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Original Article

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

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Article Info

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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.

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-y) 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 Epsten-Barr virus (EBV). The most important IL 12 producing cells are cytophages, 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-y. In fact, extensive antiviral response in liver and kidney is initiated by endogenous IL12 and INF-7. Production of TNF and INFα/ß 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 HBcAb-positive for at least six months and their body was not able to clear the viruses) and 120 healthy controls (HBsAg- and HBcAb-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 electrophorese 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 NIaIII 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 NIaIII restriction enzyme in 25 microliters final volume, and incubated at 37 °C for 16 hours. The enzyme digestion solution was observed by electrophorese 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 1. Sequence of the designed forward and reverse primers

IL12A-123F	5- CTAAAGTCAATGTGTCAGCAGAGC -3
IL12A-123R	5- CCTCCAAAGACATCCACCTCC -3

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 2. Demographic characteristics of study groups

Study variables	Patient	Control	P-value
Male	46 (38.3)	57 (47.5)	
Female	74(61.7)	63 (52.5)	0.95
Age	14.68±41.4	13.21±36.79	0.11

Table 3. Frequency (%) of genotypes and alleles obtained in both study groups

Genotype	Patients (%)	Controls (%)	OR (CI = %95)	P-value
GG	77 (65)	82 (68.3)	Reference	
AG	40 (33.3)	35 (29.2)	0.7 (0.11-4.3)	0.56
AA	3 (1.7)	3 (2.5)	0.6 (0.09-3.6)	0.65
A	44 (18.3)	41 (17.1)	Reference	
G	196 (81.7)	199 (82.9)	0.91 (0.57-1.46)	0.72

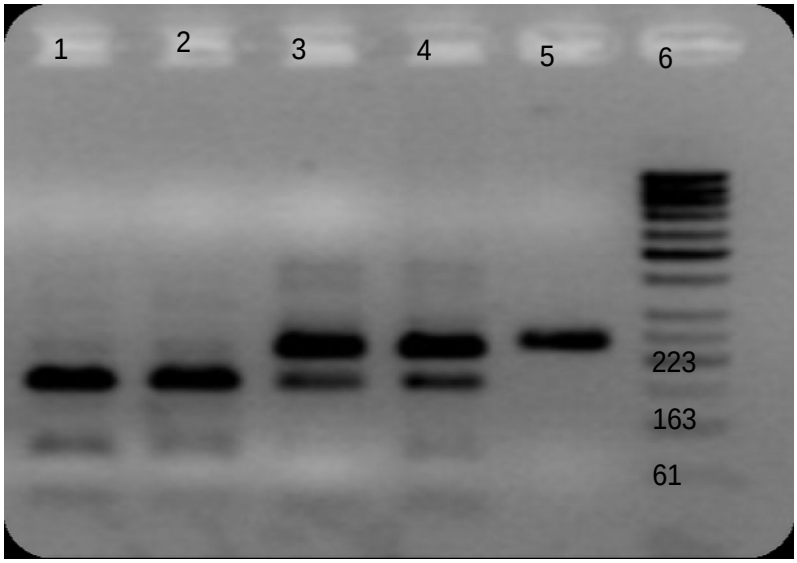

Fig. 1. Electrophorese results of cutting NIaIII enzyme on IL12A gene limiting rs568408G/A to three genotypes GG (wild homozygote), AG (heterozygote) and AA (mutant homozygote)
Well 1 is the sectioned control sample (GG) that is previously sequenced and run in every working set for accuracy of the performance of restriction enzyme. Well 2 represents GG genotype, wells 3 and 4 show AG genotype, well 5 indicates AA genotype and well 6 represents molecular weight.

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, IL12 is involved in the development of TH1 and TH1/TH2 balance in favor of TH1 cells. IL12 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 IL12 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 (9).

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 (10). The relationship of single nucleoid polymorphisms with cancers (11, 12), inflammatory and self-immune diseases (13, 14), and control of chronic diseases has been investigated (15, 16). Also, epidemiologic studies have shown that IL12A and IL12B polymorphisms are associated with several infectious and noninfectious inflammatory diseases like hepatitis, psoriasis, arthritis and asthma (17, 18).

Single nucleoid polymorphisms are located at promoter regions, coding sequences, and non-coding region 3′UTR (19). The number of polymorphisms is limited in IL12A and IL12B 3′UTR genes, and affect IL12 level so that Crohn's disease, a chronic inflammatory bowel disorder, is accompanied by high levels of IL12 (20). Further, IL12B polymorphism (-10993C/G) increases the concentration of IL12 in response to HBc-Ag from the peripheral blood cells (21). Because IL12 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 IL12A gene and rs3212227 in IL12B gene are separately or jointly associated with Alzheimer disease in Chinese population (22). Liu et al. showed that presence of a single nucleoid polymorphism in the 3 untranslated region (rs568408G/A) increased the risk of hepatocarcinoma (23).

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 IL12A and IL12B can play a significant role in modulation of susceptibility to HCC. Very few studies have analyzed the correlation of IL12 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 asscociation between IL12B rs3212227 AC/CC and hepatocarcinoma (24). Mohebi et al. performed the first study on the relationship of IL12B gene diversity with hepatitis in Iran and showed no significant association between IL12 (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 IL12. In addition, assuming that high production of IL12 induces an effective primary immune response against viral infection, this cytokine is believed to be protected against viral infections (7). On the other hand,

Song et al. showed that IL12 serum level is increased in clinical forms of Hepatitis B (27) and is partially reduced in the latent form of the virus. An interesting point is that IL12 serum level is reduced as the clinical symptoms are reduced. In general, it seems that IL12 increase clears the virus, on the one hand, and impairs the liver hepatocytes by activating the immune cells, on the other hand (28).

Given the importance of this single nucleoid polymorphism (rs568408) in terms of its location in IL12 genome, which can be effective in expression of this cytokine, and its association with development or improvement of various chronic inflammatory diseases (18, 29) as well as cancers (30, 31), this study was aimed at evaluating the relationship of this polymorphism with susceptibility to chronic hepatitis B in Iranian patients, whose results indicated no significant difference in the emergence of hepatitis B. Therefore, the possible presence of this polymorphism cannot be a 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.

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