

Analysis of Association between IL-17 gene rs2275913 Single Nucleotide Polymorphism and Chronic Hepatitis B Infection

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ABSTRACT

BACKGROUND AND OBJECTIVE: Hepatitis B disease is one of the main causes of inflammation and liver damage that can lead to chronic hepatitis B virus infection. Single nucleotide polymorphism in the cytokines gene can affect the host immune response. Interleukin 17 produced by Thelper17 cells has been shown to play a role in immune function in infectious and inflammatory diseases. This study was conducted to investigate the association between polymorphism in IL-17 gene (rs2275913) and chronic hepatitis B infection.

METHODS: This case-control study was performed on 130 chronic patients as a case group and 130 healthy individuals as control. Patients with positive result of ELISA test for HBsAg and Anti-HBc Ab and control subjects with negative result of this test were enrolled. PCR-RFLP was used to genotype extracted DNA from blood samples.

FINDINGS: The genotype frequencies of rs2275913 did not show significant difference between patients and control groups. Distribution of genotypes in patients were, 40.8% GG, 41.5% AG, 17.7% AA and in control group were, 42.3% GG, 45.4% AG, 12.3% AA (p=0.469).

CONCLUSION: The results of study showed no relation between IL-17 gene polymorphism rs2275913 and chronic HBV.

KEY WORDS: Hepatitis B virus, Interleukin-17, Single Nucleotide Polymorphism.

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Introduction

Hepatitis B virus infection is one of the most important infectious diseases in the gastrointestinal tract. Hepatitis B infection was more common in the past. Today, 350 million people in the world are still infected by HBV (1).

The clinical status of HBV infection is affected by many viruses and host factors, including HBV genotype, infection pathway, age at infection and sex. HBV infection in adults is usually acutely self-limiting and in 90% to 95% of cases, the infection is likely to be eradicated. Hepatitis B infection in liver cells is non-cytopathic, and liver damage is associated with chronic hepatitis B infection, which is the result of the continuation of the host immune system to remove the virus from the liver in the process of the disease (2). Genetic differences in the genes associated with inflammation, especially cytokines, appear to play a role in the chronic process of hepatitis B disease. Recent studies have been conducted on the role of cytokine genes involved in inflammation such as IL-1, IL-2, IL-4, IL-6, IL-18, IFN, and TNF- α (3).

IL-17 is a family of cytokines that respond to chronic and acute inflammation. So far, 6 members of this family have been identified based on the similarity of the amino acid sequences. IL-17A cytokine has been studied more in this family and has the most similarity to IL-17F in its protein sequence.

IL-17A is a pro-inflammatory cytokine that plays a key role in the host's defense against microbial infections and contributes to various inflammatory conditions such as autoimmune diseases, metabolic disorders and cancer. (4) The location of rs2275913 in the IL-17 gene is in the promoter region of the gene, which is why the polymorphism in this region can affect the expression of this gene (5). IL-17 is part of the cytokines that (are not made by Thelper1 and Thelper2), are made by the T cells producing IL-17 called Thelper17 (Th17).

Th17 secretes pro-inflammatory cytokines such as IL-17 and IL-22 which have a close relationship with antimicrobial immunity and inflammation (6). Most immunological studies conducted on this group of T cells have been studied their function in removing a specific group of infectious microorganisms and their role in inducing inflammation and molecular reactions that contribute to differentiation (7). Much has been

reported in recent years in relation to the functions of IL-17A in cell biology and its association with various diseases (8-10). Some studies have shown that chronic inflammation can be the cornerstone for cancer. Th17 cells, due to the role they play in the development of inflammation, in particular chronic inflammation, can be associated with the advent and development of cancer with IL-17 (11).

In an analytical study from several studies in China has shown that polymorphism in IL-17A gene rs2275913 is associated with an increased risk of cancer (12). It has been reported in studies that the percentage of Th17 cells in peripheral blood increases significantly in patients with chronic hepatitis B infection and is associated with increased hepatic injury in these patients. IL-17 can be widely involved in the pathogenesis of chronic liver disease and antiviral immunity (13).

In this study, the relationship between single-nucleotide polymorphism rs2275913 in the IL-17 gene and chronic hepatitis B infection was studied, according to studies that investigated the relationship between polymorphism in different cytokines and hepatitis B disease.

