

## Lack of association between Single Nucleotide Polymorphism (rs1400986) in Interleukin-20 Gene and Chronic Hepatitis B Virus Infection

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### ABSTRACT

**BACKGROUND AND OBJECTIVE:** Infection with hepatitis B virus is a major cause of chronic liver diseases and variations within cytokine genes may affect the host immune response to hepatitis B infection. This study was designed to investigate whether IL-20 rs1400986 C/T single nucleotide polymorphism is involved in the progression of chronic hepatitis B infection.

**METHODS:** In this case-control study a total of 150 chronic hepatitis B patients (Anti-HBc Ab positive and HBsAg positive for more than 6 months) and 146 healthy subjects (Anti-HBc Ab and HBsAg negative) among people who were referred to Tehran Taleghani hospital during 2003 to 2005, were examined for differences in genotype and allele frequencies in this case-control study. Polymerase Chain Reaction- Restriction Fragment length Polymorphism (PCR-RFLP) method was applied for analyzing the polymorphism site.

**FINDINGS:** Genotypes Frequency in patients group were CC 32.0% and TT 4.0% in comparison to CT 28.1% and TT 2.7% in control group; however no statistically significant difference was observed in the frequency of IL-20 gene polymorphism (rs1400986) between chronically infected patients and healthy controls for neither allele ( $P=0.549$ ) nor genotype ( $p=0.599$ ) frequencies.

**CONCLUSION:** No association was detected between rs1400986 single nucleotide polymorphism within IL-20 gene and chronic hepatitis B infection; thus, this polymorphism appears to have no influence on susceptibility to chronic hepatitis B.

**KEY WORDS:** Cytokine, Hepatitis B virus, Polymorphism, Single nucleotide, Interleukin 20.

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## Introduction

**H**epatitis B is one of the most common chronic infections in the world. It is estimated that 2 billion people worldwide are infected with hepatitis B (HBV), which may be a large group of people lose their life as a result of complications caused by chronic infection with HBV, including cirrhosis and liver cancer (1,2). HBV, is a member of the Hepadna viridae family is cause of acute or chronic Hepatitis B. When infected with HBV, patients may develop chronic infection, which can result in several stages. Most adults with acute hepatitis B, the virus is cleared from the body by itself; while most infants and children, there is a high probability of progression to chronic infection (1,3).

Mechanisms that clearing the virus or survive infection in patients is yet unknown; however, it seems that the difference in clinical outcome of disease is associated with major factors of host (such as age, gender and ability and response of the immune system person).

It seems that the Human Genome Diversity affects the individual immunological status (3). In case of infection with the hepatitis B, induced inflammatory response causes regeneration and tissue repair in acute phase and prolonged liver damage (due to poorly designed responses of CD8<sup>+</sup> and CD4<sup>+</sup>T cells) ends to fibrosis, cirrhosis and to eventually liver cancer (5 and 4). These inflammatory processes strongly influenced by balance between pro- inflammatory cytokines and anti-inflammatory cytokines (6,7).

In patients with chronic hepatitis B the ratio of Th2 to Th1 is dominant, which leads to increased production of anti-inflammatory cytokines. IL-20 is a primary pro-inflammatory cytokine belonged to IL-10 cytokine family. The region of gene cluster that encodes this interleukin is 195 kb on 1q32 chromosome, and has 28% similarity with human IL-10 gene (5, 8, 9). IL-20 causes the balance of Th1/Th2 and acts with shared IL-10 receptors (10). The signaling of this interleukin occurs by 2 type receptor complex IL-20R1/IL20-R2 and IL-22R1/ IL-22R2 (10) and STAT3 is as a key oppressed transcription factor (5, 8,10,11). IL-20 is produced mainly by stimulating

myeloid cells and epithelial cells (6 and 9). Overexpression of IL-20 in transgenic mice causes skin lesions similar to psoriasis (6).

IL-20 plays role in inflammation, angiogenesis, atherogenesis and chemotaxis, and all factors are involved in the pathogenesis of many inflammatory diseases and autoimmune like Atherosclerosis, psoriasis, rheumatoid arthritis, inflammatory bowel disease (IBD) and lupus nephritis, and is a factor of maintaining the integrity of epithelial tissues during inflammatory responses (9 and 12-15). IL-20 induces production of IL-6 and TNF in monocytes also stimulates proto-oncogene tyrosine kinase ROS in CD8<sup>+</sup>T cells. This IL targets, keratinocytes, endothelial cells, mesenchymal cells and a variety of tumor cells (16, 17).

