Original Article

Iranian Journal of Immunology
https://iji.sums.ac.ir

IL-17 Genetic Variations Increase the Risk of Cirrhotic/Hepatocellular Carcinoma in Patients with Hepatitis B Virus Infection

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ABSTRACT

Background: Genetic variation in immune regulatory genes might influence the HBV infection outcome.

Objective: This study aimed to determine the association of IL-17A rs2275913 (G197A), IL-17F rs763780 (A7488G), and IL-23R rs10889677 (C2370A) gene polymorphisms, as well as the emerged haplotypes in the individual infected by HBV and to investigate their association with the infection outcome.

Methods: 300 chronic HBV infected cases with Cirrhotic/Hepatocellular carcinoma (C/HCC), chronic active (CA), or asymptomatic carrier (AC) and 38 individuals whose infection was spontaneously cleared (SC) were enrolled. Genomic DNA was extracted, and IL-17A/F and IL-23R genotyping were performed by using the PCR-RFLP method.

Results: Out of 338 subjects, 238 and 100 were respectively male and female with a mean age of 47.61±13.41. The frequency of GA genotype (P=0.01) and A alleles (P=0.001) of IL-17A rs2275913 (G197A), as well as the frequency of AA genotype (P=0.014) and A alleles (P=0.018) of IL-17F rs763780 (A7488G) gene locus, was found to be significantly higher in the C/HCC than CA and AC groups. Furthermore, the frequency of GA and AG haplotype in CA individuals was higher than those with C/HCC and AC (P=0.003). Also, the GG haplotype was higher in AC individuals than those with C/HCC (P=0.022), and the AA haplotype was higher in C/HCC individuals than the CA patients (P=0.001).

Conclusion: Our findings suggest that A allele and GA genotype at IL-17A rs2275913 (G197A), as well as A allele and AA genotype at IL-17F rs763780 (A7488G) locus, might be associated with increased risk of C/HCC among patients with hepatitis B virus infection.

Keywords: Cirrhotic/hepatocellular carcinoma, HBV, IL-17, Genetic variation

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Cite this article as:
Gheshlaghi A, Haghshenas MR, Safarpour AR, Hosseini SY, Fattahi MR, Malekhosseini SA, Sarvari J. IL-17 Genetic Variations Increase the Risk of Cirrhotic/Hepatocellular Carcinoma in Patients with Hepatitis B Virus Infection. *Iran J Immunol.*2021; 18(2):130-140, doi: 10.22034/iji.2021.88020.1852.

Received: 2020-09-18
Revised: 2021-05-22
Accepted: 2021-05-31

INTRODUCTION

Hepatitis B virus (HBV) infection is among the major health problems worldwide, leading to about 1 million deaths annually (1). The outcome of chronic HBV infection could vary from a mild asymptomatic carrier (AC) status to a more severe chronic active (CA) form that might progress to cirrhosis(C) and hepatocellular carcinoma (HCC). Among the critical factors in determining the infection outcome, the host immune response has a great impact on the disease progression process (2). Cytokines are key elements of the immune system that could be influenced by single nucleotide polymorphisms (SNPs) in their regulatory and/or coding region. Many SNPs have been reported in different cytokines that might influence the outcome of the disease including cancers, autoimmunity as well as viral infection such as HBV and HCV infection (3). IL-17 mature protein is encoded by exon 3 of the relevant gene located on chromosome 6p12 (4). It is produced by a CD4 effector T cell population, in response to the immune system stimulation resulting from tissue inflammation and pathogen clearance (5). Th17 cells showed an important effect on the development of liver cirrhosis, especially in those with chronic HBV infection in which an increased number of Th17 cells in CA patients is measurable (6, 7). There are two types of IL-17 including IL-17A , and IL-17F, that might play roles in determining the HBV infection outcome by inducing T cell-mediated immune response (8). IL-17 preserves the virus niches, the hepatocytes, through increasing the anti-apoptosis molecules and prevention of damage by cell-mediated immunity (9). IL-17F induces the inflammation process by expression of different cytokines, chemokines , and adhesion molecules, but with less activity in comparison with IL-17A (6).

