RESEARCH ARTICLE

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Polymorphism Analysis of Interleukin-18 and Interleukin-37 Genes in Hepatitis B Infections with Different Outcomes: A Preliminary Report from an Iranian Population

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Abstract

Objective: Given the vital role of cytokines in influencing the outcomes of hepatitis B virus (HBV) infections, this study aimed to investigate the association between polymorphisms of interleukin (IL)-18 and IL-37 and the outcomes of HBV infection. **Methods:** In this study, we enrolled 300 subjects with chronic HBV infection, including those with cirrhosis/hepatocellular carcinoma (C/HCC), chronic active hepatitis B (CAH) infection, or asymptomatic carriers (AC), and 58 individuals whose infection was spontaneously cleared (SC). Genomic DNA was extracted, and IL-18/IL-37 genotyping was performed using PCR-RFLP and ARMS-PCR. **Results:** The frequency of genotypes and alleles of IL-18 single nucleotide polymorphisms (SNPs) at positions rs1946519, rs1946518, and rs187238 and IL-37 at position rs4241122 were not statistically different among the four studied groups (P>0.05). Furthermore, the frequency of different haplotypes was similar among the studied groups (P>0.05). **Conclusions:** Polymorphisms of IL-18 SNPs at positions rs1946519, rs1946518, and rs187238 and variation of IL-37 at position rs4241122 do not appear to influence the outcome of HBV infection.

Keywords: HBV- Polymorphism- IL-37- IL-18

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Introduction

The hepatitis B virus (HBV), a member of the Hepadnaviridae family, is a leading health threat that kills around 800,000 people annually (Ward et al., 2019). An acute HBV infection may follow a spontaneously self-limited pattern or proceed to chronic infection. There are different disease outcomes following chronic infection, ranging from being an asymptomatic carrier (AC) to having chronic active hepatitis B (CAH) and developing cirrhosis/hepatocellular carcinoma (C/HCC) (Hosseini et al., 2019; Liaw et al., 2009; Motavaf et al., 2014). In addition to infections, genetic variations in the tumor suppressor genes and oncogenes such as P53 and murine double minute 2 (MDM2) might increase genetic susceptibility to HCC (Hosen et al., 2021; Khazaei et al., 2018). In spite of the availability of an effective subunit vaccine, approximately 250 million people are still suffering from persistent HBV infection globally (Lavanchy et al., 2016). The interaction between immune response elements (e.g., cytokines) and viral factors can determine the clinical outcome of infection with HBV (Trépo et al., 2014). Therefore, single nucleotide polymorphisms (SNPs) in genes involved with immune responses are among the host factors that determine the disease's outcome (Gheshlaghi et al., 2021; Hirankarn et al., 2007; Zhou et al., 2006).

Interleukin-18 (IL-18), a potent pro-inflammatory member of the IL-1 superfamily (Barbier et al., 2019), is mainly produced by activated immune cells such as monocytes, macrophages, Kupffer cells, and immature dendritic cells (Lu et al., 2015). IL-18 has a pleiotropic role and participates in both innate and adaptive immune responses against viruses (Motavaf et al., 2014). It performs some activities related to viral clearance due to its synergistic effect with IL-12 in stimulating CD8+ cells, natural killer (NK) cells, B cells, dendritic cells, and macrophages to produce IFN-γ (Yasuda et al., 2019). The activated CD8+ cells induce apoptosis in the HBV-infected cells and produce IFN-γ and tumor necrosis factor-alpha (TNF-α), leading to intercellular inactivation of HBV (Hirankarn et al., 2007; Mahoney, 1999). Due to these multiple functions, IL-18 may either play an important role in HBV clearance (Hirankarn et al., 2007) or be associated

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with chronic viral hepatitis (McInnes et al., 2000).

The IL-18 gene is located on chromosome 11q22.2–q 22.3, which consists of six exons and five introns. There are three SNPs in the promoter region of the IL-18 gene: -656 G/T (rs1946519), -607 C/A (rs1946518), and -137 G/C (rs187238) (Bao et al., 2015). At positions -607 and -137, changes from A to C and C to G disrupt the cAMP-responsive element-binding protein (CREB) and H4TF1 binding site, respectively (Giedraitis et al., 2001; Yasuda et al., 2019).

The association of SNPs of the IL-18 gene and various diseases has been studied. Two SNPs at position -137 and -607 are associated with various diseases such as chronic HBV infection (Motavaf et al., 2014), systemic lupus erythematosus (SLE) (Song et al., 2013), diabetes (Mojtahedi et al., 2006), and rheumatoid arthritis due to the change in IL-18 expression level (Gracie et al., 2005).

