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Original Article

IL-6 polymorphism in patients with hepatitis B virus infection

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Abstract

Previous studies reported an association between some cytokines polymorphisms and human viral infections. In HBV infection, IL-6 plays a role in the induction of immune tolerance by inhibiting IL-1 and tumor necrosis factors, which exert anti-inflammatory properties and prevent cell damage. IL-6 has a potential role in HBV-induced hepatocellular carcinoma, liver fibrosis, and liver cirrhosis. IL-6 levels increased in subjects infected with hepatitis B virus, and genetic susceptibility was reported in viral infection. This study aims to determine IL-6 polymorphism in humans infected with hepatitis B virus. In this case-control study, 50 individuals with hepatitis C virus infection were included. The -174G/C promoter polymorphism of the IL-6 gene by Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) procedure. There were highly significant differences in (-174G/C) promoter polymorphism of IL-6 between the control group and the patients group. Two homozygous GG and CC genotypes were in patients, while one heterozygous (GC) and one homozygous (GG) genotype pattern was detected in controls. The GG and CC IL-6 (-174G/C) genotypes were predominant in patients compared to controls. In contrast, the GC IL-6 genotype was present in 52.5% of the controls, and none was found in HBV-infected patients. IL-6 GG genotype and G allele may be associated with HBV infection susceptibility in Iraqi subjects, and IL-6 gene plays a potential role in the pathogenicity of HBV infection. However, a large-scale study that included many positions was warranted.

Keywords: hepatitis B virus; HBV, IL-6, -174G/C, hepatocellular carcinoma, tumor necrosis factor.

Introduction

Hepatitis viral infection is common worldwide with health impacts as it was possible to lead to subsequent hepatocellular carcinoma [1]. The viral common etiology of hepatitis include hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), hepatitis F virus (HFV), hepatitis G virus (HGV) and SEN-V [2]. In Iraq, the prevalence of HBV was categorized within intermediate endemicity [3]. The most serious hepatitis is caused by hepatitis B virus and hepatitis C virus as they are associated with disease chronicity, liver cirrhosis and hepatocellular carcinoma development [4, 5]. However, the above complications were not induced by HBV's direct pathogenic effects but mainly as an outcome of host immune response [6, 7]. The risk factors for human viral infections are either environmental and/or genetic susceptibility. Previous studies reported an association between some cytokines polymorphisms and human viral infections [8].

IL-6 is an inflammatory cytokine that has potent multifunctional effects in inflammatory response induction and regulation [9]. IL-6 regulates both humeral and cellular immunity, potentially influencing the determination of viral infection outcomes [10, 11]. In HBV infection, IL-6 plays a role in the induction of immune tolerance in HBV infection through the inhibition of IL-1

Table 1: Patient and controls characteristics.

Variable	Patients	Controls
Number	50	40
Mean age, year	33.12±12.88	29.25±7.08
Male/Female	28/22	20/20

and tumor necrosis factor, which exert anti-inflammatory and prevent cell damage. IL-6 is with potential role in HBV-induced hepatocellular carcinoma, liver fibrosis and liver cirrhosis [12]. Previous studies reported increased serum levels of IL-6 in subjects infected with hepatitis B virus [13–15]. Thus, this study was conducted to determine IL-6 polymorphism in humans infected with hepatitis B virus.

Material and methods

Study population

In a case- control study, 50 individuals with hepatitis C virus infection were included in the study. The patients were recruited from an outpatient internal medicine clinic in Samara General Hospital. Forty healthy age and sex-matched subjects were selected as the control group. PCR confirmed the cases diagnosed clinically by specialist physician and diagnosis of HBV infection. Patients and control characteristics are shown in Table 1.

IL-6 gene (-174G/C) polymorphism detection

Genomic DNA extracted from blood samples of patients and controls according to the procedure described previously [16] using the Thermo Scientific NanoDrop system, Germany. The -174G/C promoter polymorphism of the IL-6 gene by Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) procedure. The primers used are shown in Table 2. The kit (Go Taq Master Green) was purchased from Promega, USA.

Ethical Committees of Tikrit University College of Medicine approved the study protocol. Informed consent was taken from each individual before their enrollment in the study.

Statistical analysis

The gene type is presented as frequency, and a Chi-square is applied to determine the difference in significance between groups using the SPSS package (version 20). A p-value of <0.05 is regarded as significant.

Results

There were highly significant differences in (-174G/C) promoter polymorphism of IL-6 ($P<0.01$) between the control group and the patients group. The ARMS-PCR technique shows 2 homozygous GG and CC genotypes in patients with HBV infection. The technique shows one heterozygous (GC) and one homozygous (GG) genotype pattern in the control group Table 3.

Forty-two (84%) of patients were with GG IL-6 (-174G/C) genotype, while the corresponding value in the control group was 19 (47.5%) ($P<0.001$). Eight patients (16%) had the CC IL-6 genotype, while controls did not show such genotype ($P=0.008$). In contrast, the GC IL-6 genotype was present in 21 (52.5%) of the controls, and none were found in HBV-infected patients ($P<0.0001$). These findings indicated significant differences in the frequency of GG, GC, and CC IL-6 genotypes between patients and control groups Table 3.

