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Original Article

Single Nucleotide Polymorphism of the Interferon-γ Gene (IFN-γ +874 T/A) and the Prognosis of Hepatitis B Infection

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Introduction: Chronic Hepatitis B virus infection is a multifactorial disease with a variety of clinical outcomes. Since interferon-gamma (IFN-γ) is a significant immune factor in antiviral defense, this case-control study aimed to investigate the potential relationship between single nucleotide polymorphism of rs2430561 and hepatitis B infection outcome in a population of Birjand city, eastern Iran. **Methods:** Blood samples were collected from 60 chronically HBV- infected patients and 60 healthy subjects with the history of HBV infection. Genomic DNA was extracted from whole blood by the salting-out method. The first intron of IFN-γ with a length of 264 bp was amplified by Amplification Refractory Mutation System Polymerase Chain Reaction (ARMS-PCR) followed by sequencing. **Results:** Our results exhibited a statistically significant difference between patients and control individuals (p-value<0.001). The frequency of the allele A was 73.3% in HBV- infected patients, whereas in controls (individuals with a history of HBV infection) it was 46.7%. **Conclusion:** A statistically significant relationship was found between the IFN-γ (+874T/A, rs2430561) single nucleotide polymorphism (SNP) and chronic HBV infection in the studied population. The obtained results showed that HBV infected individuals with T allele have less risk of progressing to chronic HBV infection. It also suggests that the homozygous carriers of the A allele are more vulnerable to chronic HBV infection. *J Med Microbiol Infec Dis, 2018, 6 (2-3): 43-47.*

Keywords: Hepatitis B, Chronic, Interferon-gamma, Single Nucleotide Polymorphism, Iran.

INTRODUCTION

Hepatitis B virus (HBV), an enveloped double-stranded DNA virus [1], is the primary cause of acute hepatitis and chronic liver illnesses worldwide, particularly in Asia and Africa [2]. HBV is not cytopathic; some patients become asymptomatic carriers, while others develop liver diseases, such as chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) [3, 4]. The pathogenesis of liver disease in chronic HBV infection remains unclear, but the interactions between the host's immune system and the virus ultimately determine the outcome of the disease. The pattern of cytokine production by innate immune cells like the NK cell and the adaptive immune Th1 significantly contribute to the initial control of HBV infection. Interferon-gamma (IFN-γ) is among the principal mediators inducing a host resistance against HBV and the clearance of this virus from hepatocytes [5, 6]. IFN-γ as a pro-inflammatory Th1 cytokine has a vital role in the antiviral activity, and genetic variation of individuals affects immunity in response to a pathogen [7, 8].

The human IFN-γ gene is located on chromosome 12q24 spanning ~5.4 kb and consists of four exons with three introns [9]. The single nucleotide polymorphism (SNP) of IFN-γ gene +874 A/T (rs2430561) in the first intron is suggested to affect the level of IFN-γ production and thus results in altering the performance of regulatory factors by adjusting their affinities to transcription components [10]. Previous studies investigated the association of 874 T to A transition with several human illnesses, including HBV infection [11]. It is suggested that individuals with T allele at rs2430561 might have an increased level of IFN-γ and the less chance of developing chronic HBV infection [12]. There are reports on variation in genotype and the allele frequencies of cytokine genes in relation to ethnicity and race and the inheritance of particular alleles controlling cytokine production [13, 14]. The Amplification Refractory Mutation System Polymerase Chain Reaction (ARMs-PCR) is a unified DNA amplification-based method used for typing bi-allelic cytokine gene polymorphisms that either directly or indirectly influence gene expression [15]. The object of this study was to highlight the possible relationship between an SNP (rs2430561) in IFN-γ gene

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and chronic HBV infection in a population of Birjand city, eastern Iran.