Polymorphism in the IL-17 gene may influence the amount of transcription/ translation as well as the function of proteins. It has been reported that the replacement of adenine (A) to guanine (G) at the A7488G rs763780 loci of IL17F led to an amino acid substitution of histidine to arginine (H161R). This variant neither induces cytokines and chemokines production in bronchial epithelial cells nor activates the mitogen-activated protein kinase pathway (4). Polymorphisms of IL-17A rs2275913 (G197A) , and IL-17F rs763780 (A7488G) have recently been identified to be associated with the HBV infection outcome (10, 11).

The Interleukin-23 receptor (IL-23R) gene which is located on chromosome 1p31 plays an effective role in the stimulation and accelerating the IL-23/IL-17 signaling axis. In addition, previous studies have shown that IL-23R may decrease immune surveillance by CD8 T-cells (12). Its ligand, IL-23, as a pro-inflammatory cytokine is a major component of the immune regulatory pathways and promotes autoimmunity through T-cell-mediated inflammation by affecting the Th17 cell response (13). Polymorphisms in the regulatory sequences of IL-23R might influence its translational level. In this regard, IL-23R production is down-regulated by binding of miRNA let-7f to its 3'UTR sequence and halting from the mRNA translation process. Interestingly, the transversion of rs10889677 A>C resulted in 3'UTR change; therefore, it interfered with miRNA let-7f binding/interaction with 3'UTR. They also showed a higher expression level of IL-23R in the peripheral blood mononuclear cells (PBMCs) of individuals with rs10889677) C2370A)AA genotype than those with AC or CC genotype (14). Chen et al. suggested that the polymorphism of IL-23R could affect the IL-23 mediated immune signaling, Th17 expansion , and ultimately susceptibility to a group of autoimmune and inflammatory diseases (15). More importantly, some recent studies reported that genetic variants of IL-23R may also accelerate the liver pathological progress from chronic hepatitis status to HCC (16, 17). A recent meta-analysis conducted by Liu et al. has also shown that rs1884444 (G6644T) polymorphism in this gene could

be related to HCC progression in patients (18).

In addition, the inflammatory IL23/IL17 axis has been reported to trigger some inflammatory and autoimmune diseases (19). The IL23/IL17 axis is affected by IL-23R and increases the production and expression of IL-17A/F/A in Th17 cells (20). Wang et al. reported that HBV could induce inflammation of the liver by increasing IL-23 expression, leading to liver injury through the IL-23/IL-17 axis (21). Despite numerous efforts to draw a correlation between HBV infection fate and polymorphisms of cytokines, it needs more investigations to claim that. Therefore, this study was conducted to investigate IL-17A rs2275913 (G197A), IL-17F rs763780 (A7488G), and IL-23R rs10889677 (C2370A) polymorphisms in different groups of subjects infected with HBV and their possible role in HBV infection outcome.

MATERIALS AND METHODS

Subjects

Total, 338 subjects were recruited from the Gastroenterohepatology research center, at Motahari Clinical Center, and organ transplantation research center at Abu-Ali Sina hospital affiliated to Shiraz University of Medical Sciences, 2016-2019. Out of 338 subjects, 38, 100, 100, and 100 were from those with SC, AC, CA, and C/HCC, respectively. Their disease status was located under the liver specialist and was confirmed based on biochemical, virological, and imaging records according to the patients' medical files. Those subjects with spontaneously cleared HBV infection were selected from the national Kavar cohort study regarding their serological findings (22) and further confirmed by ELISA assays (Dia.Pro.Milano, Italy) for detection of HBsAg and HBcAb according to manufacturer's instruction. The ethics committee of Shiraz University of Medical Sciences (SUMS) has approved the study protocols (IR.SUMS.REC.1398.602) while informed consent was obtained from each participant before sampling.

