (1.20-3.50) and 1.41 (1.02-1.96), respectively. We found that the GA+AA genotype of IL-10 rs1800896 had higher risk of liver cirrhosis in individuals with chronic hepatitis B when compared with the GG genotype (OR = 1.95, 95% CI = 1.01-3.59). In conclusion, we found that IL-10 rs1800896 polymorphism was correlated with an increased risk of liver cirrhosis, especially in individuals with chronic hepatitis B.

Keywords: Interleukin-10, polymorphism, liver cirrhosis

Introduction

Liver cirrhosis is a severe public health problem worldwide, which is correlated with higher morbidity and mortality worldwide [1-3]. It is reported that about 2 billion population are infected with HBV, and 3/4 of them are in Asia-Pacific region [4]. It is well known that long-term infection with HBV is associated with the development of liver cirrhosis, and other lifestyle factors may influence the susceptibility to liver cirrhosis, such as long-term heavy alcohol drinking [5, 6]. However, not all of the individuals who exposed to long-term infection with HBV and heavy alcohol drinking would suffer from liver cirrhosis, which suggests that molecular and cellular may contribute to the development of liver cirrhosis.

It is reported that cytokines play a fundamental role in the immunopathogenesis of HBV related diseases [7-9]. Previous studies have reported that cytokines gene polymorphisms, such as Interleukin-1β (IL-1β), IL-28B, IL-17 and IL-35, are associated with the development of HBV-related liver cirrhosis [10-12]. Interleukin-10 (IL-10) is an immunoregulatory cytokine, which is produced by Th2 cells, regulatory T cells, and monocytes/macrophages. The encoding gene of IL-10 is located on chromosome 1 (1q31-1q32). IL-10 is an anti-inflammatory cytokine, which could inhibit the synthesis of cytokines such as IL-6, IL-1β, IL-1α and TNF-α in activated macrophage and IFNγ by T cells [13]. Few studies investigated the role of IL-10 gene polymorphisms in the susceptibility to liver cirrhosis, and the results were inconsistent [14-17]. Therefore, we conducted a case-control study to investigate the association between three common SNPs in IL-10 gene (rs1800896, rs1800871 and rs1800872) and the development of liver cirrhosis in a Chinese population.

Materials and methods

Study subjects

A case-control study was conducted in our study. Between January 2013 and December 2014, a total of 352 patients with liver cirrhosis
were enrolled into our study. Liver cirrhosis was diagnosed either by histopathologically diagnosis, ultrasound, computer tomography (CT) or magnetic resonance imaging (MRI). Patients who had organ transplantation were excluded from this study. Finally, 318 patients were enrolled in our study, and the participation rate was 90.35%.

For the frequency-matched controls on sex and age, 318 health control subjects were randomly selected from individuals who came to our hospital for health check-up. Controls that had a history of liver cirrhosis and other HBV-related diseases were excluded from our study. All patients with liver cirrhosis and control subjects signed written informed consents. The signed written informed consents were obtained from patients with liver cirrhosis and control subjects. Our study was approved by the ethics committee of our hospital.

Genotyping assays

Each subject was asked to provide 5ml peripheral blood samples, and the blood samples were kept in -80°C before DNA extraction. Genomic DNA was selected from 5 mL peripheral blood of each sample, using the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China). Therefore, the DNA was stored at -80°C for following genotype analysis. DNA was extracted from peripheral blood samples 100 collected from patients and controls using the TIANamp Blood DNA Kit (Tiangen, Beijing, China), according to the manufacturer's instructions. After extraction, genomic DNA was diluted to a final concentration of 15-20 ng/μl for the genotyping assays. The IL-10 rs1800896, rs1800871 and rs1800872 polymorphisms were analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). Primers for amplification and extension reactions were designed using Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA). The Primers for IL-10 rs1800896, rs1800871 and rs1800872 were as follows: for IL-10 rs1800896, 5'-CTACTAAGGCTTCTTTGGAG-3' (forward) and 5'-ACTAAGGCTTCTTTGGGA-3' (reverse); for rs1800871, 5'-TCATTCTATGTGCTGGAGATGG-3' (forward) and 5'-TGGGGGAAGTGGGTAAGAGT-3' (reverse); for rs1800872, 5'-GGTGAGCACTACCTGACTAGC-3' (forward) and 5'-CCTAGGTCACAGTGACGTGG-3' (reverse). The restriction enzymes for IL-10 rs1800896, rs1800871 and rs1800872 were BseRI, MslII and Rsal, respectively. The amplification conditions were 95°C for 5 min, then 30 cycles of 94°C for 0.5 min, 60°C for 0.5 min and 72°C for 1 min, at last 72°C for 10 min. Digestion products were separated by electrophoresis on ethidium bromide stained agarose gel and visualized under UV light.

