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Original Article

Correlation between Polymorphism of -56 SNP (T/C) Interferon-γ Receptor 1 Gene and Chronic HBV Infection

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Abstract

Background and Aims: chronic HBV infection is one of the most common viral infections in worldwide which many factors such as genetic factors are involved in pathogenesis of disease. Gamma interferon (IFN-γ) and its receptor (INFGR) play a critical role in the immune response to HBV infection. Single nucleotide polymorphisms (SNPs) are effective on level of gene expression, The aim of this study is explore the effect of -56T/C(SNP) located in promoter of gamma interferon receptor1 (INFGR1)gene on chronic HBV infection.

Methods: Genomic DNA from peripheral blood samples of 150 chronically HBV infected patients and 150 healthy controls was extracted by phenol-chloroform method and DNA analysis and genotyping was performed by PCR-RFLP method.

Results: According to obtained genotyping and also statistical analysis, it was observed that between the patients and control group a significant difference existed and the genotypes of TC and CC were high in control group compared to the patients group.

Conclusion: The host genetic factors can plays an important role in pathogenesis of HBV infection, Genetic variations in INFGR1 was related to several diseases, in this study we surveyed association between -56T/C (SNP) in INFGR1 and chronic HBV infection, the results of our study showed that presence of C and TC alleles in our population is related to decrease risk susceptibility to chronic infection.

Keywords: Hepatitis B Virus; RFLP; gamma interferon receptor

Introduction

Infection with hepatitis B virus (HBV) is one of the most prevalence viral infections, About 400 million chronic HBV infection is in worldwide. HBV infection causes several clinical forms of the disease such as; acute infection which during disease infection is cleared from body within 6 months of and chronic infection that the patient cannot be cleared from the virus during this of period. Chronic infection can led to serious consequences such as hepatocellular carcinoma and liver cirrhosis, Approximately 90% of adults that are infected with the virus to toward the acute infection and indeed the infection in them is self-limited, whereas about 5 to 10% of individual's progress to the chronic infection. The various factors are involvement in formations of clinical courses HBV infection, in following are some of the most important of factors which could be determinant of various clinical forms, such as: viral factors (genetically and genotyping diversity, viral

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load), immunological factors, genetic host factors and environments factors (1).

In recent years many studies have been conducted on the viral and immunological factors, whereas studies on genetic factors is in early stages. Many epidemiological evidences indicated which host genetic background plays an important role in the emergence of clinical outcome of HBV infection. Also between different countries from the geographical point view have been seen variations in HBV prevalence. In chronic infection about 20 to 30% of cases progress to liver cirrhosis and 5% to toward liver cancer. Also 70 to 90% of babies that are infected from mothers progress to chronic infection. (2)

Cytokines played an important role in immune response to viral infection, and directly or indirectly can inhibit viral replication (3). one of the most cytokines which play a determinant role in immune responses to viral infections specifically persistent infections such as chronic HBV infection is IFN-γ, this cytokine is produced majorly by TCD8 cells but other immune cells such as APCs (antigen processing cells) and NK cells also can be producer it (4). IFN-γ has many biological functions in immune system including; increase antigen processing and activation of macrophages, inhibition of activation of Th2 cells, increase expression of MHC molecules on cell surface, leukocyte migration and stimulation of NK cells (4)

Variation in cytokine production is associated with polymorphism in within or closely related gene. The host genetic background especially single nucleotide polymorphisms (SNPs) are is considered as one of the determinants of clinical heterogeneous in pathogenesis of HBV infection (5). In transgenic mice infected with HBV has been observed which a large amount of IFN-γ and TNF-α against virus antigens released by TCD8 cells (5). Effect of IFN-γ through its receptor, the gamma interferon receptor (INFGR) made up of INFGR1 and INFGR2. INFGR1 plays a vital role in ligand binding, signal transduction and receptor trafficking (6). It has been reported which polymorphism in IFN-γ and its receptor is related to pathogenesis of chronic HBV infection (7-9). With to regards reports and evidences in studies on the host genetic factors can provide valuable and crucial information in association with different clinical course and susceptibility to HBV infection. In the present report, we explored the relationship between -56SNP(C/T) marker in the IFNGR1 gene and susceptibility to chronic HBV infection in Iranian population.

Methods

The study included 150 chronic patients positive for HBsAg, including all of the patients referred to taleghani hospital during 2010-2011, with history of impaired liver function test (transaminases over two times higher than the limit of normal for at least a 6month period), detectable IgG anti-HBc and/or sonographic and clinical findings compatible with chronic liver disease. 150 blood donors, who were negative for both HBsAg and anti-HBc, were included control subjects. Table 1 shows characteristics of in study participants. They were matched to the chronic HBV cases for age and gender. None of them had history of excessive alcohol consumption or intravenous drug abuse, and all were negative for anti-HCV and anti-HIV anti body. Patients with another cause of liver disease (Wilson's disease, hemochromatosis, α-1 antitrypsin deficiency) or history of

Table 1. Characteristics of primer and restriction enzyme for genotyping.

SNP(position)	PCR primer sequence(5′-3′)	RE	Allele phenotype
-56C/T(p)	F: TGCATGACAAGGGGTAGGAG	AfeI	T:430bp
	R: CAACCAGGTGAAGTCCAAGAG		C:339+91bp

P*:* promoter, **RE***:* restriction enzyme

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receiving immunosuppressive and anti-viral drugs were excluded.

