Interleukin-6 (-174G>C) promoter polymorphism in patients infected by hepatitis B virus

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Summary

Objective: Hepatitis B virus (HBV) infection is one of the most important health problems in the world. Interleukin-6 (IL-6) has been shown to be a major inflammatory cytokine, inducing cell proliferation and expression of acute response genes, such as fibrinogen and C-reactive protein in hepatocytes. IL-6 levels are elevated in patients acutely infected with HBV and have been associated with progression of infection to chronic hepatitis.

Methods: In the present study, the role of IL-6 (-174G/C) polymorphism was investigated on individuals which are diagnosed as hepatitis B virus carrier (n=19), chronic hepatitis (n=10) and cirrhosis (n=13), and non-infected individuals with HBV (n=8). In order to determine IL-6 polymorphism, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied using genomic DNA extracted from paraffin embedded liver needle biopsy specimens.

Results: The genotype frequencies in 50 samples were observed as follows: 76% homozygote typical (GG), 22% heterozygote (GC) and 2% homozygote atypical (CC). In this study, limited statistical association was observed between IL-6 polymorphism (GG, GC and CC genotype) and chronic hepatitis/cirrhosis compared to hepatitis B virus carriers and non-infected individuals with HBV (p=0.045).

Conclusion: As observed in only one patient with chronic hepatitis, IL-6 (-174C/C) genotype is very rare in this study group. Because of lower frequency of -174C/C phenotype, studies in larger series can give statistically more precise conclusions.

Key words: Cytokine; Hepatitis B; Interleukin-6; Liver; Polymorphism

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Introduction

Hepatitis B virus (HBV) is a hepatotropic, non-cytopathic DNA virus (3.2 kb partially double-stranded DNA) that causes a large range of clinical results, from acute and chronic infection to cirrhosis and hepatocellular carcinoma [1]. HBV infection is one of the main infectious diseases with more than 400 million chronic carriers in the world [2]. Cytokines play a key role in the defense against viral infections. Cytokine gene polymorphisms are associated with the importance of the liver disease in patient with HBV infection, which may help to explain the mechanism for the progression of viral hepatitis [3].

Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of biological activities [4]. It is one of the major inflammatory cytokines, and in some types of target cells it affects a variety of biological responses including changes in cell differentiation, growth, apoptosis and the induction of acute-phase responses [5, 6]. In an animal study, Galun et al [7] showed that human IL-6 facilitates hepatitis B virus (HBV) infection in vivo and in vitro. They developed an animal model in which mice transplanted with human liver tissue fragments or with human hepatocellular liver carcinoma cell lines (HepG), which had been previously infected ex vivo with HBV in the presence or absence of human IL-6 and in the presence of anti-IL-6-neutralizing antibodies.

The IL-6 gene is a single copy gene located on chromosome 7p15-p21 for human [8]. The IL-6 gene is polymorphic in both 5’ and 3’ flanking regions [9]. Whether the IL-6 genetic polymorphism at the -174 region may influence the clinical and/or serological outcome in patients with chronic HBV infection is not fully understood. In the present study, we investigated the IL-6 gene polymorphisms in various pathologies of HBV infections including HBV carriers, chronic hepatitis and cirrhosis, and compared their results with noninfected individuals.

Materials and methods

Subjects

The study population comprised 50 patients who were classified into four groups as follows: hepatitis B virus carrier (n=19), chronic hepatitis (n=10), cirrhosis (n=13), and non-infected
individuals (n=8). All of these patients had histopathological confirmation with a biopsy or autopsy at the time of diagnosis. Tissues were obtained from autopsy for three non-infected cases. Their clinical and laboratory findings were consistent with their diagnosis. None of the chronic hepatitis patients was HCV or HDV positive. Our research protocol was approved by the ethics committee of Gulhane Military Medical Academy.

**DNA extraction from paraffin embedded liver needle biopsy samples**

Genomic DNA was isolated from paraffin embedded liver needle biopsy tissues using the DNA isolation kit (QIAamp DNA Mini Kit, QIAGEN Sciences, Germantown, MD, USA) according to the method recommended by the manufacturer. DNA samples were quantified and subjected to specific PCR reaction as described.

