



RAPID COMMUNICATION

Polymorphisms of interleukin-1R receptor antagonist genes in patients with chronic hepatitis B in Iran

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Abstract

AIM: To investigate the relationships between polymorphisms of interleukin-1R receptor antagonist genes and susceptibility to chronic hepatitis B in Iran population.

METHODS: Genomic DNA was extracted from the peripheral blood of 80 patients with chronic hepatitis B (57 males, 23 females) aged 12-77 years (mean 36.1 ± 13.8 years) and 147 normal controls (96 males, 51 females) aged 6-75 years (mean 41 ± 18.7 years) who referred to a liver clinic of Tehran and then subjected to polymerase chain reaction (PCR) amplification. PCR products were resolved on a 3% agarose gel and stained with ethidium bromide.

RESULTS: Only three of the five kinds of polymorphism (2/2, 2/4, and 4/4) were found in this study. The frequencies of 2/2, 2/4, and 4/4 were 12.5%, 17.5%, 70% respectively in chronic hepatitis B patients and 6.8%, 24.5%, and 68.7% respectively in controls. IL-1 R allele 2 was detected in 30% of chronic hepatitis B patients and in 31.3% of controls, while IL-1 R allele 4 was detected in 87.5% of chronic hepatitis B patients and in 93.2% of controls. The frequency of IL-1R alleles 2 and 4 was detected in 21.25% and 78.75% of the patients and 19.04% and 80.96% of the controls, respectively.

CONCLUSION: Our results suggest that the carriage of IL-1R receptor antagonist alleles 2, 4, 6 may not play any role in the development of HBV infection. Large population-based studies are needed to investigate the role of IL-1 polymorphisms in the pathogenesis of developing chronic hepatitis B.

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the most important chronic viral diseases in the world. An estimated 400 million people worldwide are carriers of HBV, and approximately 250 000 deaths occur each year as a consequence of fulminant hepatic failure, cirrhosis, and hepatocellular carcinoma^[1]. According to the last health and disease survey held in Iran in 1999^[2], the prevalence of HBV carriers is 1.7%. Therefore over one million people in Iran are HBV carriers. When HBV is acquired in adulthood, the majority of infections are cleared, with chronic infection occurring in 5%-10% of cases. However, the dynamic interaction of the host inflammatory response to HBV and the subsequent impact of this interaction on the clinical outcome of HBV infection, are not yet fully understood, nor are the underlying mechanisms for persistence of the virus.

Cytokines play an important role in defense against viral infection, indirectly through determination of the predominant pattern of the host response and directly through inhibition of viral replication^[3]. Interleukin (IL)-1 is one of the most pro-inflammatory agents and has a central role in inflammation and destruction^[4]. The most important members of the IL-1 family are the IL-1 α , IL-1 β , and IL-1 receptor antagonists (ra). IL-1ra is an IL-1 natural competitive inhibitor, acting by occupancy of cell surface receptor without triggering signal transduction^[5]. IL-1ra plays a role as an important regulator of inflammation and is currently evaluated in clinical trials. Genes encoding IL-1 are located on the 430 kb region of chromosome 2q13-21, consisting of three homologous genes: IL-1A, IL1B and IL-1ra (IL-1RN)^[6]. Biallelic polymorphisms at positions IL-1A -889, IL-1B -511, and

Table 1 Comparison of IL-1R intron 2 polymorphism between chronic hepatitis B patients and controls

	<i>n</i>	Genotyping (%)						Allele frequency (%)			
		2,2	<i>P</i>	2,4	<i>P</i>	4,4	<i>P</i>	2	<i>P</i>	4	<i>P</i>
IL-1R											
Controls	147	6.8	0.148	24.5	0.225	68.7	0.840	19.04	0.840	80.96	0.148
Chronic hepatitis	80	12.5		17.5		70.0		21.25		78.75	

+ 3953 have been described, all representing a C/T single nucleotide polymorphism (SNP). IL-1RN contains an 86 bp variable number tandem repeat (VNTR) polymorphism in intron 2^[7]. These polymorphisms are located within the regulatory regions of the genes and have a potentially functional importance by modulating IL-1 protein production, and are related with the development of some diseases^[8].

