Short Communication

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Lack of Genetic Association between Interleukin-18 Gene Polymorphism (rs1946518) and Chronic Hepatitis B Infection

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ABSTRACT

BACKGROUND AND OBJECTIVE: Interleukin 18 is a member of the cytokines that play an important role in the Th1-mediated immune response by inducing interferon-gamma activity in collaboration with Interleukin-12 (IL-18). Interleukin 12 and Interleukin 18 can play an important role in purifying viruses. Considering the importance of IL-18, this study was conducted to investigate the relationship between Interleukin-18 Gene polymorphism (-607 C/A: rs1946518) and the susceptibility to chronic hepatitis B infection.

METHODS: In this case-control study, the genomic DNA of 115 patients with chronic hepatitis B (with positive results of HBsAg and Anti-HBcAb serology testing) and 115 non-HBV-infected controls (negative results of HBsAg and Anti-HbcAb serology testing and no history of liver disease) was extracted by salting-out method and the genotype of single-nucleotide polymorphism (-607 C / A: rs1946518) was sequenced using PCR-RFLP method.

FINDING: The genotype frequency of TT, GT, and GG in patients was 40%, 49.6%, and 10.4% in patients, and 41.7%, 42.6%, and 15.7% in the control group, respectively. No significant difference was found between the patients group and the control group.

CONCLUSION: Based on the results of this study, there was no clear relationship between IL-18 polymorphism and the potential for chronic hepatitis B infection. Therefore, this polymorphism cannot be a potential factor for chronic hepatitis B.

KEY WORDS: *Interleukin 18, Chronic Hepatitis, Hepatitis B Virus, Single-Nucleotide Polymorphism.*

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Introduction

Interleukin 18 is a member of the cytokines that play an important role in the Th1-mediated immune response by inducing interferon-gamma activity in collaboration with Interleukin-12. Interleukin 12 and Interleukin 18 activate the inherent and acquired immunity, and their excessive production cause immune system impairment through activation of macrophages (1).

The interleukin 18 mRNA gene is expressed in a wide range of cells, including hepatic kupffer cells, macrophages, T cells, B cells, osteoblasts, keratinocytes, dendritic cells, astrocyte cells, and microglial cells. This cytokine has the same properties as interleukin 12, such as the stimulation of interferon-gamma, increasing the production and effect of natural killer cells, and stimulating and differentiating Th1 cells. Despite its functional similarity to interleukin 12, this cytokine is structurally similar to Interleukin 1, and the receptor system and its signaling pathway are similar to Interleukin 1 receptor (2). Interleukin 18 is produced in the form of inactive precursors and is converted to active form by caspase 1 (converting enzyme of Interleukin 1) ICE = IL 1B Converting Enzyme (3).

Interleukin 18 and interleukin 1 use different receptors, but have a common intracellular signaling system (4). Interleukin 18 binds to its receptor to perform its function. The Interleukin 18 receptor includes two α and β chains, both of which are required to initiate and transmit signaling. The α chain is used to connect the ligand and the β chain to send a signaling message (5).

The production of pro-inflammatory cytokines such as interleukin 1, interleukin 12, and interleukin 18 represents early events of the body's defense mechanism against pathogens, but excessive production itself is responsible for tissue injuries (6). Because interleukin 18 is a member of the inflammatory cytokine network, it can play an important role in the removal of infectious agents or the progression of inflammatory diseases toward becoming chronic. Recent findings indicate that the promoter region of the Interleukin gene regulates the expression of this gene (7).

Two polymorphisms have been proposed in the -137 G / C and -607 C/A positions for altering the activity of Interleukin 18 promoter (8). The production of cytokines is under the control of the genetic system, and their increased concentration indicates the activity of the cytokine pathways associated with inflammation or disease progression (9). One of the most important viral diseases in the world is hepatitis B infection, which is a major issue for global health. Despite the vaccination against it, hepatitis B infection is still widespread worldwide and is responsible for the death of many people (10).

About a third of the world's population is infected with hepatitis B and six per cent are chronic carriers and more than six hundred thousand people die each year due to the complications of this disease (11). Genetic diversity in host populations including single nucleotide polymorphisms in genes involved in the immune response, especially the cytokines genes, may have important implications for various diseases, and several studies have been conducted on chronic diseases (12, 13) and cancers (14, 15). The aim of this study is to evaluate the effect of interleukin 18 gene polymorphism (-607 C / A: rs1946518) on patients referred to Ayatollah Taleghani Hospital in Tehran who are susceptible to chronic hepatitis B infection.

Methods

After being approved by the ethics committee of Shahid Beheshti University of Medical Sciences with ethics code IR.SBMU.RIGLD.REC.94.145, and obtaining informed consent from patients, this case-control study was conducted among 115 patients and 115 healthy controls referred to Ayatollah Taleghani Hospital in Tehran. The sample size was selected according to similar articles (16, 17) and considering that this study is a pilot study. The control group had negative HBs Ag and Anti HBc Ab serological test results and did not have any history and symptoms of inflammatory liver disease, and in the case group patients had chronic hepatitis B and had positive HBs Ag and Anti HBc Ab for at least 6 months, their body was not able to clear them and was approved by ELISA (Diapro Diagnostics, Italy) (18).