DNA Extraction and Cytokine Polymorphisms Analysis

The blood sample was collected in EDTA anticoagulant and total DNA was extracted from PBMCs using the salting-out method (23). The quality of the extracted DNA was then tested by Nanodrop™ densitometry (Thermo Fisher Scientific, USA). IL-17A rs2275913 (G197A), IL-17F rs763780 (A7488G) , and IL23R rs10889677 (C2370A) genotyping were done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and a set of primers, as described before (15, 24). After gene amplification, the products were digested overnight at 37°C with *XagI* for IL-17A rs2275913 (G197A), *NlaIII* for IL-17F rs763780 (A7488G) , and *MnlI* for IL23R rs10889677 (C2370A) genotypes (all from Thermo Fisher, Waltham, Massachusetts, USA), and then run and stained on 3% agarose for genotyping (25, 26). Furthermore, to confirm the results of PCR-RFLP, some randomly selected PCR amplified samples were also introduced into Sanger sequencing. The sequences of primers, length of digested fragments, and the cycling conditions are summarized in Table 1.

Statistical Analysis

SPSS software package (version 25; SPSS Inc, Chicago, IL, USA) was used for data collection and statistical analysis. The distribution of alleles and genotypes among four study groups was analyzed using the chi-square test. The Hardy–Weinberg equilibrium, as well as haplotype analysis, was done by the Arlequin software package. P-value less than (0.05) was considered a significant level.

RESULTS

Characteristics of the Study Population

Out of 338 patients, 238 were male (70.4%).

Table 1. IL-17 and IL-23R gene-specific primer sequences and reaction conditions for genotyping of IL-17 and IL-23R polymorphisms using PCR-RFLP

Locus	Primer Sequence	The cycling condition(40 cycles)	Restriction Enzyme	Length of Digested Fragments	Ref.
IL- 17A G197A	F:5`-CAAGTAAGAATGAAAAGAGGACATGGT-3` R:5`CCCCCAATGAGGTCATAGAAGAATC-3`	94°C,5min 94°C,45s 65°C,45s 72°C,45s 72°C10min	*XagI*	AA:102 bp AG:102,68 and 34bp GG:68 and 34 bp	(24)
IL-17F A7488G	F: 5`-ACCAAGGCTGCTCTGTTTCT-3` R: 5`-GGTAAGGAGTGGCATTTCTA-3`	94°C,5min 94°C,45s 65°C,45s 72°C,45s 72°C10min	*NlaIII*	GG:143bp GA:143, 80 and 63bp AA:63 and 80 bp	(24)
IL23R C2370A	F:5`-CTGTGCTCCTACCATCACCA-3` R: 5`-TGCTGTTTTTGTGCCTGTATG-3`	94°C,5min 94°C,45s 58.5°C,45s 72°C,45s 72°C10min	*MnlI*	AA:152bp AC:152،80, and 72bp CC:80 and 72 bp	(15)

The mean age (SD) of the SC, AC, CA, and C/HCC groups was 52.68±15.48, 43.14±13.41, 43.37±13.66, and 51.26±11.60, respectively, that was not significantly different (P=0.96). The male/female ratio of SC, AC, CA, and C/HCC was 16/22, 71/29, 72/28, and 79/21 which were significantly different among the studied groups (P=0.01).