MATERIAL AND METHODS

Patients and Controls. This case-control study aimed to determine the relation between IFN-γ polymorphism and chronic HBV infection in Birjand city, the east of Iran. We chose 60 patients with chronic hepatitis B (CHB), 40 males and 20 females, aged 19 to 70 years (mean: 45.1 ± 13.71). The CHB was defined as the persistent presence of HBsAg for more than six months. The HBsAg was detected by an enzyme-linked immunosorbent assay (ELISA) (Dia lab ® HBs Ag, Austria) following the manufacturer's instructions. The control group included 60 healthy participants, 44 males, and 16 females, aged 15-70 years (mean: 47.88± 13.84). The controls had a history of HBV infection with spontaneous seroclearance of HBsAg and the presence of anti-HBc antibody. Both CHB patients and the control individuals were selected from the Regional Hepatitis Comprehensive Plan conducted by the Infectious Diseases Research Center affiliated to Birjand University of Medical Sciences (2013-2014) [16].

The HCV or HDV co-infected patients, and individuals with autoimmune and other liver diseases, *e.g.*, HIV, cirrhosis, and cancer, were excluded from this study. Also, taking immunosuppressive medication in the last six months was another exclusion criterion.

The local ethics committee of Birjand University of Medical Sciences approved the study (code: Ir, bums.REC.1395.54) and the informed consent was obtained from all the individuals who participated in the study.

Genotyping. Amounts of 2 ml of whole blood were collected by heparinized syringes, and DNA extraction from samples was performed by a modified salting out method as described by others [17]. DNA concentration and its purity were analyzed by a Nanodrop instrument (Spectrophotometric). The SNP genotyping related to +874 region of the IFN-γ gene was performed by Amplification Refractory Mutation system (ARMs) Polymerase Chain Reaction (PCR). Amplification of each allele (A or T) was performed with the primers, IFN-γ generic primer, 5'-tcaacaaagctgatactcca-3', IFN-γ primer T allele, 5'-ttcttacaacacaaaatcaaatct-3', IFN-γ primer A allele, 5'-ttcttacaacacaaaatcaaatca 3'. As an internal control, the reactions included primer 1, 5'-gccttcccaaccattccctta-3'; and primer 2, 5'-tcacggatttctgttgtgttt-3' designed by others that amplified the human growth hormone gene. [11].

DNA was amplified in 25 µl final reactions containing 2 pmol of each primer (KBC, Iran), 4 µL of template DNA, 0.125 µL of 5u/µl Taq DNA polymerase (KBC, Iran), 2.5 µL of 10X reaction buffer (KBC, Iran), 0.5 µL of 100 mM MgCl2, 0.125 µL of 100 mM dNTP (KBC, Iran) and 12.95 µL ddH2O. The protocol for amplification in the Eppendorf PCR master cycler (AG-5345, Germany) included an initial denaturation at 95°C for 4 min followed by 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s with a final extension at 72°C for 2 min.

Six µL of PCR products were mixed with 2 µL of loading buffer (KBC, Iran) and run on a 1.5% agarose gel in TBE buffer. The amplicons were visualized under UV in a gel documentation device (Uvitec, Cambridge, UK), and then were sequenced in both directions (Bioneer, Republic of Korea)

Statistical Analysis. Allele and genotype frequencies were calculated in the patients and control subjects by direct gene counting. The odds ratio (OR) and range with 95% confidence interval (CI) were calculated for the presence of the reference genotype and the alleles using a logistic regression model. The ORs for individuals' genotypes and allele frequencies were calculated using the general model (analysis of each allele, and genotype separately), the major allele homozygotes (the most frequent alleles) versus a combination of heterozygotes with homozygotes. Descriptive data were presented as the mean and standard deviation (SD). Statistical evaluation was carried out using the Statistical Package for the Social Sciences (SPSS), version 18. A probability value of $P<0.05$ was considered statistically significant.

RESULTS

Our study included 120 individuals, including 60 with CHB and 60 controls. There was no significant difference between the two groups regarding age ($P=0.271$) and gender ($P=0.315$).

