Lack of association between Interleukin-12 gene polymorphism (rs568408 G/A) and susceptibility to chronic hepatitis B virus infection

Seddigh Heidarian¹, Farzaneh Sabahi¹*, Seyed Reza Mohebbi²*, Mohammad Reza Zali³

¹. Department of Virology, Tarbiat Modares University, Tehran, Iran.
². Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
³. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Introduction

Hepatitis B is a potentially life-threatening infection that causes acute infection and chronic hepatitis with progression to cirrhosis and hepatocellular carcinoma (HCC). Interleukin-12 (IL12) is responsible for activation of Th1 immune responses, leading to possible clearance of HBV infection from the host’s body. The host’s immune-genetic background plays an important role in the pathogenesis of infectious diseases. The aim of the present study was to investigate the association of interleukin 12A single nucleotide polymorphism (rs568408 G/A) with chronic HBV infection.

Methods: In this case-control study, 120 chronic HBV patients and 120 healthy controls were studied from 2013 to 2015. Genotype analysis was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: The genotype distribution of IL12 rs568408 G/A was not significantly different between the chronic HBV patients and healthy controls. The frequency rates of IL12 gene polymorphism at position rs568408 included GG (64.2%), AG (33.3%), and AA (2.5%) in the HBV patients and GG (68.3%), AG (29.2%), and AA (2.5%) in healthy controls (p=0.728).

Conclusion: The results suggested no significant association between IL12A rs568408 G/A genotypes and chronic hepatitis B virus infection.

Abstract

Introduction: Hepatitis B is a potentially life-threatening infection that causes acute infection and chronic hepatitis with progression to cirrhosis and hepatocellular carcinoma (HCC). Interleukin-12 (IL12) is responsible for activation of Th1 immune responses, leading to possible clearance of HBV infection from the host’s body. The host’s immune-genetic background plays an important role in the pathogenesis of infectious diseases. The aim of the present study was to investigate the association of interleukin 12A single nucleotide polymorphism (rs568408 G/A) with chronic HBV infection.

Methods: In this case-control study, 120 chronic HBV patients and 120 healthy controls were studied from 2013 to 2015. Genotype analysis was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: The genotype distribution of IL12 rs568408 G/A was not significantly different between the chronic HBV patients and healthy controls. The frequency rates of the IL12 gene polymorphism at position rs568408 included GG (64.2%), AG (33.3%), and AA (2.5%) in the HBV patients and GG (68.3%), AG (29.2%), and AA (2.5%) in healthy controls (p=0.728).

Conclusion: The results suggested no significant association between IL12A rs568408 G/A genotypes and chronic hepatitis B virus infection.

Keywords: Chronic hepatitis B infection, single nucleotide polymorphisms, Interleukin 12, Iran

*Corresponding Author: Department of Virology, Tarbiat Modares University, Jaleh Ale Ahmad Blvd, Tehran, Iran. Tel: +9821-82883880, Email: sabahi_f@modares.ac.ir

Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel: +9821-22432514, Email: sr.mohebbi@sbnu.ac.ir

Received: 27 October, 2015
Accepted: 05 April, 2016


Introduction

Hepatitis B is an inflammatory liver disease which is induced by Hepatitis B Virus (HBV). Over 400 million people in the world are infected with chronic hepatitis, and 15 million people die annually due to the complications associated with HBV. About 2-10% of patients suffer from chronic clinical complications with different consequences, and 15-40% of patients are at risk of liver cirrhosis and cancer (1).

Cytokines and chemokines, which are produced from macrophages and lymphocytes, regulate antiviral humoral and cellular immune responses and act coordinately in elimination of viral and bacterial infections (2). For example, Tumor Necrotic Factor (TNF) and Interferon gamma (INF-γ) directly inhibit virus proliferation (3). Interleukin 12 (IL12) is a 70 kDa heterodimer cytokine that is located on 3p12-152 chromosome and connected to disulfide bonds. These dimers are produced by IL 12A and IL 12B genes. IL12 was first identified in 1989 in B lymphocyte cell lines contaminated with Epstein-Barr virus (EBV). The most important IL 12 producing cells are cytokocytes, dendritic cells and Th1 lymphocytes, which cause activation and proliferation of immune cells like macrophages, Natural Killer Cells (NKs), Th1 lymphocytes and T cytotoxic lymphocytes. On the other hand, production of antiviral cytokines stimulate these cells (4).