IL-17A, IL-17F and IL-23R Gene Polymorphisms among Different Groups

The polymorphisms of IL-17A at rs2275913 (G197A), IL-17F at rs763780 (7488) and IL-23R in rs10889677 (C2370A) loci were successfully genotyped in 338 individuals in four study groups. Genotype frequencies in all four groups were in according to Hardy-Weinberg equilibrium (P>0.05). The distribution of genotypes and alleles of IL-17A, IL17F and IL-23R in SC, AC, CA, and C/HCC is shown in Table 2. Our results showed that the frequency of GA genotype (53(53%) vs. 35(35%) OR=2.09, 95% CI 1.18—3.85, P=0.01) and A alleles (75(37.5%) vs. 45(22.5%), OR=2.06, 95% CI 1.33—3.20, P=0.001) of IL-17A G197A gene locus was significantly higher in the C/HCC than the CA group. It means that individuals carrying the rs2275913 A allele and GA genotype at IL-17A were more likely to get C/HCC than those with G allele and GG genotype. Genotypic distributions of IL-17F rs763780 (A7488G) represented a significantly higher frequency of the AA genotype (92(92%) vs. 85(85%), OR=2.88, 95% CI 1.20—6.88, P=0.014) and A alleles (190(95%) vs. 177(88.5%), OR=2.46, 95% CI 1.14—5.33, P=0.018) of IL-17F gene locus was significantly higher in the C/HCC patients in comparison to AC individuals. In addition, the frequency of the AG genotype of IL-17F rs763780 (A7488G) in AC individuals was higher than those with C/HCC (17(17%) vs. 6(6%), OR=3.20, 95% CI 1.20—8.52, P=0.014). This finding showed that subjects with the AG genotype are 3.20 times more possible to progress toward an asymptomatic state. In IL-23R rs10889677 (C2370A), the results showed that the frequency of genotype and allele among the four studied groups was not statistically significant (P>0.05).

Haplotype Analyses of IL-17 Gene Polymorphisms and SC, AC, CA and C/HCC Risk

It is well known that the haplotype analysis exhibits a greater impact than SNP genotyping in clinics. Herein the haplotype

Table 2: The frequency of IL-17A/F and IL-23R genotypes/alleles polymorphism in 4 studied groups. P-value < 0.05 was considered to be statistically significant.

POSITION	GENOTYPES/ ALLELES	SC	AC	CA	C/HCC	SC AND AC	SC AND CA	SC AND C/HCC	AC AND CA	AC AND C/HCC	CA AND C/HCC
IL-17A G197A	Genotypes					P value OR CI					
	GG	16 (42%)	48 (48%)	60 (60%)	36 (36%)	0.53 0.78 0.37to1.67	0.59 0.48 0.22-1.03	0.39 0.78 0.14-1.10	0.088 0.61 0.35-1.07	0.08 0.6 0.34-1.07	0.001 0.37 0.21-0.66
	GA	19 (50%)	45 (45%)	35 (35%)	53 (53%)	0.59 1.22 0.57-2.58	0.10 1.85 0.87-3.96	0.67 1.12 0.64-1.94	0.14 1.51 0.85-2.68	0.25 1.37 0.7-2.40	0.01 2.09 1.18-3.69
	AA	3 (8%)	7 (7%)	5 (5%)	11 (11%)	0.85 1.13 0.27-4.65	0.51 1.62 0.36-7.17	0.46 1.42 0.54-3.69	0.55 1.43 0.43-4.66	0.32 1.64 0.60-4.42	0.11 0.42 0.14-1.27
	Alleles										
	G	51 (67.1%)	141 (70.5%)	155 (77.5%)	125 (62.5%)	0.58 0.85	0.07 0.59	0.47 1.22	0.11 0.69	0.09 1.43	0.001 2.06
	A	25 (32.9%)	59 (29.5%)	45 (22.5%)	75 (37.5%)	0.48-1.50	0.33-1.06	0.70-2.13	0.44-1.08	0.94-2.17	1.33-3.20
IL-17F A7488G	Genotypes										
	AA	35 (92%)	80 (80%)	85 (85%)	92 (92%)	0.12 2.91 0.81-10.46	0.39 2.05 0.56-7.56	1.0 1.01 0.35-2.77	0.35 0.70 0.33-1.47	0.014 2.88 1.20-6.88	0.18 2.02 0.81-5.02
	AG	3 (8%)	17 (17%)	12 (12%)	6 (6%)	0.27 0.41 0.11-1.52	0.76 0.62 0.16-2.36	0.57 0.73 0.24-2.19	0.31 1.50 0.67-3.35	0.014 0.31 0.11-0.82	0.21 0.46 0.16-1.30
	GG	0 (0%)	3 (3%)	3 (3%)	2 (2%)	0.56 0.36 0.01-7.17	0.56 0.36 0.01-7.17	1.0 0.79 0.24-107	1.0 1.0 0.19-5.08	1.0 1.51 0.24-9.27	1.0 0.65 0.10-4.03
	Alleles										
	A	73 (96.1%)	177 (88.5%)	182 (91%)	190 (95%)	0.06 3.16	0.20 2.40	1.0 0.79	0.40 0.76	0.018 2.46	0.40 1.87
	G	3 (3.9%)	23 (11.5%)	18 (9%)	10 (5%)	0.92-10.86	0.68-8.42	0.20-3.04	0.39-1.45	1.14-5.33	0.60-5.82