PCR amplification yielded two bands of different sizes in each lane, a 429 bp band indicative of human growth hormone gene (the control), and a 264 bp band indicative of either A or T allele (Fig. 1).

The allele and genotype frequencies of this IFN-γ SNP (rs2430561) were determined in patients and controls (Table1).

Our results showed that individuals with the allele T had less risk of progression to chronic HBV infection (OR: 3.14, 95% CI: 1.83-5.39) (Table 1). Also, the results revealed the higher risk of chronic HBV in individuals with the homozygote AA genotype compared to the TT genotypes (OR: 16, 95% CI: 3.27-78.28) (Table 1).

DISCUSSION

This study was conducted to investigate the relationship between a common SNP (rs2430561 T>A) of the IFN-γ gene and the vulnerability to HBV chronic infection in the Birjand city, eastern Iran. Our findings demonstrated a positive relationship between IFN-γ SNP (+874T/A, rs2430561) and chronic HBV infection in the studied population.

T lymphocytes and natural killer cells are the only cells that are responsible for secreting IFN-γ. These cells release IFN-γ once activated by antigens, alloantigens, or mitogens [18]. IFN-γ induces antiviral responses by the enhanced expression of major histocompatibility complex (MHC) antigens on hepatocytes [19]. This cytokine also seems to be involved in the clearance of HBV infection as it suppresses the replication of HBV-infected cells and adjusts the antiviral capacity of cytotoxic lymphocytes to, directly and indirectly, diminish the viral load [20].

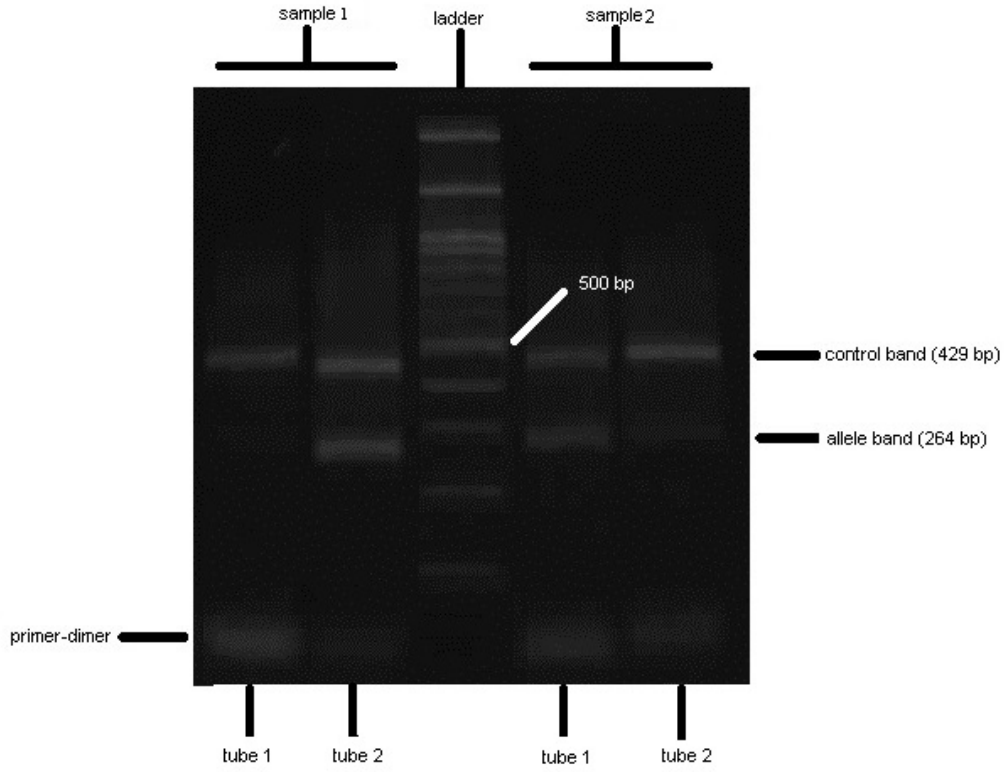

Fig. 1. PCR amplification of IFN-γ Alleles. Middle lane is ladder 100 (CMG, Isfahan, Iran). The 429 bp band in all lanes is indicative of amplification of human growth hormone gene (internal control). A 264 bp band shows amplification of either allele A or T.