This IL highly expresses on hepatocytes of damaged liver tissues in patients with fibrosis, cirrhosis and liver cancer. Single nucleotide polymorphisms in the genes of cytokines and their receptors genes can affect their performance through changing in gene expression, mRNA stability and protein structure (2, 17).

In several studies to date have been trying to determine association between nucleotide changes in different genes with certain diseases and their impact on disease progression and response to treatment (2, 18-20).

The importance and correlation of genetic changes and in particular single nucleotide polymorphism in genes that involved in immune response and cytokine genes with different diseases, including cancers (21,22) inflammatory diseases (23) and viral diseases (24-26) Have been evaluated and new studies are in progress to determine the effect of host genetic factors on the pathogenesis and persistence of hepatitis B infection and to develop new drugs and diagnostic and therapeutic procedures (27).

The results of several studies proved that single nucleotide polymorphisms in genes could affect the susceptibility to chronic infection (29,28) or clearing the hepatitis B virus from the body by the immune response (30,31) or progression of disease (32). So checking on the different polymorphisms in various

genes and their association with disease has become more valuable. The aim of this study was to evaluate the relationship and the role of single nucleotide polymorphism rs1400986 C/T of IL-20 gene in susceptibility to chronic hepatitis B infection.

## Methods

This study was cross-sectional and case-control study. After approval by the Digestive Disease Research Center of Shahid Beheshti University of Medical Sciences, a total of 296 healthy volunteers and patients with chronic hepatitis B among patients referred to Taleghani Hospital in Tehran during the years 1392 to 1394 were selected for this study, including 150 chronic hepatitis B patients with positive HBsAg ELISA and AntiHBc-Ab (ELISA kit bioprobes srl Diapro diagnostics, Italy) for more than 6 months (33), and blood samples were also collected as control group from 146 individuals who were healthy (serum HBsAg negative and AntiHBc Ab negative).

This project approved by the Digestive Disease Research Center of Shahid Beheshti University of Medical Sciences and has approved project code (code 719) and written consent was received from all participants. Genomic DNA from 5 ml blood in EDTA-containing glass collected and extracted through the standard method of saturated salt was according to the instructions of Miller and colleagues (34). IL-20 gene rs1400986 polymorphism were selected from information contained in GenBank and genotyped by PCR-RFLP method.

In summary, by using software Gene Runner version 4 primers were designed for amplification of a region of the gene polymorphisms. PCR reaction mixture with a final volume of 25 ml, containing 2.5 ml of 10X PCR Buffer with Mg<sup>2+</sup>, 0.5 mM dNTP, 10 pmol of each specific primers and 1.5 unit Taq polymerase enzyme was ready. Forward primer sequences 5'CTGGCAGTAGGCTTGTTATGAAATC3' and reverse primer 5'CCACGACCTGTGCCACCAA3' region of the genome that contains the position of the

polymorphism (C/T) 1807 to replicate-out. PCR reaction by adding 100 ng of DNA template to the reaction mixture and heat cycles were applied as follows:

Denaturation at 94 °C for 10 min, with 35 cycles of 94 °C for 45 seconds, connecting at a temperature of 61 °C for 40 seconds and elongation at 72 °C for 45 seconds, followed by a long final at 72 °C for 10 minutes.

PCR product by restriction enzyme Eco130 I (StyI) was digested with an enzyme recognition sites for 5'C ▼ CWWGG3 ' and TT genotype is not recognized by the enzyme and were not cut, and thus made piece of 437 nucleotides, but CC cutting genotype and caused two pieces of 278 and 179 bp and the CT heterozygous genotype created 3 bands in sizes 437, 278 and 159 bp on 3% agarose gel. Significant differences obtained in the genotype and allele frequencies between the two groups by chi-square test (2χ), respectively.

For data analysis, SPSS version 22 was used. P value less than 0.05 was considered significant. Odds Ratio was calculated at 95%.

## Results

C to T nucleotide substitution in the 2 groups of patients and controls were observed with low frequency (in patients (32%) 48 people CT and (4%) 6 people TT, (28.1%) 41 people CT and (2.7%) 4 people TT in the control group).