POSITION	GENOTYPES/ ALLELES	SC	AC	CA	C/HCC	SC AND AC	SC AND CA	SC AND C/HCC	AC AND CA	AC AND C/HCC	CA AND C/HCC
IL-23R C2370A	Genotypes										
	CC	12 (31.6)	37 (37%)	39 (39%)	34 (34%)	0.55	0.41	0.68	0.77	0.73	0.46
						0.718	0.72	1.12	0.91	0.90	0.80
						0.35-1.74	0.32-1.59	0.62-2.03	0.51-1.62	0.50-1.61	0.45-1.48
	CA	20 (52.6)	51 (51%)	50 (50%)	55 (55%)	0.86	0.78	0.77	0.88	0.81	0.47
						1.06	1.11	1.08	1.04	1.06	1.22
						0.50-2.25	0.52-2.34	0.62-1.89	0.59-1.81	0.60-1.87	0.70-2.13
	AA	6 (15.8)	12 (12%)	11 (11%)	11 (11%)	0.55	0.44	0.30	0.82	0.88	1.0
						1.37	1.51	0.64	1.10	1.04	1.0
						0.47-3.97	0.51-4.44	0.28-1.47	0.46-2.63	0.59-1.84	0.41-2.42
	Alleles										
	C	44 (57.9%)	125 (62.5%)	128 (64.0%)	123	0.48	0.34	0.66	0.75	0.83	0.76
					(61.5%)	0.82	0.77	0.88	0.93	1.04	1.09
	A	32 (42.1%)	75 (37.5%)	72 (36.0%)	77 (38.5%)	0.48-1.41	0.45-1.32	0.50-1.55	0.62-1.40	0.69-1.56	0.61-1.93

SC: spontaneously cleared; AC: asymptomatic carrier; CA: chronic active; C/HCC: Cirrhotic/hepatocellular carcinoma

