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RESEARCH ARTICLE

Association of IFN-γ (+874A/T) with Chronic Hepatitis B Virus Infection

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Abstract

Background: Chronic hepatitis B virus (HBV) infection is a disease of worldwide distribution. IFN-γ is one of the key regulatory cytokine that influence the clearance of HBV. The production of this cytokine can be influenced by the presence of certain single nucleotide polymorphisms (SNPs) in *IFN-γ* gene.

Subjects and Methods: This case-control study involved 62 chronic HBV outpatients and 40 apparently healthy individuals. Blood samples were collected from each participant, and DNA was extracted from each sample. *IFN-γ* gene was amplified by PCR technique using specific primers. The polymorphism in the gene of this cytokine was assessed by sequencing.

Results: There was a significant difference in the frequency of AA genotype between patients (33.87%) and control (20%) (OR=3.341, 95%CI=1.075-10.387). Furthermore, allele A had higher frequency among patients (58.06%) compared with control (42.5%) with significant difference (OR= 1.873, 95%CI=1.06-3.309).

Conclusion: allele A of the SNP+874A/T may be a predisposing risk factor for chronic HBV infection.

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Introduction

Chronic hepatitis B virus (HBV) infection is one of the most common viral infections worldwide, with about 350-400 million chronic carriers (1). More than 90% of HBV infections in adults are self-limited within 6 months, whereas, 5%-10% of these infections progress towards chronic infection (2). Such infection can lead to serious clinical consequences such as liver cirrhosis and hepatocellular carcinoma (3).

Beside the pathogenesis of the virus, the characteristic immune response of the host is among the most important factors which determine the sequelae of the infection (4). However, the immune determinants of successful clearance of HBV are not fully understood, but both cellular and humoral immune responses are important (5).

Interferon-γ is a Th_1 cytokine involved in the activation of cellular immunity, particularly cytotoxic CD8+T cells (6). During an acute self-limited HBV infection, high level of IFN-γ is secreted by T-lymphocytes (7), which has significant role in HBV clearance (8). In a recent study, Conde *et al*. (9) reported decreased serum levels of IFN-γ in Brazilian patients with chronic HBV carrier in relation to acute self-limited patients, which indicates the crucial role of this cytokine in the course of this disease.

The *IFN-γ* gene is located on chromosome 12q24.1, covering approximately 5.4 Kb (6). Many studies demonstrated an association between the SNP in the first intron (+874A/T) of the gene with different diseases such as pulmonary tuberculosis (10, 11), age-related cataract (12), oral lichen planus (6), asthma (13), leishmaniasis (14), and brucellosis (15). To our knowledge, there is no previous study that investigates the relationship between *IFN-γ* polymorphism and HBV infection in Iraqi patients. Hence, this study aimed to investigate the impact of IFN-γ +874 A/T on the susceptibility to chronic HBV infection.

Materials and Methods

Subjects and Samples

This study included 62 (21-53 years old, mean 37±1.17 years, 48 males and 14 female, four of whom are pregnant) chronic HBV patients from outpatients of Al-Kadhimiya Teaching Hospital/Baghdad/Iraq during the period from August 2012 to March 2013. These patients were positive for HBsAg, with symptoms of impaired liver function (transaminases are more than two-fold the normal level for at least six months period, clinical results are compatible with chronic liver disease). Forty apparently healthy individuals who were negative for HBsAg (26-59 years old, mean 39±2.1 years, 31 males and 9 females) from the College of Medicine/ Al-Nahrain University were recruited as control group. Venous blood samples (3 mL) were collected from each participant in the EDTA vacutainer tubes.

DNA Extraction and Genotyping

Genomic DNA was extracted from leukocytes using ready kit (gSYNC^{TM} DNA Mini Kit Whole Blood Protocol/ Geneaid/ Korea) according to the manufacturer's instructions. The +874A/T SNP in the first intron of *IFN-γ* gene was amplified using two primers: forward 5'-TGATTCTGGCTAAGGAATGT-3', and reverse 5'-AATTGCAATGTCACAAATGA-3' with an expected 462 base pair size product. Polymerase chain reaction (PCR) was performed in a total volume of 50 µL master mix (Bioneer/Korea). Template DNA (10 µL) from each sample and primers (5 µL from each) were added to master mix tube. The mixture then put in shaker and spinner for 10 cycles for better mixing. After mixing, the master mix tubes were transferred to the thermocycler (MyGenie 32 thermal block/Bioneer/Korea). The reaction mixture was denaturated for 2 min at 94 ^{o}C, and then subjected to 35 cycles of denaturation at 94 ^{o}C for 1 min, annealing at 55 ^{o}C for 30 sec, extension at 72 ^{o}C for 2 min, and 7 min for final elongation at 72^{o}C. The amplified products were separated by electrophoresis on 1% agarose gel stained with ethidium bromide (Biobasic/Canada)(0.5 µg/ml). The gel was visualized under an ultraviolate transluminator with a 100 bp ladder (Kappa Biosystem/USA). A nanodrop (ACTGene/USA) was used to estimate the concentration of the PCR products which were all had concentrations >100ng/µl. These products were sent to Bioneer company/Korea for automated DNA sequencing.

