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Association of P2X7 Gene Polymorphisms with Chronic Hepatitis B Virus Infection in Duhok, Iraqi Kurdistan

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Abstract

Host and viral factors are important determinants in the pathogenesis of chronic HBV infection. To date, polymorphisms in several genes such as tumor necrosis factor-alpha, tripartite motif-containing 22, have been found to contribute to the risk of developing chronic HBV infection. The polymorphism in P2X7 gene has recently been demonstrated to be associated with susceptibility or resistance to infectious diseases. However, still no clear association between P2X7 gene and chronic HBV infection has been reported in the literature. Hence, this study aimed to investigate whether two polymorphisms of P2X7 (1513 and -762) genes confer susceptibility to chronic HBV infection. In a case control study, single nucleotide polymorphisms (SNPs) in P2X7 (1513, -762) genes were assessed using allele-specific PCR and PCR- RFLP. Thereafter, frequency of the genotypes and alleles in patients and control groups were compared and analyzed. For the 1513 loci, the heterozygosity (AC) was higher in patients (73; 50.0%) than controls (14; 23.3%) [P = 0.001, OR 3.286, 95% CI 1.587-6885]. Furthermore, we found that the C allele was a risk factor for predisposition to chronic HBV infection (P = 0.006, OR 2.247, 95% CI 1.207-4.231). For the -762 loci, there were no significant statistical differences between the case and control groups in the genotype and allele frequencies (P > 0.05). In conclusion, in our study population, the P2X7 gene polymorphisms could be associated with susceptibility to chronic HBV infection.

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Keywords: P2X7 gene; polymorphism; chronic hepatitis B.

1. Introduction

Although a safe and effective vaccine is available, chronic hepatitis B virus (HBV) infections remain a major public health problem affecting around 400 million people in the world [1]. Fifteen percent to 40% of chronic HBV infection will eventually develop serious complications in term of cirrhosis, hepatic decompansation and hepatocellular carcinoma (HHC) [2]. Host and viral factors are important determinants in the pathogenesis of chronic HBV infection. To date, polymorphisms in several genes such as tumor necrosis factor-alpha (TNF-alpha), tripartite motif-containing 22 (TRIM22), have been found to contribute to the risk of developing chronic HBV infection [3, 4]. The polymorphism in P2X7 gene has recently been demonstrated to be associated with susceptibility or resistance to infectious diseases [5]. The P2X7 encodes for the P2X7 receptor, a plasma membrane receptor which mediates adenosine triphosphate (ATP) induced autophagy [6]. Activation of P2X7 by ATP causes immediate opening of a cation selective channel, permitting the influx of Ca2+ and Na+ and the efflux of K+. This process induces caspase cascade with resultant apoptosis and activation of phospholipase D, which promote phagosome-lysosome fusion, causing intracellular death of microorganisms [7]. There are more than 30 single-nucleotide polymorphisms (SNPs) in the P2X7 gene. The most common variant is 1513AC that produces a change of glutamic acid to alanine at residue 496 (Glu496 to Ala) in the C terminus, which impairs multiple P2X7 receptor functions [8]. Another polymorphism in the promoter region of -762TC has been suggested to alter P2X7 gene expression; however experimental evidence is lacking to find a clear association with infectious diseases [9]. The P2X7 receptor appears to be a necessary component of hepatitis B virus entry into susceptible cells during infection [10]. However, still no clear association between P2X7 gene and chronic HBV infection has been reported in the literature. Hence, this study aimed to investigate whether two polymorphisms of P2X7 (1513 and -762) genes confer susceptibility to chronic HBV infection.

2. Materials and methods:

2.1. Specimen collection:

The study included 146 chronic HBV infected patients who were managed in hepatitis clinic, Azadi Teaching Hospital, Duhok from November 2011 to November 2012. Additionally, sixty healthy individuals were studied as controls. The study was approved by the ethics committee of Faculty of Medical Sciences, University of Duhok, Iraqi Kurdistan. Patient and control subjects were matched for age, gender, race and nationality. The frozen plasma specimens were shipped to Mycobacteriology Research Center (MRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Tehran, Iran for genomic DNA isolation and genotyping.