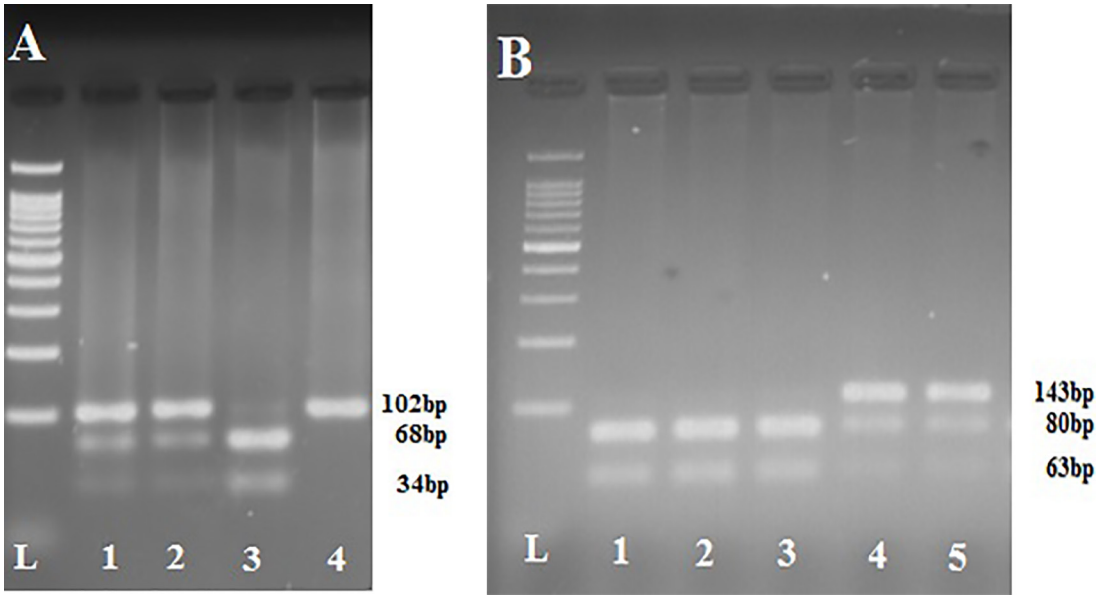

Figure 1. Results of PCR-RFLP gel electrophoresis in IL-17A (rs2275913) and IL-17F (rs763780) polymorphisms. (A) Genotypes in the IL-17A was determined as the AG (columns 1 and 2) (102 bp, 68 bp and 34 bp), GG (column 3) (68 bp and 34 bp), and AA (column 4) (102 bp). (B) Genotypes in the IL-17F was presented as AA (columns 1, 2, and 3) (63 bp and 80 bp), and GA (columns 4 and 5) (143 bp, 80 bp and 63bp). L: 100 bp ladder

analysis of IL-17 SNPs was performed to estimate the frequency of the haplotypes in SC, AC, CA, and C/HCC cases.

A total of four haplotypes including GA, AA, GG, and the AG were obtained after investigation of the observed genotypes. The most frequent haplotype was GA with a frequency of 63%, 60%, 72%, and 58% in SC, AC, CA, and C/HCC individuals, respectively (Figure 1). In addition, the findings demonstrated that the frequency of GA haplotype was significantly higher in the CA group in comparison with C/HCC (OR 1.86, 95% CI 1.22–2.82, P=0.014) and AC (OR=1.71, 95% CI 1.12—2.60, P=0.011) groups. Moreover, the frequency of the AA haplotype in the C/HCC group was higher than those with CA (OR 2.50, 95% CI 1.58–3.94, P=0.001). Furthermore, the frequency of GG haplotype was higher in AC subjects in comparison to the C/HCC group (OR 2.49, 95% CI 1.11–5.58, P=0.022). In the case of AG haplotype, CA individuals were harboring this haplotype more frequently than AC (OR 7.21, 95% CI 0.87-59.24, P= 0.03), and C/HCC (OR 7.21, 95% CI 0.87-59.24, P=0.03) individuals (Table 3).

DISCUSSION

Virological, environmental, and host genetic factors including immune-related ones are crucial contributors to the variations in the clinical outcome of HBV infection (27). Cytokines and their cognate receptors such as IL-17 A/F, and IL-23R might be involved in HBV infection outcome, due to the leading role in inflammation and cell immunity. Herein, the frequency of genotypes and alleles of IL-17A/F and IL-23R in individuals encountered with HBV was investigated in different groups of patients.

Analysis of the results revealed the higher frequency of GA genotype and A allele distribution of IL-17A + 197G/A in C/HCC than in CA individuals. Our data demonstrated that GA genotype, as well as the A allele showed about 2 fold increased risk of C/HCC in comparison with other genotypes and alleles. Conversely, the G allele and GG genotype might be considered as protective factors that prevent the progress of C/HCC, while they increase the risk of CA. This polymorphism that is located in the promoter region has also been reported by

Table 3. The frequency of different haplotypes of IL17A and IL-17F among four studied groups. A; Asymptomatic carrier (AC) versus chronic active (CA). B; Asymptomatic carrier versus Cirrhotic/ hepatocellular carcinoma (C/HCC). C; Asymptomatic carrier versus spontaneously cleared (SC). D; Chronic active versus Cirrhotic/hepatocellular carcinoma. E; Chronic active versus spontaneously cleared. F; Cirrhotic/hepatocellular carcinoma versus spontaneously cleared.