This study was to discuss the relationship between polymorphisms of IL-1R gene and susceptibility to HBV in the Iranian population, and to reveal the correlation between the genotype and phenotype distributions, in order to provide a certain scientific basis for prevention and treatment of chronic hepatitis B.

MATERIALS AND METHODS

Subjects

A total of 80 patients with chronic hepatitis B (57 males, 23 females) aged 12-77 years (mean 36.1 ± 13.8 years) were recruited in a liver clinic of Tehran. The diagnosis of all the patients was confirmed according to the criteria for chronic hepatitis B, and the patients did not have other viral hepatitis. One hundred and forty-seven control subjects (96 males, 51 females) aged 6-75 years (mean 41 ± 18.7 years) were selected in a liver clinic of Tehran (HBsAg negative, anti-HBe negative, and anti-HBc negative). Liver, renal, endocrine and cardiovascular disorders were excluded. There was no statistical difference in sex between case and control groups, and we also saw a statistically significant difference in age between two groups ($P = 0.026$) but it was not clinically important. AST was over 40 U/L in 24.1% of cases and none of controls, and over 40 U/L in 31.6% of cases and none of controls, respectively.

PCR preparation

Two microliter peripheral venous blood was collected in an EDTA tube. Genomic DNA was extracted from peripheral blood leukocytes as previously described^[9]. Each PCR was carried out in 25 μ L reaction mixture containing 100-200 ng genomic DNA, 100 μ mol/L dNTP, 25 mmol/L $MgCl_2$, 20 pmol/L primers and 1U TaqDNA polymerase (DNA Technology)^[10].

The sequence of the forward primer is 5'-CTCAGCAACACTCCTAT-3'. The reverse primer sequence is 5'-TCCTGGTCTGCAGGTAA-3'. PCR conditions included an initial denaturing step at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, at 60°C for 1 min, at 70°C for 2 min, and a final extension at 70°C for 4 min. Using this PCR strategy, the common allele (allele 1) generated a 410-bp band (including four copies of an

86-bp repeat). The uncommon alleles generated a 240-bp band (two copies of the same repeat, allele 2), a 500-bp band (five copies of the same repeat, allele 3), and a 325-bp band (allele 4). The PCR products were resolved on a 3% agarose gel and stained with ethidium bromide.

HBV-DNA measurement

Serum HBV-DNA levels in patients with chronic hepatitis B were detected with the real-time fluorescent quantitative PCR method (reagents supplied by Sinagen Co. Ltd.). Results were considered abnormal when HBV-DNA > 200 copies/mL.

Statistical analysis

IL-1R (2-6) allele and genotype frequencies were calculated in patients with chronic hepatitis B and control subjects. Comparison of allele and genotype frequencies between groups, and association of IL-1R polymorphisms with HBV-DNA replication and other variables were examined for statistical significance with chi-square test. Analysis was completed with SPSS11.5. $P < 0.05$ was considered statistically significant.

RESULTS

IL-1R (2-6) allele frequencies were measured. IL-1R 3, 5, 6 were negative in all cases and controls. Therefore we deleted them in our results.

IL-1R genotypes

The intron 2 of IL-1RN polymorphism contained VNTR of 86 bp. There were five alleles in humans, including allele 1 (four repeats, 410 bp), allele 2 (two repeats, 240 bp), allele 3 (five repeats, 500 bp), and allele 4 (four repeats, 325 bp). Only three of the five kinds of polymorphism of IL-1R (2/2, 2/4, and 4/4) were found in this study.

Frequencies of IL-1R genotypes in both groups

The genotype and allele frequencies of IL-1RN in patients with chronic hepatitis B and control subjects were determined, and explored with 2×2 chi-square test (Table 1).

Only three of the five kinds of polymorphism of IL-1R (2/2, 2/4, and 4/4) were found in this study. The frequencies of 2/2, 2/4, and 4/4 were 12.5%, 17.5%, 70% respectively in chronic hepatitis B patients, and 6.8%, 24.5%, and 68.7% respectively in controls. IL-1R allele 2 was detected in 30% of chronic hepatitis B patients and 31.3% of controls, while IL-1R allele 4 was detected in 87.5% of chronic hepatitis B patients and 93.2% of controls. Allele frequency of IL-1R alleles 2 and

4 was detected in 21.25% and 78.75% of the patients and 19.04% and 80.96% of controls respectively.