2.2. DNA Isolation:

Genomic DNA was extracted using the standard protocol with slight modifications [11]. Briefly, peripheral blood leukocytes (PBLs) were separated from two milliliters of the whole blood using RBC lysis buffer (0.155 M NH4Cl, 0.01 M NaHCO3). Thereafter, PBLs was re-suspended in 500 µL of SE buffer (NaCl 3M, EDTA

0.5M, PH = 8), containing 40 µL of 10% SDS and 3 µL of 20 mg/mL of proteinase K. The suspension was incubated at 600°C for 30 minutes. After incubation, 200 µL of equilibrated phenol (PH = 8) was added to the mixture and centrifuged for 10 minutes at 12000 g. The aqueous phase was transferred to a new tube and the DNA was precipitated using cold propanol [11].

2.3. P2X7 Genotyping

P2X7 gene polymorphism was studied using allele-specific PCR and PCR- RFLP. For P2X7 gene polymorphisms at -762, two outer primers [P2X73 (5'-GAAACAGGGCCCTGGGTCCTC-3', forward) and P2X74 (5'-TGGTGGGGGTGGAGGGGC- 3', reverse)] and two inner primers [P2X75 (5'-GGTGTCCCTCACTGAATAGGTCAAT-3', forward and P2X76 (5'-GGCAGTCCAACAAAGTTAGGTTTG-3', reverse)] were used. For the -762°C allele, a 235 bp fragment was amplified using the outer forward (P2X73) and inner reverse (P2X76) primers. For the -762 T allele, a 186 bp fragment was amplified using the inner forward (P2X75) and outer reverse (P2X74) primers [12, 13]. The amplification was accomplished by an initial denaturation at 94°C for 5 minutes and 30 cycles at 95°C for 30 seconds, at 65°C for 30 seconds, at 72°C for 45 seconds, followed by an extension at 72°C for 10 minutes. The amplified PCR products for CC, CT and TT had the following sizes 235 bp, 186+235 bp and 186 bp, respectively (Figure 1). For P2X7 gene polymorphisms at 1513, the primers 5′ACTCCTAGATCCAGGGATAGCC3′ and 5′TACAGACGTGA GCCACGGT 3′ were used to amplify the 417 bp product (9, 10). The PCR product was digested with 4U enzyme of Hae II. The digested PCR products were run on 8% polyacrylamide gel, which was stained with ethidium bromide. The digest pattern for AA, AC and CC are as follows; 209 + 143 + 65 bp; 209 + 143 + 117 + 92 + 65 bp and 143 + 117 + 95 + 65 bp, respectively (Figure 2).

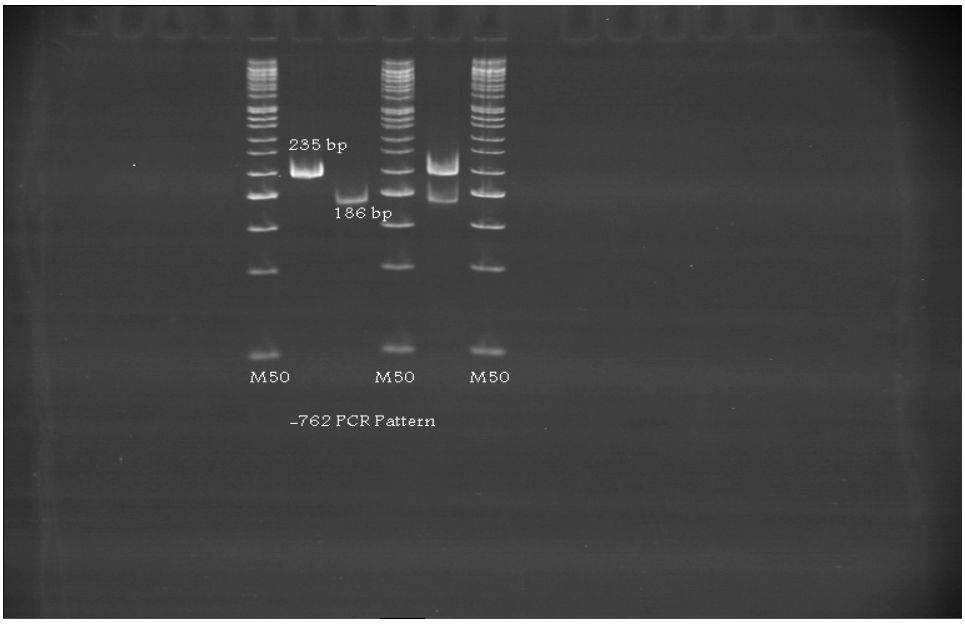

Figure 1: The -762 Amplified PCR Products, CC, CT and TT genotypes had 235 bp, 186+235 bp and 186 bp, respectively