Table 1. Allele and genotype frequencies in the case and control group

Gene Profiles	Case (n=60) (%)	Control (n=60) (%)	OR (95% CI)	*P*-value
Allele A	88 (73.3)	56 (46.7)	3.14 (1.83-5.39)	<0.001
Allele T	32 (26.7)	64 (53.3)	1	
Genotype TT	4 (6.7)	8 (13.3)	1	
Genotype AT	24 (40)	48 (80)	0.31(0.18-0.54)	<0.001
Genotype AA	32 (53.3)	4 (6.7)		
Genotype TT (nominal)	4 (6.7)	8 (13.3)	1	
Genotype AT (nominal)	24 (40)	48 (80)	1 (0.27-3.65)	0.61
Genotype AA (nominal)	32 (53.3)	4 (6.7)	16 (3.27-78.28)	<0.001

OR, Odds Ratio; CI: Confidence Interval

The difference in IFN-γ level among individuals may not be the only contributing factor responsible for the clearance of HBV infection, and variation in the genetic components of the host also affects the clinical outcome of the disease [21-23]. Previously, studies showed that SNPs influenced HBV infection in the cytokine genes that resulted in changes in the cytokine secretion amount among individuals with different genetic background. This variation showed association with the SNPs in the regulatory regions of cytokine genes [24-25]. HBV elimination in the infected individuals is related to coordination between innate and adaptive immune responses. During this procedure, cytokines play an important role in adjusting almost all phases of the host immune response. Some studies indicated that various immune-regulatory cytokines like IFN-γ inhibit HBV replication by the non-cytolytic process [26, 27].

Our results suggested that the A allele carriers of SNP at +874 position, especially homozygous individuals were more prone to chronic HBV infection and carriers of wild-type allele T at position +874 had a more chance of recovery from HBV infection [28]. Therefore, the low IFN-γ expression may reduce the host immune response to HBV, and these individuals are more prone to the infection. A study conducted by Sun and colleagues (2015) in a Chinese population revealed that IFN-γ +874T haplotype is potentially protective against persistent HBV infection [29]. Also in agreement with our results, in a rural area of Northern China IFN-γ+ 874 AA genotype showed to be associated with an increased risk of chronic HBV infection [30]. On the other hand, a study from Iran in 2016 showed no association between this polymorphism and HBV clearance in a population from Golestan Province [31]. The difference in results might be attributed to the ethnic background of the studied populations, sample size, and the genotyping method. Despite conflicting findings, the results of this work and many previous reports suggest the existence of a positive association between IFN-γ +874

T>A polymorphism and the risk of chronic HBV infection in Asian and European populations [13, 14] Indeed, the genotype and allele frequency are distinct among different populations. It is possible that the association of the SNP, IFN-γ T>A, with the development of chronic HBV infection is more evident in Asians due to the higher frequency of the allele A in this people compared with other populations. However, further studies with larger sample sizes are required to gain insight into the underlying mechanisms. In overall, our findings indicated that IFN-γ +874 AA genotype was associated with accelerated disease progression as this genotype was more frequent in CHB cases. However, the pathogenic mechanisms by which the IFN-γ rs2430561 T>A polymorphism facilitates the development of HBV infection remains to be investigated. The predisposition to an infection or severity of illness cannot exclusively be attributed to the virulence of an organism. In chronic viral hepatitis, the genetic composition of the host seems to be linked with the viral persistence and disease progression towards chronic liver diseases and knowledge on this issue may help us to provide novel therapeutic approaches.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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