In the case of dissatisfaction of people to participate in research, the history of infection with HIV, HCV or HDV, the patients were excluded. 4 ml of peripheral blood was collected from each person and EDTA anticoagulant was added to prevent blood clotting, and the salting out method was used for genomic DNA purification (19). Genomic DNA was stored in Tris-EDTA buffer and stored at 20 °C until genotyping was performed. For determination of genotype in individuals, the restriction fragment length polymorphism (PCR-RFLP) technique was used. The

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Gene Runner and Primer3 applications were used to design the primer, while the BLAST method, obtained from the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov), was used to validate the primer. The primer sequences are listed in Table 1. Two microliters genomic DNA was added to the reaction mixture, which contains 2.5 µl Taq DNA polymerase buffer, 0.75 µl magnesium chloride ($MgCl_2$), 0.3 µl Taq DNA polymerase enzyme, 0.5 µl dNTP and 0.5 µl of each primer, and distilled water was added to reach a final volume of 25 µl of the mixture in the microtube, and the mixture was placed in an automatic Thermal Cycler (Eppendorf, Germany) to carry out PCR reaction cycles.

The program used for PCR was as follows: First, the initial denaturation was performed for 5 minutes at 94 °C for separation of the two DNA strands, followed by 39 cycles, each cycle containing three temperature cycles of 94 °C for 45 seconds, 63 °C for 35 seconds (primer binding temperature), 72 °C for 45 seconds (to produce the complementary strand and elongate the DNA strand), and finally, 72 °C for 10 minutes for final replication of the DNA strand was applied. In order to detect PCR product by electrophoresis, 1% agarose gel (PeQ Lab, Germany) was prepared using TBE 1X buffer and Green Viewer staining with ultraviolet illuminator.

Then, the PCR product reacted with Tru1I (MseI) restriction enzyme (from Fermentas Biotechnology Company, which cuts the position of the target SNP) as follows: 10 µl PCR product was added to a mixture containing 2 µl Buffer Red and 0.3 µl Tru1I (MseI) restriction enzyme, and reached a final volume of 30 µl by distilled water and the mixture was incubated at 65 °C for 16 hours and the target enzyme was obtained by the NEBcutter program. The enzymatic digestion product was detected by electrophoresis on a 3% agarose gel prepared by TBE 1X buffer. To confirm the accuracy of the PCR-RFLP method, 10% of the samples were selected randomly and sequenced by direct sequencing method.

Data were analyzed by SPSS software version 19 and $p < 0.05$ was considered significant. T-test and Chi-square tests were used to examine the samples.

Table 1. Specifications of primers and restriction enzyme

Polymorphism	Primer sequence	Restriction enzyme	Allele phenotype
-1946518 C/A	F: 5- GATTTACTTTTCAGTGGAAGAGG -3 R: 5- AGT CTT TGC TAT CAT TCG AGG -3	Tru1I (MseI)	G: 196 bp+35 bp T: 98 bp+98 bp+35 bp

Results

The length of the fragment produced by the PCR is 231 bp, and after adjacent to the restriction enzyme, the cut of the PCR product is observed in three ways; if the cut is from the 196 and 35 locations, it is homozygote (GG), if the cut is from the 196, 98, 98, 35 locations, it is heterozygote (GT), and if the cut is from the 98, 98, 35 locations, it is homozygote (TT), shown in Figures 1 and 2.

Of all 230 people included in the study, 70 people (30.4%) were male (35 patients - 35 controls) and 160 people (69.6%) were female (80 patients - 80 controls). The age of people in the two groups was matched and the age range was between 18 to 70 years old and the mean age was 36.63±13.25 years. Genotype GT had the highest frequency (49.6%) in the patients and control groups (Table 2). Based on the results of the study, there was no significant difference between the subjects with chronic hepatitis B and healthy individuals in terms of Interleukin 18 genotypes. However, according to the results, the heterozygote GT genotype in patients with chronic hepatitis B (49.6% vs 42.6%, p = 0.49) and homozygous TT genotype in the control group (15.7% vs. 10.4%, p=0.39) were observed as dominant genotypes.

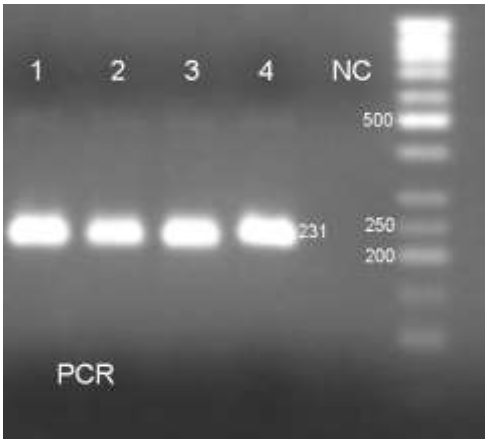


Figure 1. PCR product electrophoresis with a size of 231 bp (wells 1 to 4). The well 5 is negative control samples and well 6 is related to Ladder 50 bp (Fermentas).