The IL-37 gene is located on the short arm of chromosome 2 (2q14.1). It can bind to and act as a ligand for the IL-18 receptor (IL-18R1/IL-1Rrp). This cytokine also binds to the IL-18 binding protein (IL-18BP), an inhibitory binding protein of IL-18, and subsequently forms a complex with the IL-18 receptor's beta subunit, thereby inhibiting IL-18 function. Thus, the polymorphisms related to this gene may also alter the physiological processes related to IL-18 (Lu et al., 2015). Also, another role of this cytokine is to ameliorate the inflammatory response through negative feedback mechanisms (Ding et al., 2017). Sakai et al. showed that IL-37 could reduce inflammatory liver injury via effects on hepatocytes and non-parenchymal cells (Sakai et al., 2012). Moreover, Zhao et al. stated that decreased IL-37 production is associated with HCC progression (Zhao et al., 2014). According to several studies, certain genetic variations within the IL-37 gene are associated with several diseases such as tuberculosis (Allam et al., 2016; Liu et al., 2017), coronary artery disease (CAD) (Yin et al., 2017), and autoimmune-based thyroid diseases (Yan et al., 2015).

Given the role of cytokines in different outcomes of HBV infection, this study aimed to investigate the association of IL-18 [-656 G/T (rs1946519), -607 C/A (rs1946518), and -137 G/C (rs187238)] and IL-37 (rs4241122) SNPs with HBV infection outcomes.

Materials and Methods

Study population

From July 2016 to Feb 2020, a total of 358 subjects were recruited consecutively from the Organ Transplantation Research Center of Abu-Ali Sina Hospital in cooperation with the Gastroenterohepatology Research Center at Nemazee Hospital, both affiliated to Shiraz University of Medical Sciences, Shiraz, Iran. The status of HBV infection in the subjects was determined by gastroenterologists and confirmed based on biochemical and virological markers as well as imaging records according to the patients' medical files. Those who had spontaneously cleared (SC) HBV infection were also selected from the national Kavar cohort study (Fattahi et al., 2014). The recency of HBV infection was also checked through ELISA assays for HBsAg and HBcAb. Participants were categorized into four groups: group 1: 58 subjects with SC infection; group 2: 100 ACs; group 3: 100 patients with CAH; and group 4: 100 patients with C/HCC. The study was approved by the local ethics committee of Shiraz University of Medical Sciences (IR.SUMS.REC.1399.194). Furthermore, written informed consent was obtained from each participant.

Evaluation of HBs antigen and anti-HBc antibody

HBsAg and HBcAb of sera from the SC group were assayed using commercial ELISA kits(Dia.Pro.Milano, Italy) according to manufacturers' instructions,.

DNA extraction and molecular genetic analysis

Peripheral venous blood (5 mL) Was taken from the subjects was used to extract DNA via the salting-out method described in the literature (Sarvari et al., 2018). An ultraviolet spectrophotometer was used to check the quantity and quality of the DNA.

Single nucleotide polymorphisms (SNPs) at position -656 of IL-18 were genotyped by an in-house polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay (Figure 1). At positions -607 and -137 of IL-18 and position rs4241122 of IL-37, SNPs were checked by the amplification of specific alleles (ARMS) PCR method (Figiure 1). For RFLP-PCR, 0.6 μL (300 ng) of extracted DNA was amplified in each 20.8 μL reaction reagent containing 9 μL of distilled water, 10 μL of master mix (2x) (Parstous, Tehran, Iran), and 0.5 μL (10 pM) of each primer (Metabion, Germany). For ARMS-PCR, 300 ng extracted DNA was amplified in each 15.7 μL reaction including 7 μL of distilled water, 7 μL of master mix (2x) (Parstous, Tehran, Iran), and 0.3 μL (10 pM) of each primer (Metabion, Germany). As indicated in Table 1, PCR temperature cycling programs were specific for each SNP. The reaction conditions including primer sequences and product size for both PCR-RFLP and PCR-ARMS methods are summarized in Table 1.

Statistical analysis

Data from medical records were collected and then analyzed by SPSS Statistics for Windows (version 25, IBM Corp., Armonk, NY, USA). The chi-squared test was used for analyzing the frequency of alleles and genotypes among the study groups. Hardy–Weinberg equilibrium, as well as haplotype analysis, was performed using the Arlequin software package. P-values less than 0.05% were considered significant.