**Determination of IL-6 promoter-174G>C polymorphism by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method**

The -174G/C single-nucleotide polymorphism (SNP) in promoter region of IL-6 gene (SNP rs1800795; gene access number NM 000600; gene ID 3569) was PCR-RFLP method previously reported by Mazzatti et al [10] In order to screen for -174G/C polymorphism of IL-6 gene, 303 bp fragment containing the whole core promoter region was amplified by PCR with the following primers: forward 5’-TTG TCA AGA CAT GCC AAA GTG CT-3´ and reverse 5’-GCC TCA GAC ATC TCC AGT CC-3´. Amplification was carried out on PCR System (CLP Apollo ATC401 thermal cycler, Apollo Instrumentation, San Diego, CA, USA) in a 50 μl reaction mixture containing 2 mM of dNTPs, 10 pmol each of forward and reverse primers, 2.5 U Taq DNA polymerase, 10x PCR buffer (Fermentas, Vilnius, Lithuania) and 50 ng genomic DNA. The PCR cycling conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 40 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and final extension step at 72°C for 5 min. Then the PCR product (303 bp) was digested with NlaIII (New England Biolabs, Hertfordshire, UK) and incubated at 37°C for 3-5 hours.

Digestion of PCR product by NlaIII yields 233 bp (G/G), 233/122 bp (G/C) and 122 bp (C/C) (Figs.1&2). The undigested polymerase chain reaction product and digested products were separated on a 2% agarose gel electrophoresis, visualized by ethidium bromide staining under an ultraviolet illuminator, scanned and photographed.

**Figure 1.** DNA isolation and PCR detection of IL-6 -174 G/C polymorphism. M, 100 bp ladder; Lane 1, undigested PCR product (303 bp); Lane 2, homozygote typical genotype (233 bp); Lane 3 and 4, heterozygote genotype (233 bp and 122 bp); Lane 5, homozygote atypical genotype (122 bp).

**Figure 2.** Digestion of PCR product by NlaIII using the Gel Logic 200 Imaging System (Eastman Kodak Company, Rochester, NY, USA).

**Statistical analysis**

The statistical analysis was done with SPSS software (version 13.0). Chi-square test was used for the comparison of groups, and p<0.05 was considered to be statistically significant.

**Results**

As a result of this study, the genotype frequencies in 50 samples were observed as 76% homozygote typical (G/G), 22% heterozygote (G/C) and 2% homozygote atypical (C/C). The G and C allele frequencies in the different groups were reported in Table 1. Statistically limited association was observed between IL-6 polymorphism (G/G, G/C and C/C genotype) and chronic hepatitis and cirrhosis compared to hepatitis B virus carriers and non-infected individuals with HBV (p=0.045).

All of the non-infected individuals had IL-6 G/G genotype. In the hepatitis B virus carrier group, two of nineteen cases were G/C genotype and 17 had G/G genotype. The chronic hepatitis group included three G/C, six G/G, and one C/C genotype. In the cirrhosis group, six cases were G/C genotype and seven had G/G genotype.
Table 1. The representation of IL-6 -174 G/C polymorphism in patients infected by hepatitis B virus.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>n (%)</th>
<th>Disease type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>IL-6 G/C genotype</td>
<td>11 (22%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>IL-6 G/G genotype</td>
<td>38 (76%)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>IL-6 C/C genotype</td>
<td>1 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>50 (100%)</td>
<td>8 (100%)</td>
</tr>
</tbody>
</table>

1, non-infected individuals with HBV; 2, hepatitis B virus carrier; 3, chronic hepatitis; 4, cirrhosis

Discussion

Within the past few years, the focus on cytokine SNP function and association with human diseases has increased considerably. There is also on-line database that include the positive associations of cytokine SNPs in human diseases described in articles. Both IL-6 and the IL-6 (-174G/C) promoter polymorphism have been linked to various diseases associated with inflammation. IL-6 has been associated with psoriasis [11], rheumatoid arthritis [12], systemic-onset juvenile chronic arthritis [13], Kaposi sarcoma in HIV-positive men [14] and atherosclerosis [15]. It has also been shown that IL-6 (-174G/C) genetic polymorphisms, involved in insulin resistance and non-alcoholic steatohepatitis [16]. On the other hand, IL-6 plays a role in carcinogenesis by promoting it through multiple signal pathways [17, 18].

In a few recent studies, the relation between IL-6 promoter (-174G>C) polymorphism and hepatitis B was investigated [19-21]. No relationship was detected in previous studies performed in Far East Asian patients [19, 20]. Using the single base extension method, Park et al [20] studied the three known (-597G>A, -572C>G, -174G>C) SNPs in the IL-6 promoter region in a large chronic hepatitis B cohort to evaluate the effects of IL-6 promoter variants. They found no significant associations between IL-6 promoter variants and disease outcome in chronic hepatitis B. However, it must be noted that both of these studies by Migita et al [19] and Park et al [20] included only a few IL-6 (-174G/C) and none IL-6 (-174C/C) genotype.