A

Haplotypes		Frequencies		OR	CI	P-value
IL-17A	IL-17F	AC 2 n= 200	CA 2n= 200			
G	A	120	144	0.58	0.38 to 0.88	0.011
A	A	58	38	1.74	1.09to 2.77	0.019
G	G	21	11	2.01	0.94 to 4.03	0.065
A	G	1	7	0.13	0.016 to 1.13	0.03

B

Haplotypes		Frequencies		OR	CI	P-value
IL-17A	IL-17F	AC 2 n= 200	C/HCC 2 n= 200			
G	A	120	116	0.92	0.61 to 1.37	0.76
A	A	58	74	1.43	0.94 to 2.18	0.11
G	G	21	9	0.40	0.17 to 0.90	0.035
A	G	1	1	1.0	0.062 to 16.11	1.0

C

Haplotypes		Frequencies		OR	CI	P-value
IL-17A	IL-17F	AC 2 n= 200	SC 2 n= 76			
G	A	120	48	0.87	0.50 to 1.510	0.63
A	A	58	25	0.82	0.47to 1.470	0.52
G	G	21	3	2.85	0.82 to 9.86	0.09
A	G	1	0	1.15	0.046 to 28.57	1.0

D

Haplotypes		Frequencies		OR	CI	P-value
IL-17A	IL-17F	CA 2 n= 200	C/HCC 2 n= 200			
G	A	144	116	0.53	0.35 to 0.81	0.004
A	A	38	74	2.50	1.58to 3.94	0.001
G	G	11	9	0.80	0.32 to 1.99	0.81
A	G	7	1	0.13	0.01 to 1.13	0.03

E

Haplotypes		Frequencies		OR	CI	P-value
IL-17A	IL-17F	CA 2 n= 200	SC 2 n= 76			
G	A	144	48	1.50	0.85 to 2.62	0.15
A	A	38	25	0.47	0.26to 0.86	0.014
G	G	11	3	1.41	0.38 to 5.22	0.59
A	G	7	0	5.93	0.33 to 10.52	0.19

F

Haplotypes		Frequencies		OR	CI	P-value
IL-17A	IL-17F	C/HCC 2 n= 200	SC 2 n= 76			
G	A	116	48	0.80	0.46 to 1.38	0.43
A	A	74	25	1.19	0.68to 2.09	0.52
G	G	9	3	1.14	0.30 to 4.35	0.84
A	G	1	0	1.15	0.04 to 28.57	1.0

Espinoza et al. to provide a better settlement for NFAT transcription factor, resulting in a higher expression of IL-17 as well as immune inflammation (28). In agreement with our finding, Hejr et al. showed a higher frequency of GA genotype and A allele of IL-17A rs2275913(G197A) polymorphism in acute rejected kidney transplant patients in comparison to non-rejected transplant patients infected with HBV (29). This study indicated the importance of IL-17 polymorphism in inflammation and rejection as demonstrated by others (30). Moreover, Li et al. reported a higher frequency of GG genotype and G allele of rs2275913(G197A) in HCC patients than CA subjects (10). Another study reported a higher frequency of allele T and T containing genotypes of rs8193036

(C4309T) in those whose HBV infection had cleared spontaneously compared to chronic active individuals or asymptomatic carrier group. In a poly-variant analysis, they also determined that IL-17A expression level and related AG and GG of genotypes of IL-17A rs2275913(G197A) were the factors that contributed to an increased risk of HCC (10). In a case-control study by Ren et al., it was demonstrated that the GG genotype and G allele of IL-17A rs2275913(G197A) polymorphism are related to HBV infection in the Chinese population (31). Contrary to these findings, Xi et al. reported no significant difference in the genotype and allele frequencies of rs2275913 (G197A) polymorphisms between the HCC patients and asymptomatic HBV subjects. (11) Also, Wang et al. reported no differences in allele and genotype distributions of polymorphisms at IL-17A rs2275913(G197A) between CA and HCC patients in the Chinese population (32). In addition, it has been shown that this kind of polymorphism might be associated with progression and response to therapy of rheumatoid arthritis in Polish patients (33), susceptibility to gastric cancer in a Japanese population (34) , and an acute rejection of kidney transplant in HBV-infected patients (29).