Results

Characteristics of the study population

The mean age of the participants was 47.61 ± 13.41 years. Out of 358 subjects, 244 were male and 114 were female. A total of 58 individuals were in the SC group, while the remaining three groups (AC, CAH, and C/HCC) each had 100 patients. The frequency of males and females was 44.8% and 55.2% in the SC group, 71% and 29% in the AC group, 70% and 30% in the CAH group, and 77% and 23% in the C/HCC group, respectively. There was no significant difference between

Table 1. *IL-18* and *IL-37* Gene-Specific Primer Sequences, Reaction Conditions, Methods and Product Size

Gene	SNPs	Primer	Primer sequence	The cycling condition (40 cycles)	Method	Product size
IL-18	rs1946519	F	5′- AGGTCAGTCTTTGCTATCATTCCAGG-3′	95°C,40s	PCR-RFLP	G:96 and 24-bp
		R	5′-TGCAACAGAAAGTAAGCTTGCGGAGAGG-3′	61°C,45s	(MwoI)	T:120-bp
				72°C,40s		
IL-18	rs1946518	FI	5′ -GTTGCAGAAAGTGTAAAAATTATTAC -3′	95°C,45s	ASO-PCR	Main band: 196-bp
		FII	5′ -GTTGCAGAAAGTGTAAAAATTATTAA -3′	55.5°C,40s		Control band: 301-bp
		R	5′ -TAACCTCATTCAGGACTTCC -3′	72°C,40s		
		IC	5′ -CTTTGCTATCATTCCAGGAA -3′			
IL-18	rs187238	F	5′ -CCCCAACTTTTACGGAAGAAAAG -3′	95°C,40s	ASO-PCR	Main band: 261-bp
		FII	5′ - CCCCAACTTTTACGGAAGAAAAC -3	63.5°C,40s		Control band: 446-bp
		R	5′ -AGG-AGGGCAAAATGCACTGG-3′	72°C,40s		
		IC	5′ -CCAATAGGACTGATTATTCCGCA-3′			
IL-37	rs4241122	FI	5′ -CAGGCTCTAGACTGACTCCA-3′	95°C,45s	ASO-PCR	Main band: 355-bp
		FII	5′- CAGGCTCTAGACTGACTCCG-3′	61°C, 45s		Control band: 110-bp
		R	5′ -TCAAACTATCAACATCAAGGCACA-3′	72°C,40s		
		IC	5′-ACACAACTGTGTTCACTAGC-3′			
			5-′CAACTTCATCCACGTTCACC-3′			

F, forward; R, reveres; IC, internal control; RE, restriction enzyme; ASO-PCR, Allele-Specific Oligonucleotide PCR; PCR-RFLP, PCR Restriction Fragment Length Polymorphism

the groups in gender distribution (P = 0.556). Also, the mean age of the participants of the above groups was 55.60 ± 16.71, 43.69 ± 13.11, 45.26 ± 12.48, 50.88 ± 11.21 years, respectively, representing no significant differences (P = 0.435).

ELISA results

The results of ELISA assay showed that all 58 sera samples of the SC group were negative for HBsAg and positive for HBcAb, confirming the spontaneous clearance of HBV infection.

IL-18 and IL-37 gene polymorphisms in different groups

As shown in Table 2, the four groups of disease phases (SC, AC, CAH, and C/HCC) showed no differences in the frequencies of both genotypes and alleles of IL-18 polymorphisms (-607 C/A, -137 G/C, and -656 G/T). Also, a comparison of the frequency of the genotypes and