In 2009, Fabris et al [21] reported for the first time a relationship between IL-6 (-174G>C) polymorphism and the course of chronic HBV infection. For the analysis of polymorphism, they performed a polymerase chain reaction based restriction fragment length polymorphism assay. Interleukin-6 -174G>C promoter polymorphism identify two phenotypes: the high producer phenotype, comprising the -174G/G and -174G/C genotypes, characterized by higher circulating IL-6 levels, and the low producer phenotype accounted by the -174C/C genotype [9]. In their study, IL-6 (-174C/C) genotype occurred according to the following trend: patients with chronic hepatitis B (4/80, 5%), HBsAg inactive carriers (3/27, 11%), and HBeAb positive control subjects (6/25, 24%). They concluded that possessing an IL-6 low producer phenotype may provide some advantage to older patients with chronic HBV infection [21].

In the present study, only one case of IL-6 (-174C/C) genotype was present in the study group of 50 cases (2%). Ethnic differences have been demonstrated in the frequency of the -174 G allele, with non-Caucasian populations exhibiting much higher frequencies than Caucasian populations [22]. Although the number of cases in our study is very limited, IL-6 (-174C/C) genotype seems to be rare in our study group compared to the study with Caucasians [21]. However, it is not an exactly similar situation to the almost absence of the C allele of -174G>C polymorphism in East Asian populations. Instead, it might be somewhere in between these two extremes. The only one case of IL-6 (-174C/C) genotype was detected in the patient with chronic hepatitis B (1/10, 10%). On the other hand the frequency of IL-6 (-174G/C) genotype was much higher than IL-6 (-174C/C) genotype. Of all, in II (22%) patients, IL-6 (-174G/C) genotype was detected. IL-6 (-174 G/C) genotype occurred as follows in the present study: 46.2% (6/13) in patients with cirrhosis due to HBV infection, 30% (3/10) in patients with chronic hepatitis B, 10.5% (2/19) in HBsAg inactive carriers, and none (0%, 0/8) in non-infected individuals. We used PCR-RFLP assay similar to the study of Fabris et al. [21].

In a small number of studies, the relation between IL-6 polymorphisms and hepatitis C was also investigated. IL-6 promoter polymorphisms probably influence the development of chronic HCV infection, too. With the permissive effect of male gender, haplotypes represented by the wild-type allele for -597 and -174 loci appear to favor a worse evolution of the disease [23]. Faleti et al [24] investigated the role of IL-6 polymorphisms on the
disease progression of patients with hepatitis C virus (HCV) infection. Among patients whose grading and staging scores increased at the end of the follow-up ≥ 2 Ishak points, IL-6 -174G>C genotype frequencies were GG 37/66, GC 21/45, CC 2/10 (p = 0.041) and GG 18/66, GC 8/45, CC 0/10 (p = 0.040), respectively. Grading progression was independently associated with carriage of the G allele in -174G>C polymorphism. They concluded that IL-6 polymorphisms influence histologic progression of HCV in patients with persistently normal transaminases.

The mechanism by which the polymorphism influences disease risk is unclear. It is unknown if the association between the -174G/C polymorphism and disease risk is a direct or indirect result of IL-6 as there have been discrepancies on the effect of the polymorphism on circulating IL-6 levels [25-27]. It seems that the SNP in the promoter region of the IL-6 gene at position -174 may regulate the plasma concentrations of IL-6 in vivo [4-6]. The presence of lysine (allele C) at the guanine site (allele G) is associated with low levels of plasma IL-6 in healthy subjects. The CC genotype is considered to be a low producer and GG a high producer of IL-6 [9].

On the other hand, Endler et al [26] did not find this association and Brull et al [25] found the opposite, i.e., patients with the CC allele had higher plasma IL-6 levels. Furthermore, other haplotypes cooperate with the transcriptional regulation of IL-6 influencing its production [28]. Additional studies of the genotype–phenotype association of this polymorphism are necessary to clarify its role in disease susceptibility [27].

In conclusion, we have found that the occurrence of IL-6 (-174C/C) genotype is very rare in this study group. Although we hypothesized to find low producer -174C/C genotype more frequent in the patients with clinical and laboratory findings consistent with hepatitis B virus carrier, the only -174C/C genotype was observed in a chronic hepatitis patient. Nevertheless, our study has several limitations. Because of lower frequency of -174C/C phenotype, studies with larger number of cases can give statistically more reliable conclusions. The measurement of serum IL-6 levels could contribute to clarify the relation of it with IL-6 (-174G>C) polymorphism. Additionally, the advantage of present study is the inclusion of cases only if they have histopathological confirmation.

References