In IL-17F rs763780 (A7488G), the frequency of the AA genotype and A allele was found to be higher in C/HCC patients than in AC individuals. Also, the frequency of IL-17F rs763780 (A7488G) the AG genotype was significantly higher in AC subjects in comparison with C/HCC patients. Patients with A allele and AA genotype were found to be about 2.3 times more susceptible to C/HCC, while the G allele might play a protective role against C/HCC. Additionally, subjects carrying the AG genotype at IL-17F were found to develop an asymptomatic state up to 3.2 times. In contrast to our findings, in China, Xi et al. reported no significant differences in the genotype and allele frequencies of these polymorphisms in HCC patients in comparison with asymptomatic controls (11). Another study reported no differences in allele and genotype of IL-17F rs763780 (A7488G) distribution among the CA, HCC , and healthy control groups in the Chinese population (32). Moreover, polymorphisms of IL-17F at rs763780(A7488G) loci might be related to increased risk of rheumatoid arteritis (33), acute myeloid leukemia (35), and gastric cancer (24).

From dozens of studies, it could be suggested that haplotype analysis may have a greater impact than SNP genotyping in clinical prediction. Haplotype analysis showed that AA, GG, and AG haplotypes could be associated with the progression of HBV infection to CA, and C/HCC status. In this regard, Li et al. reported that HCC patients had more frequent haplotypes CG and TG of rs8193036(C4309T) and rs2275913(G197A) (10). Also, Xi et al. reported that ACA and GGG haplotypes derived from IL-17A rs4711998(A4169G), IL-17A rs2275913(G197A), and IL-17F rs763780(A7488G) were significantly associated with increased or decreased risk of HBV-related HCC, respectively(11). Moreover, it has been reported that GCT haplotypes derived from IL-17A rs4711998(A4169G), IL-17A rs2275913(G197A) , and IL-17F rs763780(A7488G) were among the genetic risk factors for HCC (32).

The current study showed that the frequency of genotype and allele of IL-23R rs10889677 (C2370A) was not statistically significant among the groups. However, Labib et al. found that the frequency of GA and AA genotype of IL23R gene rs11209026(G78790A) was significantly higher in HCV patients without HCC than those with HCC (36). Xu et al. reported that IL-23R rs6682925 (C4094T) and rs1884444(G6644T) were related to the risk of HCC when compared with both the HBsAg positive and negative controls (17). Furthermore, a meta-analysis revealed that IL-23R polymorphisms at rs1884444 (G6644T) might be associated with the increased risk of HCC (18). In addition, it has been reported that polymorphisms of IL-23R at rs10889677(C2370A) might be associated with acute rejection kidney

transplant in HBV infected patients (29) and dilated cardiomyopathy (15). Moreover, Karimi et al. reported that the frequency of AC carriers and C allele of IL-23R10889677 (C2370A) was significantly increased in acute liver rejection than successful transplantation, indicating that AC carriers and C allele have the predictive value for acute rejection (37).

In conclusion, our results indicated that individuals carrying the A allele and GA genotype of IL-17A rs2275913 as well as A allele and AA genotype of IL-17F rs763780 were more likely to proceed toward C/HCC than those with G allele and GG genotype. Furthermore, the AA haplotype might be associated with spontaneous clearance of HBV infection and those with GA and AG haplotype might prolong chronic state and slow progression into C/HCC end stages.

ACKNOWLEDGMENTS

The present study was extracted from the thesis written by Abolfazl Gheshlaghi, which was financially supported by Shiraz University of Medical Sciences (Grant No. 97-176704).

Conflicts of Interest: None declared.

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