Statistical methods

The statistical difference between cases and controls was analyzed by A Chi-squared test and t test. The Hardy-Weinberg equilibrium (HWE) was tested by Fisher’s exact test for each SNP in controls. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression models adjusted for
IL-10 gene polymorphisms and liver cirrhosis

We conducted analysis on the association between IL-10 rs1800896 polymorphism and risk of liver cirrhosis stratified by drinking and chronic hepatitis B status (Table 3). We found that the GA+AA genotype of IL-10 rs1800896 had higher risk of liver cirrhosis in individuals with chronic hepatitis B when compared with the GG genotype (OR = 1.95, 95% CI = 1.01-3.59). However, no significant interaction was found between IL-10 rs1800896 polymorphism and alcohol drinking in the risk of liver cirrhosis.

Discussion

Genetic susceptibility to cancers has obtained a growing attention to investigate the gene polymorphisms in the development of several diseases. Inflammation and related cytokines play an important role in activating stellate cells and causing liver fibrosis [17], and the inflammatory responses of immune cells and following cytokine expression in the liver could contribute to the development of liver cirrhosis. In our study, we found that IL-10 rs1800896 polymorphism was correlated with an increased risk of liver cirrhosis, and had interaction with chronic hepatitis B in the pathogenesis of liver cirrhosis.

Table 2. Association between IL-10 rs1800896, rs1800871 and rs1800872 polymorphisms and risk of liver cirrhosis

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Patients</th>
<th>%</th>
<th>Controls</th>
<th>%</th>
<th>HWE</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1800896</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>125</td>
<td>39.31</td>
<td>152</td>
<td>47.80</td>
<td>0.90</td>
<td>1.0 (Ref.)</td>
<td>-</td>
</tr>
<tr>
<td>GA</td>
<td>141</td>
<td>44.34</td>
<td>135</td>
<td>42.45</td>
<td>1.27 (0.90-1.80)</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>52</td>
<td>16.35</td>
<td>31</td>
<td>9.75</td>
<td>2.04 (1.20-3.50)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>GA+AA</td>
<td>193</td>
<td>60.69</td>
<td>166</td>
<td>52.20</td>
<td>1.41 (1.02-1.96)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>rs1800871</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>128</td>
<td>40.25</td>
<td>139</td>
<td>43.71</td>
<td>0.23</td>
<td>1.0 (Ref.)</td>
<td>-</td>
</tr>
<tr>
<td>CT</td>
<td>139</td>
<td>43.71</td>
<td>135</td>
<td>42.45</td>
<td>1.12 (0.79-1.59)</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>51</td>
<td>16.04</td>
<td>44</td>
<td>13.84</td>
<td>1.26 (0.77-2.07)</td>
<td>0.34</td>
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<tr>
<td>CT+CC</td>
<td>190</td>
<td>59.75</td>
<td>179</td>
<td>56.29</td>
<td>1.15 (0.83-1.60)</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>rs1800872</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>110</td>
<td>34.59</td>
<td>122</td>
<td>38.36</td>
<td>0.65</td>
<td>1.0 (Ref.)</td>
<td>-</td>
</tr>
<tr>
<td>AC</td>
<td>159</td>
<td>40.25</td>
<td>153</td>
<td>48.11</td>
<td>1.15 (0.81-1.64)</td>
<td>0.41</td>
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</tr>
<tr>
<td>CC</td>
<td>49</td>
<td>25.16</td>
<td>43</td>
<td>13.52</td>
<td>1.26 (0.76-2.11)</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>AC+CC</td>
<td>208</td>
<td>65.41</td>
<td>196</td>
<td>61.64</td>
<td>1.18 (0.84-1.65)</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

Results

Population characteristics

The demographic and clinical characteristics of patients with liver cirrhosis and controls were shown in Table 1. There were no significant differences between patients and controls in terms of age and sex (P > 0.05). By Chi-squared test, we found that patients with liver cirrhosis were more likely to be infected with chronic hepatitis B and chronic hepatitis C, and have a habit of alcohol drinking. Of 318 patients, 163 (51.26%) were at A of Child-Pugh score, 128 (40.25%) were at B and 27 (8.49%) were at C.