Methods

Population study: This case-control study was approved by the ethics committee of Shahid Beheshti University of Medical Sciences with the code 1396IR.SBMU.RIGLD.REC and after obtaining written consent from the participants, the study was conducted on 130 healthy volunteers and 130 patients with hepatitis B referred to Taleghani Hospital in Tehran during the years 2013 to 2016. Patients in the case group were included in the study based on ELISA tests of HbsAg and Anti-HBc Ab (14).

ELISA tests with the kit of Diapro Company (Italy) were carried out by the personnel of the Virus Institute of Gastrointestinal and Liver Diseases Research Institute of Shahid Beheshti University of Medical Sciences. From each patient and control, 5 ml of venous blood was taken in EDTA-containing tubes and the salting out method was used to extract genomic DNA.

Determination of the genotype: A specific pair of primers was designed to amplify the desired region of

the IL-17 by the PCR method. The primer design was based on Gene Runner and OLIGO7 software. This primer pair replicates a 425 bp fragment in the promoter of the gene (Table 1). To check the specificity and not to connect to other parts of the genome, the BLAST software of NCBI site was used. PCR was performed on Thermocycler machine (Eppendorf). In preparation of a PCR mixture for reaction in a final volume of 25 μ l, 100 ng of extracted DNA, 0.5 μ l of $MgCl_2$, 2.5 μ l of buffer, 0.5 μ l of dNTP mixture and 5 Pico mole from each primer and 2 units of Taq polymerase enzyme were used.

In order to prepare a PCR mixture, the products of Yekta Tajhiz Azma Company in Iran were used. The PCR program started in 10 minutes of initial denaturation at 95 °C, then continued with 45 seconds denaturation at 95 °C and 45 seconds at 63 °C for primers annealing and 45 seconds at 72 °C for amplification (35 cycles progressed) and completed at the end of 10 minutes at 72 °C for final amplification. The RFLP method was used to determine the genotype in rs2275913 polymorphism.

In this study, the XagI restriction enzyme was used for cutting the fragment for 16 hours at 37 °C. To view the PCR and RFLP products electrophoresis on 1% and 3% agarose gel was done. The genotype of the subjects was determined based on the separation of DNA fragments on the gel. To confirm the results of RFLP, 5% of samples were determined by direct sequencing method. To analyze the results of the sequence, Bio Edit software was used.

Statistical analysis: Analytical analysis of quantitative data (age) were analyzed by t-test, and qualitative data (genotype and gender) were analyzed by Chi-square test, using SPSS software. Due to the limited number of samples, there was no possibility of adjustment of age and sex between the control and patient groups. Therefore, logistic regression test was used to eliminate age and gender factors and $p < 0.05$ was considered significant.

Results

In this study, 130 patients with hepatitis B were studied. Of total, 72 participants were men (55.38%) and 58 participants (44.62%) were women with an average age of 42.6 ± 15.35 years old. In the 130 cases, 46 men (35.38%) and 84 women (64.62%) with an average age of 48.07 ± 15.35 years participated. Determination of genotype conducted according to cutting pattern of XagI enzyme. There is no cutting site for the enzyme in the AA genotype.

The AG genotype is composed of 425, 317, and 108 base pairs and in the GG genotype; two pieces of 317 and 108 of base pairs are created. The result of RFLP on agarose gel is considerable in Fig. Direct sequencing was performed by Gen Fanavaran Company for 5% of the samples and confirmed the findings obtained by the RFLP method for these specimens (Fig 2). There was no significant difference in the distribution of genotypes in the two groups of patients and control (Table 2) ($p=0.469$).

Table1. Characteristics of primers

Primer direction	sequences	GC count	Anneling temperature
Forward	5'-TTGACCCATAGCATAGCAGC-3'	50	53.37
Reverse	5'-CTCCATAGTCAGAACCCAGC-3'	55	53.04