Statistical Analysis

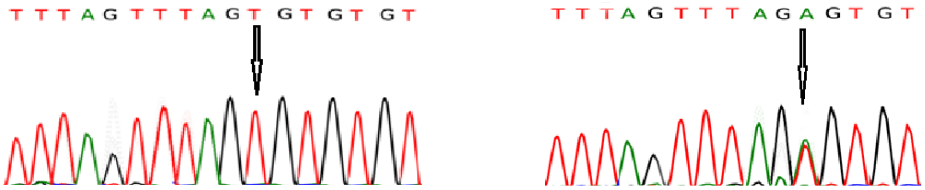
Data were analyzed using Chi-square test. SPSS software version 14.0 was used to calculate Odds Ratios (ORs) and 95% confidence interval (95%CI) by binary logistic regression.

Results

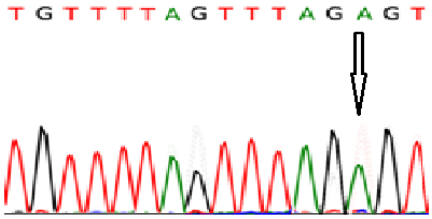
The distribution of IFN-γ+874A/T alleles in the two groups (patients and control) was in accordance with Hardey-Weinberg Equilibrium (HWE).

Results of DNA sequencing revealed three genotypes at the position of +874A/T which were TT, AT, and AA (figure 1), with frequencies of 17.7%, 48.38%, and 33.87% respectively in patients group, and 35%, 45% and 20% respectively in control (table 1). Logistic regression test revealed significant difference in the frequency of AA genotype between patients and control (OR= 3.341, 95%CI=1.075-10.387).

Allele A of the SNP had higher frequency among patients (58.06%) compared with control (42.5%) with significant difference (OR= 1.873, 95%CI=1.06-3.309). Further computation for risk estimates made under dominant and recessive model showed a significantly high risk for AA+AT genotype in recessive model (OR=2.497, 95%CI=1.0-6.265) (table 1).



(A) **(B)**



(C)

Figure 2: DNA sequencing for part of the first intron of IFN-γ gene. The arrow indicates the position of the SNP+874AlT. A: represents the genotype TT; B: AT and C: AA

Table 1: Genotypes and allele frequencies of the SNP +874A/T in patients and control

Variables	Cases N=62	Control N=40	P-value	OR	95%CI
Genotypes					
TT	11 (17.7%)	14 (35%)	0.105	1.0	
TA	30 (48.38%)	18 (45%)	0.134	2.121	0.794-5.665
AA	21 (33.87%)	8 (20%)	0.037	3.341	1.075-10.387
Alleles					
T	52 (41.9%)	46 (57.5%)	0.031	1.873	1.06-3.309
A	72 (58.06%)	34 (42.5%)			
Dominant model					
AA	21 (33.87%)	8 (20%)	0.133	2.049	0.803-5.226
AT+TT	41 (66.13%)	32 (80%)			
Recessive model					
AA+AT	51 (82.26%)	26 (65%)	0.051	2.497	1.0-6.265
TT	11 (17.74)	14 (35%)			

N: number, OR: odds ratio, CI: confidence interval

Discussion

Several immunoregulatory genes and proinflammatory cytokines take part in the host immune response to HBV infection (16). Among these is the IFN-γ which plays a role in modulating almost all the immune response, such as T-cells differentiation, anti-tumor, and antiviral activities (17).

Plasma levels of this cytokine are affected by many regulatory factors, among which is the activity of *IFN-γ* gene. Intron regions of the different genes are non-coding DNA sequences; however, these regions can influence the transcription and regulation of gene activities (18). Accordingly, SNPs in introns may affect the transcription of the gene therein, which is the case of +874T/A SNP (rs2430561, NCBI Refernce Sequence: NT_029419.12) in the *IFN-γ* gene.

Pravica et al. (19) reported that INF-g+874T/A directly influences IFN-γ production, and T and A alleles of this functional variant are associated with high and low production of IFN-γ respectively, while individuals carrying TA genotype have intermediate levels of this cytokine. This variation in IFN-γ production was attributed to the position of the SNP which lies within the transcription factor binding site of nuclear factor kappa light chain of enhanced B cell (NF-kB). Electophoretic mobility shift assay revealed specific binding of this factor to the allelic sequence containing +874T allele (19).

Based on these facts, one can deduce that individuals carrying A allele have relatively low levels of IFN-γ (aside from the other factors affecting IFN-γ levels), and thus less effective cell-mediated immune response compared with those carrying T allele. Our results confirmed this notion and indicated that individuals carrying TT genotype are less prone to develop chronic HBV infection compared with those carrying either AA genotype or AT genotype, and the presence of T allele seems to have protective characteristic against HBV infection in a co-dominance manner since the carriers of AT genotype have intermediate protection levels. These results do not agree with that of Conde et al (9) who did not find such association, may be due to the very small sample size of their study (23 chronic HBV patients), as well as the genetic variations among different populations. However, and despite the relatively small size samples, this study provided evidence for the role of the variant IFN-γ+874A/T in the susceptibility of chronic HBV infection.

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