Kawana et al. showed that specific hepatitis B virus CTL lymphocytes inhibit the proliferation of this virus in the liver and kidney cells of all transgenic mice with mediation of INF-γ. In fact, extensive antiviral response in liver and kidney is initiated by endogenous IL12 and INF-7. Production of TNF and INFα/β is increased by administration of IL12 and cause the destruction of HBV nucleocapsid, prevents the proliferation of virus in liver and kidney cells and consequently leads to HBV clearance from the blood. However, high doses of exogenous IL12 reduce the proliferation of the virus, but result in impairment of liver cells (6). Polymorphism (rs568408) is located at untranslated region 3 (3’UTR) and is a location for miRNA bonding. This region affects the stability of mRNA and consequently translation of IL12. Assuming that high production of IL12 produces an effective, primary immune response...
against virus infection, this cytokine is considered to be protected against viral infections (7).

Given the pivotal role of this cytokine, the expression of its genome is of great importance in regulation and guidance of immune system in controlling viral infections. The present study was conducted to evaluate the association of IL12A rs568408 3′UTR G with chronic hepatitis B virus infection.

Materials and Methods

Study sample

In this case-control study, the study sample was determined based on some studies carried out in this regard. It comprised of 120 patients with HBV (those who were HBsAg- and HBeAb-positive for at least six months and their body was not able to clear the viruses) and 120 healthy controls (HBsAg- and HBeAb-negative and without liver diseases), referring to the Taleghani hospital, Tehran from 2013 to 2015. The samples were selected through convenience sampling.

DNA was purified from the whole peripheral blood treated with EDTA by salting out method from the patients and controls, and genomic DNA was kept in Tris-EDTA buffer at -20 °C until the experiment was performed.

Genotyping and polymorphism

To determine the genotype of the samples in both groups, PCR-RFLP method was performed (8). First, a pair of primers with sequences presented in Table 1 were designed and optimized by Gene Runner (Hasting software Inc. Version 4) and BLAST technique, taken from the National Center for Biotechnology Information site. Then, a segment of IL12A rs568408 G was proliferated under the following conditions:

75 Ng reaction mixture containing PCR buffer along with magnesium chloride, 1.5 units Taq DNA polymerase (Super Taq, England), 0.5 microliters of each primer and 0.5 microliters dNTPs were added to 25 microliters final volume. PCR reaction was performed by an automatic thermocycler (Eppendorf, Germany) as follows: first, initial denaturation was performed at 95 °C for 5 minutes, followed by 38 replication cycles (each cycle induced three thermal stages, 95 °C for 30 seconds, 61.5 °C for 35 seconds, and 72 °C for 45 seconds). Then, the final segment was preserved at 72 °C for 10 minutes to replicate. The PCR product was detected by electrophoretic technique on 1.5% agar gel (Roche, Germany) and ethidium bromide staining against ultraviolet light. The PCR product, in enzymatic digestion response, was then reacted by NlaIII restriction enzyme (NEW ENGLAND BioLabs), cutting the given polymorphism position as follows: 10 microliters PCR product was directly added to the mixture containing buffer and NlaIII restriction enzyme in 25 microliters final volume, and incubated at 37 °C for 16 hours. The enzyme digestion solution was observed by electrophoresis on 3% polyacrylamide gel.

Statistical analysis

The statistical analysis of data obtained from the two groups was performed by SPSS-21 software using descriptive statistics (frequency, percentage and mean) and inferential statistics (chi-square). P<0.05 was considered significant.

<table>
<thead>
<tr>
<th>Table 1. Sequence of the designed forward and reverse primers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IL12A-123F</strong></td>
</tr>
<tr>
<td><strong>IL12A-123R</strong></td>
</tr>
</tbody>
</table>

Results

The demographic data (age and gender) of the patient and control groups are presented in Table 2. As indicated, both groups were not significantly different in terms of gender, but they were significantly different (P=0.01) with regard to age. This confounding factor, however, was eliminated by regression analysis.

The PCR product was a 241 base-pair segment at which polymorphism was located. Then, due to enzymatic digestion (Figure 1), two 223 and 18 base-pair segments in AA homozygote, three 61, 163 and 18 base-pair segments in GG homozygotes, and three 223, 163 and 18 base-pair segments in AG heterozygote patients were observed.

The results of statistical analysis and frequency of different rs568408A/G polymorphisms for healthy people indicated 68.3% GG homozygote, 29.2% AG heterozygote, and 2.5% AA homozygote. The genotype distribution of the patients consisted of 64.2% GC homozygote, 33.3% AG heterozygote and 2.5% AA homozygote (P=0.782).

The statistical results of logistic regression analysis, presented in Table 3, showed no significant correlation between rs568408 genotypes in IL12 and susceptibility to chronic hepatitis B virus infection.