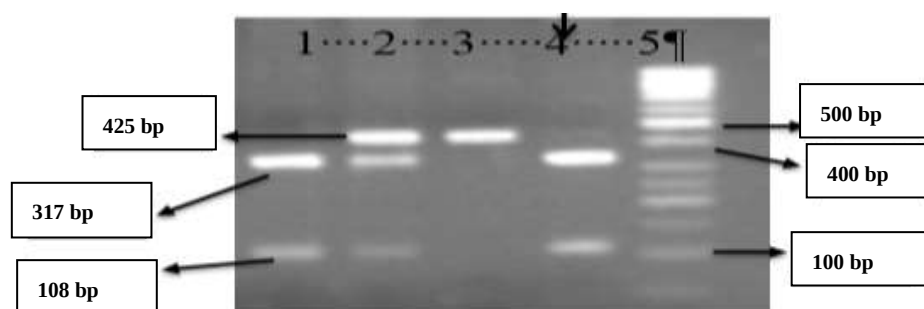


Figure 1. Sample (1, 4) for genotype AA. Sample (2) for genotype AG. Sample (3) for genotype GG. and (5) Marker with a size of 50 base pairs

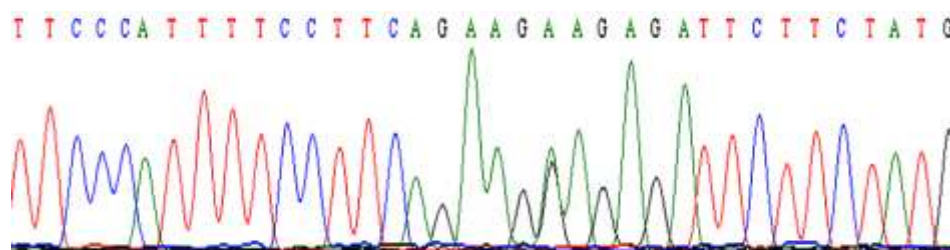


Figure 2. One sample of direct sequencing results on the PCR product's HBV patients is the AG genotype. The site of single-nucleotide polymorphism rs2275913 is indicated in the diagram with a dark arrow. The two pick A (green) and G (black) are interspersed and indicates the heterozygote genotype

Table2. Comparison of frequency and distribution of genotypes in both patient and control groups

Varialbe Groups	GG	AG	AA
Patient	53(40.8)	54(41.5)	23(17.7)
Control	55(42.3)	59(45.4)	16(12.3)
Total	108(41.5)	113(43.5)	39(15)

Discussion

In this study, the association between polymorphism (rs2275913) in IL-17A was observed in both the case and control groups. Various studies have shown the role of IL-17 in the pathogenesis of chronic liver disease and antiviral immunity.

These studies show evidence of a relation between the IL-17 activity pathway and immune mediators in liver injuries (15). It was also shown that the frequency of Th17 cells that produce IL-17 is increased in patients with chronic HBV. (16).

IL-17 and other Th17-related cytokines exacerbate liver disease. These results indicate that Th17 cells do not only contribute to the induction of antiviral immunity in acute HBV infection but also play an important role in inflammatory responses to chronic HBV infection (17).

The study of the importance and effect of IL-17 gene polymorphism in several genetic and clinical studies in different populations has been carried out and the association of IL-17A polymorphism with various diseases has been identified. In a study in China, the association between polymorphism in the IL-17 gene and the risk of gastrointestinal cancer has been shown (18).

In another study in China, it was found that in patients with chronic HBV, specifically, the Th17 cells that produce IL-17 increased (19). Previous studies on

polymorphism in different genes and hepatitis B disease also have different results. A study on the association between polymorphism in IL-20 gene and the risk of chronic hepatitis B infection in Iranian patients and a lack of correlation between them was determined (20). In another study, there was a relationship between polymorphism in interferon-receptor-1 gene and chronic hepatitis B virus (21). In a study in China, it was found that IL-17A could be a candidate gene for showing a possible risk for liver cirrhosis due to the spread of chronic hepatitis B infection (22).

In another study, in China Ren and colleagues, a study of 1208 patients and controls showed that rs763780 and rs2275913 polymorphisms in the IL-17 gene were associated with hepatitis B infection (23). In another study in Iranian population, there was no significant relationship between IL-17F rs763780 gene polymorphism and chronic hepatitis B infection (24). Unlike Ren and colleagues (23) in China, the results of this study did not show a significant relationship between the genotypes studied in IL-17A rs 2275913 and chronic hepatitis B disease.

One of the reasons can be geographical and racial differences in the population. It is also possible to find different results by examining more patients and controls or other polymorphisms in the IL-17 gene.

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