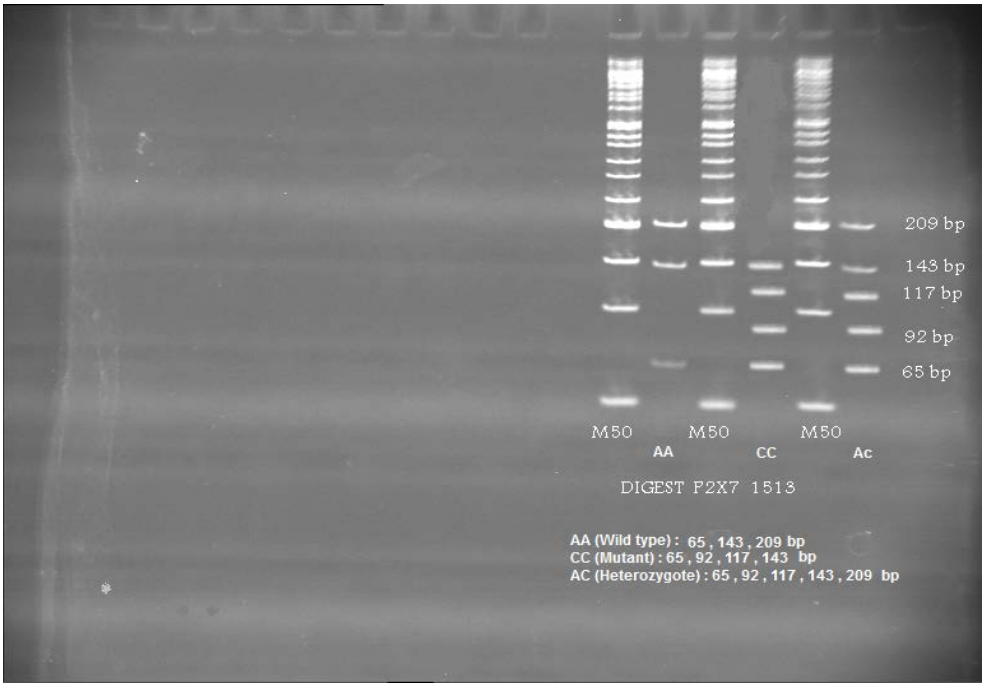

Figure 2: Digest Pattern of 1513 P2X7 Gene.

2.4. Statistical Analysis

The frequency of the genotypes and alleles in patients and control groups were estimated by direct gene counting and then the data was analyzed using SPSS version 11 (SPSS Inc., Chicago, IL, USA). All P values were two tailed. A P value of less than < 0.05 was considered significant with 95% confidence interval (CI).

3. Results:

Three genotyping patterns were reported with 1513 and – 762 P2X7 polymorphisms; frequent homozygous genotype (wild type), infrequent heterozygous genotype and infrequent homozygous genotype (mutant type). For the 1513 loci, the heterozygosity (AC) was higher in patients (73; 50.0%) than controls (14; 23.3%) and the difference was statistically significant (P = 0.001, OR 3.286, 95% CI 1.587-6885). In contrast, the frequency of wild type (AA; 75.0% versus 49.3%) was significantly higher in control cases (P = 0.001, OR 0.324, 95% CI 0.157-0.663). Furthermore, we found that the C allele was a risk factor for predisposition to chronic HBV infection (P = 0.006, OR 2.247, 95% CI 1.207-4.231). (Table 1).

With regard to the -762 loci, the genotypes were TC (142, 97.2%); TT (2, 1.4%); CC (2, 1.4%) in the patient. Whereas, in the control group, the genotypes were TC (59, 98.3%); CC (1, 1.7%); TT (0, 0.0%). There were no significant statistical differences between the case and control groups in the -762 genotype and allelic frequencies ($P > 0.05$) (Table 2).