The (-174G) alleles that were present in the GG genotype were more common (84%)in patients than in controls (73.75%). In contrast, the (-174C) allele was more common in the control group (26.25%) than in the patients group (16%) Table 4. Thus, an association

Table 2: Primers used in amplification refractory mutation system polymerase chain reaction.

Primer	Primer sequences	Annealing temperature
Common forward primer	5'-GAG CTT CTC TTT CGT TCC-3'	
Reverse 1 primer (C allele)	5'-CCT AGT TGT GTC TTG CCC-3'	54˚C
Reverse 2 primer (G allele)	5'-CCC TAG TTG TGT CTT GCG-3'	

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Table 3: Genotype frequency in patients with hepatitis B virus infection and controls.

Gene type	Patients		Controls		P-value
	Number	Percent	Number	Percent	
GG	42	84	19	47.5	<0.001
GC	0	0.0	21	52.5	<0.0001
CC	8	16	0	0.0	0.008
Total	50	100	40	100	

between IL-6 (-174G/C) promoter polymorphism and HBV infection in humans. IL-6 GG genotype and (-174G) were risk factors for HBV infection, while the (-174C) allele was protective against HBV infection.

Discussion

The present study shows highly significant differences in IL-6 (-174G/C) promoter polymorphism between patients with HBV infection and controls. In HBV-infected subjects, the GG IL-6 (-174G/C) genotype was found in 84% of the cases, while it was 47.5% in the controls. This finding suggests that the IL-6 GG (-174G/C) genotype and G allele were risk factors for HBV infection and indicate a genetic susceptibility in individuals with this gene and allele.

Previous studies reported controversial findings regarding the association of IL-6 GG (-174G/C) genotype polymorphism and susceptibility to human HBV infections. Some studies reported a negative association [13, 17–21], while others found a positive one [22–25]. In addition, other studies reported a positive association between IL-6 (-174G/C) promoter polymorphism and the development of hepatocellular carcinoma [1, 26–28], while one study reported a negative association [29]. The controversy in different studies' findings may attributed to racial differences of IL-6 alleles frequency [13, 30, 31].

This study shows only the homozygous genotype (GG and CC) in patients with HBV infection, while the control group shows both heterozygous and homozygous (GG and GC) genotype patterns. However, other studies show both heterozygous and homozygous genotype patterns in both patients with HBV infection and control groups [23, 24]. Thus, these findings are evidence that reflects geographical and racial variation in the frequency of IL-6 (-174G/C) genotypes and alleles.

GG IL-6 (-174G/C) genotype was detected in 84% of patients and 47,5%, with a highly significant difference. This finding was in agreement to that reported for Indonesia and Iran as they found that GG IL-6 (-174G/C) genotype in 63.1% of patients *versus* 42% in controls [24], 60.9% in patients *versus* 47.6% in controls [23].

The GC IL-6 (-174G/C) genotype was not detected in the present study cohort; however, it was reported in 31.6% of Indonesian studies [24] and 34.3% of Iranian studies [23]. Meanwhile, in controls, the frequency was 52.5% for this study, 38% for the Indonesian study, and 39.7% for the Iranian study.

CC IL-6 (-174G/C) genotype was detected in 16% of this study compared to 5.3% and 4.7% in the Indonesian and Iranian studies, respectively. In controls, this genotype was not found in the present study cohort, while it was detected in 12.8% of Iranian studies and in 20% of the Indonesian study [23, 24]. The variation in gene type polymorphism rate between different communities could be due to environmental factors and racial variation.

The (-174G) alleles which were present in the GG genotype were more common in patients (84%) than in controls (73.75%). In contrast, the (-174C) allele was

Table 4: Allele frequency in patients compared to controls.

Allele	Patients		Controls	
	Number	Percent	Number	Percent
G	84	84	59	73.75
C	16	16	21	26.25
Total	100	100.00	80	100.00

more common in the control group (26.25%) as compared to the patients group (16%). This finding was consistent with previous studies, which reported the same distribution patterns in both patients and control groups [23, 24]. Thus, the IL-6 GG genotype and (-174G) were a risk factor for HBV infection, while (-174C) allele was with protective against HBV infection.

The previous studies that did not find an association between IL-6 (-174G/C) promoter polymorphism were performed between 2003 and 2009. [13, 17–21]. The first study that reported an association between IL-6 (-174G/C) polymorphism and chronic hepatitis B infection in 2009 [22] and then followed by studies that indicated a positive association between polymorphism in (-174) region and susceptibility to HBV infection, course chronicity and development of HCC [1, 23–28].

The controversies in findings between the old and new studies may be attributed to the study population and/or to the improvement and development of sensitive gene polymorphism detection tools. The study limitations were only one position assessed and a small sample size.

Conclusion

IL-6 GG genotype and G allele may be associated with HBV infection susceptibility in Iraqi subjects, and IL-6 gene plays a potential role in the pathogenicity of HBV infection. However, a large-scale study that included many positions was warranted.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The approval for this study was obtained from the Ethics Committee of the Tikrit University College of Medicine Ethical Committee (approval ID: PR/TUCOM/V9 on 14/01/2019). The study was conducted in adherence with Helsinki Ethical standards.

Informed consent

Written informed consent was obtained from all participants in this study.

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