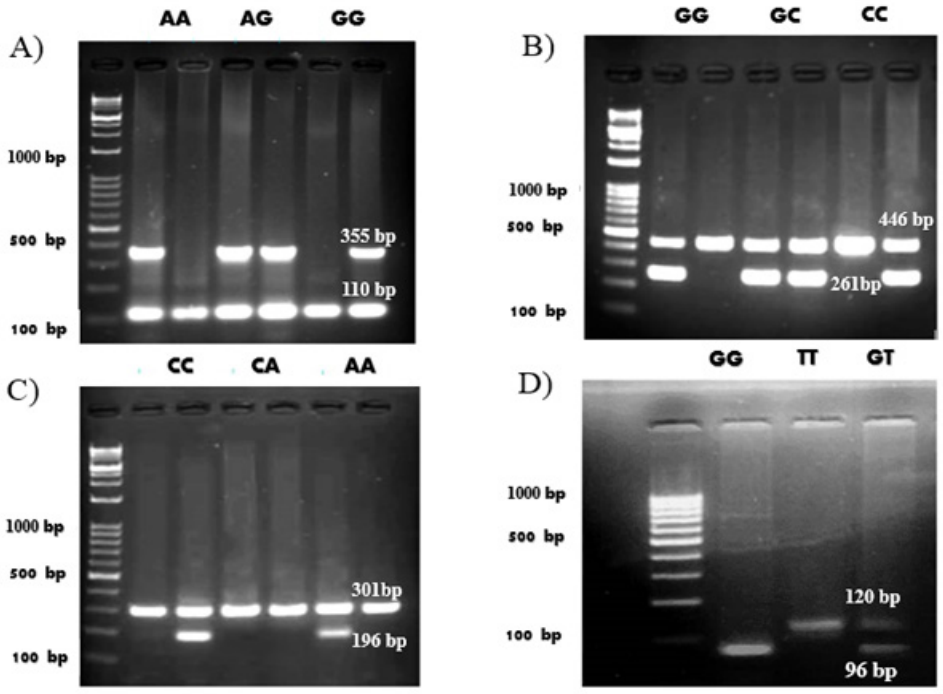

Figure 1. Results of PCR-ARMS and PCR-RFLP Gel Electrophoresis of IL-37 and IL-18 Polymorphisms. A) PCR-ARMS gel electrophoresis of IL-37 (-1122 A/G); B) PCR-ARMS gel electrophoresis of IL-18 (-137G/C); C) PCR-ARMS gel electrophoresis of IL-18 (-607C/A); D) PCR-RFLP gel electrophoresis of IL-18 (-656G/T).

Table 2. The Frequency of IL18 and IL37 Genotypes/Alleles in the 4 Studies Groups. P-value < 0.05 was considered to be statistically significant

Group	Spontaneous Clearance	Healthy carriers	Chronic active	Cirrhosis/HCC	P value
IL18 (CA-607)					
Allele A	41 (35.3)	66 (33.0)	73 (36.5)	80 (40.0)	0.537
Allele C	75 (64.7)	134 (67.0)	127 (63.5)	120 (60.0)	
CA	35 (15.0)	64 (27.4)	67 (28.6)	68 (29.1)	0.432
CC	20 (18.0)	35 (31.5)	30 (27.0)	26 (23.4)	
AA	3 (23.1)	1 (7.7)	3 (23.1)	6 (46.2)	
IL18 (GC-137)					
Allele G	80 (81.8)	151 (75.5)	150 (75.0)	138 (69.0)	0.323
Allele C	36 (18.2)	49 (24.5)	50 (25.0)	62 (31.0)	
GC	32 (18.9)	42 (26.6)	40 (23.7)	52 (30.8)	0.366
GG	24 (13.7)	53 (30.3)	55 (31.4)	43 (24.6)	
CC	2 (14.3)	2 (14.3)	5 (35.7)	5 (35.7)	
IL18 (GT-656)					
Allele T	43 (37.1)	75 (37.5)	84 (42.0)	93 (46.5)	0.229
Allele G	73 (62.9)	125 (62.5)	116 (58.0)	107 (53.5)	
GT	4 (7.8)	12 (23.5)	16 (31.4)	19 (37.3)	0.324
GG	19 (16.7)	37 (32.5)	32 (28.1)	26 (22.8)	
TT	35 (18.1)	51 (26.4)	52 (26.9)	55 (28.5)	
IL37 (AG-1122)					
Allele A	72 (62.1)	134 (67.0)	142 (71.0)	134 (67.0)	0.43
Allele G	44 (37.9)	66 (33.0)	58 (29.0)	66 (33.0)	
AG	28 (14.6)	58 (30.2)	54 (28.1)	52 (27.1)	0.093
AA	22 (15.2)	38 (26.2)	44 (30.3)	41 (28.3)	
GG	8 (38.1)	4 (19.0)	2 (9.5)	7 (33.3)	

alleles of IL-37 polymorphism (-1122 A/G) between the four groups showed no significant differences (Table 1).

Haplotype analysis of IL-18 gene polymorphisms and SC, AC, CAH, and C/HCC risk

Haplotype-based analysis has greater power than SNP genotyping. Therefore, haplotype analysis of IL-18 SNPs was performed using Arlequin version 3.5 software. We analyzed haplotype frequencies in the four study groups. After investigation of the observed genotypes, a total of eight haplotypes were attained, including GCG, TAG, TAC, TCC, TCG, GCC, GAC, and GAG. The most frequent haplotypes were, in order, GCG, TAC, and TAG. Haplotype analysis revealed no significant difference in the frequencies of different haplotypes between the studied groups ($P > 0.05$).

Discussion

The outcome of HBV infection is greatly influenced by the strength of the immune response (Trépo et al., 2014). Cytokines play a major role in establishing coordination among different parts of the immune system. Polymorphisms in cytokine genes' regulatory or coding regions might influence their function and thereby affect the outcome of infection, particularly in viral infections (Sarvari et al., 2018; Sarvari et al., 2017).