Table 1: Frequency distribution of the P2X7 - 1513 polymorphism in patients with chronic hepatitis B virus and in control subjects

Genotype/Allele		HBV Patient	Control	Odd ratio (95% CI)	P value
Genotype	AA (frequent type)	72 (49.3%)	45 (75.0%)	0.324 (0.157-0.663)	0.001
	AC (heterozygote mutant)	73 (50%)	14 (23.3%)	3.286 (1.587-6.885)	0.001
	CC (homozygote mutant)	1 (0.7%)	1 (1.7%)	0.407 (0.011-15.167)	0.499
Allele	A	217 (74.3%)	104 (86.7%)	2.247 (1.207-4.231)	0.006
	C	75 (25.7%)	16 (13.3%)		

Table 2: Frequency distribution of the P2X7 - 762 polymorphism in patients with chronic hepatitis B virus and in control subjects

Genotype/Allele		Patient HBV	Control	Odd ratio (95% CI)	P value
Genotype	TT (frequent type)	2 (1.4%)	0 (0.0%)	inf (0.023-inf)	1.000
	TC (heterozygote mutant)	142 (97.2%)	59 (98.3%)	0.602 (0.025-5.895)	1.000
	CC (homozygote mutant)	2 (1.4%)	1 (1.7%)	0.819 (0.057-23.296)	1.000
Allele	T	146 (50.0%)	59 (49.2%)	0.967 (0.618-1.513)	0.914
	C	146 (50.0%)	61 (50.8%)		

4. Discussion

Hepatitis B virus is one of the most common viral infections affecting human being. Genetic variability in the host plays an important role in the pathogenesis of HBV infection. Several genes such as TNF-alpha (3), TRIM22 (4) and mannose-binding protein (MBP) [14] have been implicated in susceptibly to chronic HBV infection. Recently, P2X7 has been suggested to be essential component of HBV entry into susceptible hepatocytes [10]. Although P2X7 has been studied extensively in infectious diseases such as susceptibility to *M. tuberculosis* [5], limited studies were performed for HBV infection. In this regard, we have investigated the possible association between P2X7 gene polymorphism and susceptibility to chronic HBV infection, which may provide a novel finding in the field of HBV infection.

In this study, we found a higher frequency of 1513AC genotype in patients (50.0%) than control (23.3%), which appeared to be a risk factor of developing chronic HBV infection. This finding supports previous studies, which indicates that appropriate expression and functionality of P2X7 could be a major contributor to the specific targeting of HDV and HBV to human hepatocytes [10]. Additionally, it is well known that the P2X7 receptor can mediate cell death, killing of infectious microorganisms, and regulation of the inflammatory cytokines [5].

In contrast, the frequency of wild type (AA genotype) was significantly higher in control group (P = 0.001), which indicates its protective effect against chronic HBV infection. Furthermore, we found that the 1513C allele is a risk factor for chronic HBV infection in our study population. Our observation was supported by other investigators who documented that the 1513C allele is a risk factor for the development of tuberculosis in the North Indian Punjabi population [15]. Hence, the present study demonstrates that polymorphism in P2X7 1513 genotype can be used as a tool for identifying genetic susceptibility to chronic HBV infection.

In our study, -762TC genotype was not associated with chronic HBV infection among the study population. Similar studies stated that P2X7 polymorphisms may or may not be associated with resistance or susceptibility to infectious diseases [16]. The polymorphism in -762 does not appear to correlate with altered receptor expression and hence does not suggest genetic factor for susceptibility to disease [17]. However, a study by Bahari et al. found a significant association between -762TC and tuberculosis [18]; whereas, other studies found a protective effect against tuberculosis [19]. With regard to -762 T allele, we did not find an association with chronic HBV infection. In contrast to this study, other researchers found a protective effect of the C allele at position -762 in the P2X7 promoter region, which supports a putative role for this gene in human immunity to infectious diseases [17]. We therefore concluded in this study that -762TC genotype and T allele are not associated risk factor of chronic HBV infection.

To the best of our knowledge, this is the first report to investigate the relation of P2X7 gene polymorphisms with susceptibility to chronic HBV infection. In conclusion, the present study supports the P2X7 gene polymorphism could be associated with susceptibility to chronic HBV infection. Further prospective studies of these polymorphisms are warranted to identify people who are at high risk of developing chronic HBV infection.

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