<table>
<thead>
<tr>
<th>Table 2. Demographic characteristics of study groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study variables</strong></td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Age</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3. Frequency (%) of genotypes and alleles obtained in both study groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td>GG</td>
</tr>
<tr>
<td>AG</td>
</tr>
<tr>
<td>AA</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>G</td>
</tr>
</tbody>
</table>
The effect of its polymorphisms has been evaluated in response to HBc levels of IL-12. Limited in IL-12 promoter regions, coding sequences, and non-coding regions, polymorphisms change the amount of this cytokine. Internal and external signals but genotype changes of conditions, while its genotype is not influenced by concentration is affected by behavioral and physical stress. This network is influenced by several factors such as blood infusion, stress, iron level, etc., so IL-12 concentration is affected by behavioral and physical conditions, while its genotype is not influenced by internal and external signals but genotype changes of polymorphisms change the amount of this cytokine.

Since SNPs are the most common type of polymorphisms that directly and indirectly affect the expression of genes and performance of cytokines, studies on diseases in the recent years have been concentrated on this domain. The relationship of single nucleoid polymorphisms with cancers, inflammatory and self-immune diseases, and control of chronic diseases has been investigated. Also, epidemiologic studies have shown that IL-12A and IL-12B polymorphisms are associated with several infectious and noninfectious inflammatory diseases like hepatitis, psoriasis, arthritis, and asthma.

Single nucleoid polymorphisms are located at promoter regions, coding sequences, and non-coding region 3'UTR. The number of polymorphisms is limited in IL-12A and IL-12B 3'UTR genes, and affect IL-12 level so that Crohn's disease, a chronic inflammatory bowel disorder, is accompanied by high levels of IL-12. Further, IL-12B polymorphism (-10993C/G) increases the concentration of IL-12 in response to HBc-Ag from the peripheral blood cells. Because IL-12 is a proinflammatory cytokine, the effect of its polymorphisms has been evaluated in infectious and noninfectious inflammatory diseases. Multiple sclerosis and Alzheimer are diseases of nervous system with vivid inflammatory response. Rs568408 polymorphism in IL-12A gene and rs3212227 in IL-12B gene are separately or jointly associated with Alzheimer disease in Chinese population. Liu et al. showed that presence of a single nucleoid polymorphism in the 3 untranslated region (rs568408G/A) increased the risk of hepatocarcinoma.

Long-term inflammation due to hepatitis B or C can lead to initiation and progression of malignancy in liver, so HBV and HCV are major risk factors for hepatocarcinoma (HCC). SNPs of IL-12A and IL-12B can play a significant role in modulation of susceptibility to HCC. Few studies have analyzed the correlation of IL-12 polymorphisms and chronic hepatitis B. Liu et al. found that rs568408 (A/G), rs2243115 and rs3212227 are associated with increased risk of HCC in the patients with hepatitis B (23); whereas, Nieters et al. reported no significant association between IL-12B rs3212227 AC/CC and hepatocarcinoma (24). Mohebi et al. performed the first study on the relationship of IL-12B gene diversity with hepatitis in Iran and showed no significant association between IL-12 (p24) 1188 C/A polymorphism and susceptibility to hepatitis B and C (25). However, another study conducted by Zargar et al. on the correlation of this polymorphism with multiple sclerosis indicated that AA genotype was increased and CA genotype was reduced in the patients. They emphasized the role of AA genotype in increased susceptibility to MS in Iranian population (26). Polymorphism rs568408 is located at 3'UTR, and this region affects the stability of mRNA and translation of IL-12. In addition, assuming that high production of IL-12 induces an effective primary immune response against viral infection, this cytokine is believed to be protected against viral infections.

Discussion

The results of this study indicated that rs568408 G polymorphism in interleukin 12 (p35) cannot possibly be a risk factor for chronic HBV infection among Iranian population. In fact, IL-12 is involved in the development of TH1 and TH1/TH2 balance in favor of TH1 cells. IL-12 is a member of cytokine network that includes anti-inflammatory and proinflammatory bioactive cytokines. This network is influenced by several factors such as blood infusion, stress, iron level, etc., so IL-12 concentration is affected by behavioral and physical conditions, while its genotype is not influenced by internal and external signals but genotype changes of polymorphisms change the amount of this cytokine.

SNPs are the most common type of polymorphisms that directly and indirectly affect the expression of genes and performance of cytokines, studies on diseases in the recent years have been concentrated on this domain. The relationship of single nucleoid polymorphisms with cancers, inflammatory and self-immune diseases, and control of chronic diseases has been investigated. Also, epidemiologic studies have shown that IL-12A and IL-12B polymorphisms are associated with several infectious and noninfectious inflammatory diseases like hepatitis, psoriasis, arthritis, and asthma.