In the present study, the relationship of three polymorphisms of the IL-18 gene (-607 C/A, -137 G/C, and -656 G/T) and one polymorphism of the IL-37 gene (-1122 A/G) with four different categories of HBV infection was investigated. The results showed no significant relationship between the outcome of HBV infection and the frequencies of the genotypes and alleles of polymorphisms at the -607 C/A, -137 G/C and -656 G/T loci of the IL-18 gene. In the literature, investigations on this controversial issue are limited in number. In a study by Lu et al., no significant relationship was observed between -607 C/A polymorphism and HBV infection outcome in the Chinese population. However, contrary to our findings, that study showed a significant relationship between the incidence of -137 G/C polymorphism and the risk of HBV-dependent liver disease (Lu et al., 2015).

In a study conducted by Haas et al., 757 patients with hepatitis and 791 healthy individuals were compared for IL-18 polymorphisms, revealing no significant difference between the groups in terms of the frequency of -607 C/A and -137 G/C. Interestingly, the occurrence of the -607 C/A polymorphism was significantly related to the response rate of the patients (Haas et al., 2009). In a study carried out by Hirankarn et al., the relationship between IL-18 polymorphisms and the incidence of chronic hepatitis was investigated. First, it was shown that the clearance rate in patients with AA genotype of

-607 C/A polymorphism was much higher than other genotypes. However, there was no relationship between the incidence of the -137 G/C polymorphism and the rate of recovery from the disease. Also, the homozygosity of haplotype -607A / -137G was higher in patients with chronic hepatitis than other haplotypes (Hirankarn et al., 2007). A meta-analysis declared no significant association between -607 C/A and -137 G/C polymorphisms and the risk of hepatocellular carcinoma (HCC) in the Asian and American races (Zhu et al., 2016). However, another meta-analysis indicated a significant relationship between the -137 G/C polymorphism and the risk of chronic hepatitis, though the association between the -607 C/A polymorphism and chronic hepatitis was still insignificant. Little research has been done on the relationship between the third polymorphism, -656 G/T, and the risk of hepatic diseases (Yang et al., 2015). In a 2013 done study by Yue et al. in the Chinese community, the occurrence of two alleles, -137C and +105C, was associated with a reduced risk of hepatitis C. Still, no association was found between the -656 G/T polymorphism and hepatitis, which is similar to our findings (Yue et al., 2013). Therefore, it can be said that regarding the relationship between different IL-18 polymorphisms and the occurrence of different types and intensities of hepatitis, such a relationship is completely dependent on race and demographic characteristics. None of these polymorphisms appear to be associated with the risk of hepatitis, cirrhosis or carcinoma.

In the present study, the frequency of genotypes and alleles of the -1122 A/G polymorphism of the IL-37 gene in the four study groups was statistically similar. To the best of our knowledge, no similar study has been done on the relationship between polymorphisms of this gene and HBV infection outcome; thus, we could not compare our results with other observations. It has been reported that IL-37 serum levels are much higher in patients with chronic hepatitis B than in healthy individuals (Yue et al., 2013). Moreover, in a study conducted by Zhao et al., the decreased expression level of IL-37 in patients with HCC was confirmed, and there was an inverse relationship between the tumor size and gene expression. However, no study was previously performed on the relationship between -1122 A/G polymorphism and liver diseases (Zhao et al., 2014).

In conclusion, this study showed no significant relationship between the frequencies of three polymorphisms of the IL-18 gene (-607 C/A, -137 G/C, and -656 G/T) and HBV infection outcomes. Moreover, the results showed that polymorphisms at the -1122 A/G locus of the IL-37 gene did not influence the outcome. Therefore, we conclude that the mentioned polymorphisms are not valuable biomarkers in predicting liver disease progression in patients with HBV infection.

Author Contribution Statement

Study concept: Sarvari J, Hosseini SY and Haghshenas MR; Sample collection: Fattahi MR and Malekhosseini SA; Bench work: Molaei V; Data analysis: Molaei V, Haghshenas MR; Manuscript drafting: Molaei V; Critical revision of the manuscript: Hosseini SY, Haghshenas MR, Fattahi MR, Malekhosseini SA and Sarvari J. All authors read and approved the final manuscript.

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Ethics approval

The present study was approved by the Medical Ethics Committee (IR.sums.rec.1394.s18) of Shiraz University of Medical Sciences and written informed consent was obtained from all participants before sampling.

Availability of data

All data presents in the article

Funding statement

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Conflict of interest

All authors declare no conflict of interest.

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