The genotype distributions of IL-10 rs1800896, rs1800871 and rs1800872 were in line with HWE, and the P values for HWE were 0.90, 0.23 and 0.65, respectively (Table 2). The genotype distribution of IL-10 rs1800896 showed significant difference between patients with liver cirrhosis and controls (χ² = 8.08, P value = 0.02).

We conducted analysis on the association between IL-10 rs1800896 polymorphism and risk of liver cirrhosis stratified by drinking and chronic hepatitis B status (Table 3). We found that the GA+AA genotype of IL-10 rs1800896 were more likely to have an increased risk of liver cirrhosis when compared with the GG genotype, and the ORs (95% CI) for the AA genotype and GA+AA genotype were 2.04 (1.20-3.50) and 1.41 (1.02-1.96), respectively. Moreover, there was no significant association between IL-10 rs1800871 and rs1800872 polymorphisms and development of liver cirrhosis.
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Table 3. Association between IL-10 rs1800896 polymorphism and risk of liver cirrhosis stratified by drinking and chronic hepatitis B status

<table>
<thead>
<tr>
<th>Variables</th>
<th>GG</th>
<th>GA+AA</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic hepatitis B No</td>
<td>69</td>
<td>137</td>
<td>1.02 (0.67-1.55)</td>
<td>0.93</td>
</tr>
<tr>
<td>Yes</td>
<td>79</td>
<td>154</td>
<td>1.02 (0.67-1.55)</td>
<td>0.93</td>
</tr>
<tr>
<td>Alcohol drinking No</td>
<td>52</td>
<td>118</td>
<td>1.86 (0.97-3.58)</td>
<td>0.07</td>
</tr>
<tr>
<td>Yes</td>
<td>114</td>
<td>135</td>
<td>2.54 (1.03-6.36)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Previous studies have reported that IL-10 gene polymorphisms are associated with the development of hepatocellular carcinoma, and could influence the long-term infection of chronic hepatitis B and C [18-21]. Gao et al. assessed the relationship between the polymorphisms of IL-10 gene at position rs1800896 and rs1800872 and long-term infection of HBV and/or HCV, and they reported that IL-10 rs1800896 and rs1800872 polymorphisms could influence the chronic infection of HBV and HCV replication [18]. Gong et al. conducted a study in a Chinese population, and they indicated that IL-10-producing regulatory B cells were associated with the development of impaired anti-HBV immunity and the pathogenesis of chronic hepatitis B [19]. Ren et al. conducted a meta-analysis with 24 studies, and they reported that IL-10 rs1800872 was associated with an increased susceptibility to HBV infection, but IL-10 rs1800896 and rs1800871 were not [20]. Saxena et al. reported that IL-10 rs1800871 and rs1800872 gene polymorphisms were associated with the progression of HBV infection related disease, and they could promote the inactive carrier state to malignancy in an Indian population [21]. In our study, we found that IL-10 rs1800896 polymorphism slightly enhanced the risk of liver cirrhosis [14]. The discrepancies of the above mentioned results could be explained by differences in ethnicities, study design and disease status as well as sample size.

There were several limitations in our study. First, selection bias may exist in our study due to the hospital-based subjects in our study, but the matched on age and sex could reduce the bias in this study. Second, the sample size of our study is relatively small, which may limit the statistical power to find difference between groups. Therefore, further large-scale studies in different ethnic groups are greatly needed to confirm our results.

In conclusion, we found that IL-10 rs1800896 polymorphism was correlated with an increased risk of liver cirrhosis; especially in individuals with chronic hepatitis B. Future studies with larger sample size may contribute to elucidate the impact of IL-10 polymorphisms on the risk of liver cirrhosis.

Disclosure of conflict of interest

None.

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References

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