However, no significant differences in allele and genotype distribution between the 2 groups (respectively p=0.549 and p=0.599). TT homozygous genotype with less frequently in patients (4% vs. 2.7%) and thus a higher frequency of CC homozygous genotype was observed in the control group (69.2% vs. 64%).

96% of patients were C allele carriers while in the control group the 97.3% were C allele carriers which are provided in table 1. CT heterozygote genotype in patients with chronic hepatitis B had the dominant genotype (32% vs 28.1%, p=0.416).

**Table 1. Genotype and allele frequency of rs1400986 polymorphism in the IL-20 gene of patients with chronic hepatitis B compared with healthy control**

| Genotype | Chronic Hepatitis B<br>N=150 (%) | Healthy control<br>N=146 (%) | P-value | OR (CI-95%)        |
|----------|----------------------------------|------------------------------|---------|--------------------|
| CC       | 96(64)                           | 101(69.2)                    | -       | -                  |
| CT       | 48(32)                           | 41(28.1)                     | 0.416   | 0.812(0.492-1.341) |
| TT       | 6(4)                             | 4(2.7)                       | 0.490   | 0.634(0.173-2.315) |
| Allele   |                                  |                              |         |                    |
| C        | 144(96)                          | 142(97.3)                    | -       | -                  |
| T        | 6(4)                             | 4(2.7)                       | 0.551   | 0.676(0.187-2.447) |

## Discussion

In this study rs1400986 genotype and allele polymorphism frequency of IL-20 gene in patients with chronic hepatitis B compared with healthy control group and no significant differences were observed. IL-20 is known as an adjustment factor of the inflammatory response. During infection, this interleukin acted on epithelial cells and improves cohesion of tissues and heals the wounds; hence it can be used as a potential marker (9).

Chiu et al (2014) to prove the therapeutic role of IL-20 in the treatment of liver fibrosis, tested its effect on liver injury induced by carbon tetrachloride in a mouse model. Based on the results, the IL-20 in mice with liver injury were significantly higher (16). Although few studies of the IL-20 gene single nucleotide polymorphism has been released however, numerous articles have been reported statistically significant relationship between polymorphisms of this gene and some inflammatory diseases (35-39).

Fife et al (2006) showed an association between rs1400986 single nucleotide polymorphism in IL-20 gene with juvenile idiopathic and also found a significant association between the disease and the haplotype IL10 rs1800896 A / IL20 rs1400986 T (40). In another study, rs1400986 polymorphisms of interleukin-20 gene by clearing hepatitis B infection in populations of European Americans was read. Based on the results of that study people who have cleared the virus from the body without treatment, are more likely to have CC genotype at polymorphism position (17). On the Iranian population Arbabi and co-workers

(2013), reported another single nucleotide polymorphisms of interleukin-20 gene (rs1518108) associated with hepatitis C infection (35).

Traks et al study (2008) showed of IL-20 and IL-24 haplotypes associated with major depressive disorder and said they are involved in the pathogenesis of the disease (41). Another study worked on the relationship between IL-20 gene Polymorphisms and psoriasis and excessive proliferation of keratinocytes (13 and 42-47). The effect of IL-20 gene polymorphism and chronic hepatitis B, IL-20 gene single nucleotide polymorphism rs1400986 in this case-control study studied.

The results showed that this single nucleotide polymorphisms statistically is not associated with chronic Hepatitis B in the study population. Despite significant differences in frequencies of genotypes and alleles of the patient and control groups was not observed. A possible explanation for this result could be probed in the difference in IL-20 gene variations and heterogeneity population genetics. Limited data population, luck factor and the difference in analysis parameters can be considered as other reasons to justify such ambivalence.

The importance of studies such as these, is the practical implications of their. It is suggested that these studies, which are examined as variations of the genes of the immune system for communicating with infectious diseases or inflammatory diseases or are considered as potential biomarkers for prognosis of the disease, are completed with other similar studies on gene expression or proteomics. To investigate the

relationship between IL-20 gene single nucleotide polymorphism with multifactorial diseases such as hepatitis B haplotype analysis is also recommended. Prediction of Chronic hepatitis B potential can lead new therapeutic strategies or "personal care", which can provide a specific treatment for each person. Our results indicate that there is no association between rs1400986 polymorphisms of interleukin-20 gene with chronic hepatitis B and this polymorphism cannot be

considered as a determining factor in the chronic disease in the study population.

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