There were no significant differences between patients and controls in these regards. However, the 2, 2 and 2, 4 and 4, 4 genotypes were not significantly different between patients and controls, which were 6.8% versus 12.5% ($P = 0.148$), 24.5% *vs* 17.5% ($P = 0.225$), and 68.7% *vs* 70% ($P = 0.840$) respectively. The genotype distribution in controls was also consistent with the Hardy-Weinberg equilibrium ($P = 0.215$).

DISCUSSION

HBV infection is a major global health problem with an estimated 300 million people chronically infected worldwide^[11,12]. Individuals with an inadequate primary immune response to HBV are at increased risk of developing chronic hepatitis B. Age is the strongest host feature associated with chronic infection with 90% infants and 5%-10% of adults developing chronic hepatitis B after exposure to HBV. In addition, people with the same age, sex and ethnical group are exposed to the same HBV strain, which could cause a broad spectrum ranging from no infection to different clinical outcomes^[13]. These data suggest that host genetic factors are responsible for the clinical outcomes of HBV infection. Clearance of HBV requires a coordinated innate and adaptive humor- and cell-mediated immune response. Cytokines are soluble polypeptide molecules that mediate cell-to-cell communication and regulate the intensity and duration of the immune response. Previous studies have shown that the maximal capacity of cytokine production varies among individuals and correlates with SNP in the promoter region of various cytokine genes^[14,15]. Furthermore, cytokine gene polymorphisms are associated with liver disease severity in patients with viral hepatitis^[16]. In the present study, we compared the distributions of interleukin-1R receptor antagonist gene polymorphisms between patients with chronic B and control subjects.

Interleukin-1R receptor antagonist gene is one of the IL-1 gene family members, and located in the proximal region of chromosome 2q13-21. Different polymorphisms have been described in interleukin-1R receptor antagonist genes, and at least two of them could influence the protein production. The IL-1ra gene is also polymorphic due to a variable number (2-6) of tandem repeats of 86 bp (VNTR) within its second intron^[17]. This polymorphism has been shown to be unambiguously functional at the level of secreted protein, as monocytes from individuals homo- or heterozygous for allele 2 (IL-1Ra A2 1, IL-1RN * 2, 2 repeats) produce significantly more IL-1ra in response to GM-CSF^[18].

Pociot *et al*^[19] reported that IL-1B polymorphisms are correlated with IL-1 β expression. IL-1B allele T carrier has higher productions of IL-1 β than IL-1B allele C carrier. In the present study, genotype distributions and allele frequencies for IL-1B (-511) promoter polymorphisms in patients with chronic hepatitis B and control subjects were not statistically different. Further analysis of the relationship between IL-1B polymorphism and HBV-DNA

replication in patients with chronic hepatitis B showed that IL-1B (-511) genotype CC was associated with HBV-DNA replication.

IL-1Ra is a naturally occurring anti-inflammatory protein, competitively blocks the binding of IL-1 α and IL-1 β type I and type II IL-1 receptors, but exerts no agonist activity, despite sharing 30% amino acid sequence homology with IL-1 β , and 19% with IL-1 α . IL-1ra has been shown to inhibit the effects of IL-1 both *in vitro* and *in vivo*^[20]. There is increasing evidence that IL-1RN polymorphisms are related with susceptibility to individual diseases, including psoriasis, systemic lupus erythematosus, and inflammatory bowel disease^[4]. By the study of interleukin-1R receptor antagonist gene intron 2 polymorphisms, our results suggested that the distributions of interleukin-1R receptor antagonist gene 2/2, 2/4, 4/4 genotype in patients with chronic hepatitis B were not significantly different from those in control subjects. A possible explanation of this result could be provided by the fact that carriage of IL-1R allele of each of these polymorphisms may be related with other production of IL-1 β ^[21], which may augment the production of other cytokines, such as IL-2, IL-6 and TNF- α , and trigger the complex immunological processes to eliminate the virus and its complex. It may be also due to the low sample size of our study (The power of study was 68%).

In summary, our results suggest that the carriage of IL-1R receptor antagonist alleles 2, 4, 6 may not play any role in the development of HBV infection. Large population-based studies are needed to investigate the role of IL-1 polymorphisms in the pathogenesis of developing chronic hepatitis B.

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