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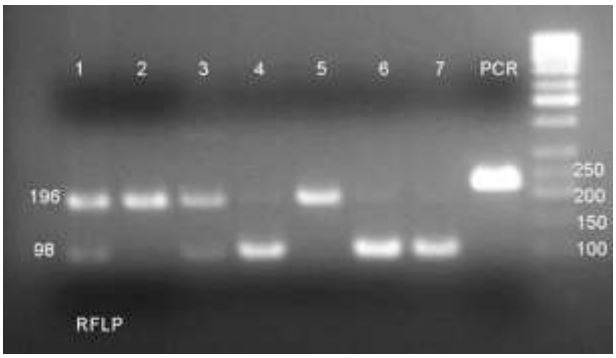


Figure 2. The result of electrophoresis of enzymatic digestion on 3% agarose gel. Fragments from RFLP enzymatic cut. As can be seen in the figure, wells 2 and 5 are homozygote (GG), wells 1 and 3 are heterozygote (GT), and wells 4, 6, and 7 are homozygote (TT). Well 8 contains PCR product and well 9 contains Ladder 50 bp.

Table 2. Frequency distribution of obtained genotypes

Genotype	Patient N(%)	Control N(%)	OR CI–95%)	P - value
GG	46(40)	48(41.7)	Reference group	
GT	57(49.6)	49(42.6)	0.82(0.47–1.4)	0.49
TT	12(10.4)	18(15.7)	1.4 (0.62–3.3)	0.39

Discussion

The present study showed that despite the importance of Interleukin 18 polymorphisms, there is no significant genetic association between rs1946518 polymorphism and chronic hepatitis B infection. The balance between Th1 and Th2 determines the immune responses involved in autoimmune and allergic diseases. In human diseases, the production of $CD4^+$ T cells plays a major role, and cytokines associated with Th1 and Th2 are involved in the stabilization of autoimmune disorders (20).

The host immune response to infections causes quick reaction from cytokines, which speeds up the mechanisms for the removal of the invasive organism, and when the risk is eliminated, cytokines stop and tissue damage is resolved. In contrast, imbalance in the production of cytokines can lead to progressive tissue damage (21). Increased levels of interleukin 18 have been associated with autoimmune diseases, and blocking interleukin 18 has been shown to have beneficial effects in improving autoimmune and inflammatory diseases.

These diseases include multiple sclerosis, myasthenia gravis, rheumatoid arthritis, lupus erythematosus, Crohn's disease, type 1 diabetes (22). The production of cytokines is under the control of the genetic system, and their increased concentration indicates the activity of the cytokine pathways associated with inflammation or disease progression (9). The gene encoding cytokines is one of the candidates among host genetic factors and polymorphism in a gene promoter can lead to the production of different levels of cytokines and a unique immune response (23). The polymorphisms in the Interleukin 18 gene have been associated with a number of diseases. In the study of Migita et al., investigation of the relationship between Interleukin 18 promoter polymorphism at positions 607 and 137 and the progression of liver disease in patients with chronic hepatitis B showed that AA genotype at position 607 and C allele at 137 in healthy subjects was significantly higher than chronic hepatitis B patients with progression of liver disease (24). In the study of Rosemary et al., the association between the Interleukin 18 gene polymorphism -607 C / A and the serum immunoglobulin E level as a risk factor for allergic retinitis was observed. AC genotype was associated with elevated levels of immunoglobulin E in the serum. This can indicate the effect of this polymorphism on the onset of the disease (25).

In a study by Nikiteas et al. in Greece, the association between heterozygous Interleukin 18 gene polymorphism -607 A / C and the susceptibility to colorectal cancer was found to be significantly higher in patients than in healthy subjects (26). In the study of Teixeira et al. in the United States, the role of interleukin 18 gene polymorphism -137 C / G: rs187238 and -607 C/A: rs1946518 in the susceptibility to hepatocellular carcinoma did not show significant difference in the frequency of the allele and genotype at position 607 in patients with hepatocellular carcinoma and healthy subjects, but analysis of position 137 showed that C allele was more common in people with hepatocellular carcinoma than in healthy subjects (27). The study of Vairaktaris et al in Germany investigated the correlation between the Interleukin 18 gene polymorphism -607 A/C and susceptibility to oral cancers, and there was no significant correlation between the two groups (28).

Considering the importance of polymorphisms in the susceptibility to diseases and their effects on the course of diseases, and given the differences in results in different populations and races, conducting studies on them are necessary. In Iran, no study has been

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conducted on this polymorphism and its relationship with chronic hepatitis B. To obtain comprehensive and measurable results, further studies are recommended with larger sample size and in other polymorphisms associated with this cytokine, and among those who have been able to completely remove the virus from the body during the acute phase of the disease. By examining the results of this study and statistical analysis, despite the relative increase of GT genotype in the patients, there was no significant difference between genotypic distribution in the patients and the genotypic distribution was similar between the two groups. There is no clear correlation between the studied polymorphism and the susceptibility of individuals to chronic hepatitis B disease, and the association of other polymorphisms involved in this cytokine is potentially susceptible to the disease.

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