Single nucleoid polymorphisms are located at promoter regions, coding sequences, and non-coding region 3'UTR. The number of polymorphisms is limited in IL-12A and IL-12B 3'UTR genes, and affect IL-12 level so that Crohn's disease, a chronic inflammatory bowel disorder, is accompanied by high levels of IL-12. Further, IL-12B polymorphism (-10993C/G) increases the concentration of IL-12 in response to HBc-Ag from the peripheral blood cells. Because IL-12 is a proinflammatory cytokine, the effect of its polymorphisms has been evaluated in infectious and noninfectious inflammatory diseases. Multiple sclerosis and Alzheimer are diseases of nervous system with vivid inflammatory response. Rs568408 polymorphism in IL-12A gene and rs3212227 in IL-12B gene are separately or jointly associated with Alzheimer disease in Chinese population. Liu et al. showed that presence of a single nucleoid polymorphism in the 3 untranslated region (rs568408G/A) increased the risk of hepatocarcinoma.

Long-term inflammation due to hepatitis B or C can lead to initiation and progression of malignancy in liver, so HBV and HCV are major risk factors for hepatocarcinoma (HCC). SNPs of IL-12A and IL-12B can play a significant role in modulation of susceptibility to HCC. Few studies have analyzed the correlation of IL-12 polymorphisms and chronic hepatitis B. Liu et al. found that rs568408 (A/G), rs2243115 and rs3212227 are associated with increased risk of HCC in the patients with hepatitis B (23); whereas, Nieters et al. reported no significant association between IL-12B rs3212227 AC/CC and hepatocarcinoma (24). Mohebi et al. performed the first study on the relationship of IL-12B gene diversity with hepatitis in Iran and showed no significant association between IL-12 (p24) 1188 C/A polymorphism and susceptibility to hepatitis B and C (25). However, another study conducted by Zargar et al. on the correlation of this polymorphism with multiple sclerosis indicated that AA genotype was increased and CA genotype was reduced in the patients. They emphasized the role of AA genotype in increased susceptibility to MS in Iranian population (26). Polymorphism rs568408 is located at 3'UTR, and this region affects the stability of mRNA and translation of IL-12. In addition, assuming that high production of IL-12 induces an effective primary immune response against viral infection, this cytokine is believed to be protected against viral infections.
Polymorphism of this gene was analyzed and a rather
of the limitations of this study was that only one
prognosis in severity or improvement of the disease in
Iranian patients with hepatitis or the healthy ones. One
of the limitations of this study was that only one
polymorphism of this gene was analyzed and a rather
limited number of patients and controls were evaluated.
Of course, genotype distribution is different among
various ethnicities. To achieve a definitive result, this
polymorphism is suggested to be studied among other
ethnicities.

Conclusion
As stated, the genetic factors of the patient are one of
the most significant elements in development or
amelioration of infectious and noninfectious diseases.
Polymorphisms are the indicators of these involving
genetic factors. A profile of SNPs of the genes encoding
the immune system path plays the role of a prognosis in
the development, improvement or severity of infectious
or noninfectious diseases in clinical medicine.
According to the studies conducted, these polymorphisms are affected by different ethnicities and
populations. Given the significance of rs568408 position
in IL12A, which can affect the expression and amount
of this cytokine, and the correlation of this polymorphism with development or improvement of
chronic, inflammatory and cancer diseases, this study
was carried out to examine the correlation of this SNP
with susceptibility to chronic hepatitis B among Iranian
patients, which revealed no significant difference in the
emergence of hepatitis B. Hence, this polymorphism
cannot probably be involved in the severity or
amelioration of the disease in patients with chronic
hepatitis B.

References
2. Gee K, Guzzo C, Che Mat NF, Ma W, Kumar A. The IL-12 family of cytokines in infection, inflammation and autoimmune
1986;323(6091):819-22.
6. Rossol S, Marinos G, Carucci P, Singer MV, Williams R, Naoumov NV. Interleukin-12 induction of Th1 cytokines is important
8. Falleti E, Fabris C, Tonitutto P, Fontanini E, Cussigh A, Bitetto D, et al. TGF-β1 genotypes in cirrhosis: Relationship with the
variants with the development of antibodies to surface antigen of hepatitis B virus in hemodialysis patients in response to
chronic hepatitis C patients (Persian)]. JBUAMS. 2011;13 (4) :26-33