The blood specimen was obtained from each participant and DNA was extracted from buffy coat using phenol-chloroform method. A region of INFGR1 gene promoter containing single nucleotide polymorphism (SNP) at position -56 was amplified by PCR method using primer sequences that were designed according to the data achieved from the NCBI data bank (http:www.ncbi.nlm.nih.gov). The resulting PCR products were digested with restriction enzymes then segregated using agarose gel electrophoresis. Table 1 shows the Primer and restriction enzymes for genotyping. In order to confirm genotyping by PCR-RFLP method, 10% from samples randomly sequenced using direct sequencing by ABI genetic analyzer 3130xl system.

Statistical methods

The data were analyzed by chi-square test according to the Hardy–Weinberg equilibrium (HWE) and independent sample t test, with a P value less than 0.05 as statistical significance. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated by binary logistic regression (SPSS software version 16.0).

Results

Characteristics of patients and healthy control subjects for age and gender index is presented in Table 2. As it is shown no difference between the patient and control groups was observed for gender and age.

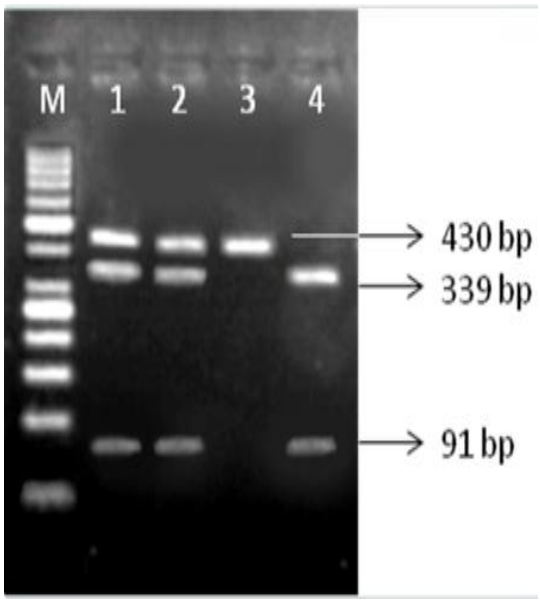

Fig. 1. the results of digestion by restriction enzymes. *M* marker 50bp, *1,2* genotype TC, *3* genotype TT, *4* genotype CC.

It PCR test a 430bp fragment containing the interested polymorphism was obtained after digestion with restriction enzyme produced several fragments including 339bp, 91bp and a 430bp unrestricted fragment (figure 1).

For candidate SNP, genotyping was performed in 150 patients and 150 healthy controls by PCR-RFLP method (figure 1).

The candidate SNP met the Hardy–Weinberg equilibrium. The results of sequencing were indicator of accuracy of genotyping by PCR-RFLP method (figure 2). There was a significant difference between the case and Control groups so that CC and TC genotypes

Table 2. Shows characteristics of study participants.

		Patient	control	p dominant
Gender	**Male**	83	74	0.876
	Female	67	76	
Mean age		42/25 ± 15/16	41/31 ± 17/11	0.625

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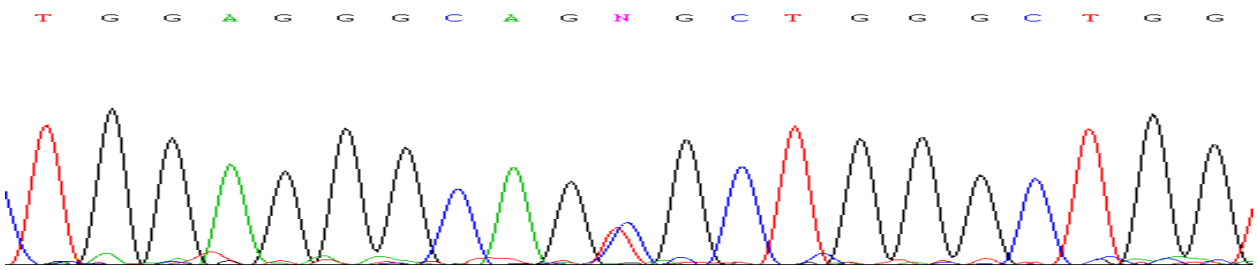

Fig. 2. The result obtained from sequencing related to a heterozygote genotype that was selected randomly in among of samples and indicator of accuracy of genotyping by PCR-RFLP method.

Table 3. Genotype distributions of SNPs in study participants.

Polymorphism	genotype	patient %	control%	OR(95%CI)	P-value
-56SNP(T/C)	TT	38%	22%	1	
	TC	44%	50.7%	1.989 (1.158-3.416)	0.013
	CC	18%	27.3%	2.623 (1.372-5.014)	0.004

were higher in the healthy control group compared to patient group.

Distribution of genotypes achieved by PCR-RFLP was analyzed for frequency, chi square test and logistic regression. Table 3 shows the frequencies of genotypes in patients and healthy controls.

Regarding the results achieved via genotyping and statistical analysis interested SNPs (Table 3), we observed a significant association (P<0.004) between patient and control groups. In the -56T/C (SNP) homozygosity for the –56C variant (CC genotype) and heterozygosity for -56TC was found to be associated with healthy control group with decrease risk to toward chronic infection.

Discussion

Ability of the immune response for control of initial infection determines the clinical course of HBV infection. Chronic HBV infection is considered as multifactorial disorder that many factors are effective on pathogenesis of it such as viral factors, environment factors and special genetic factors (10, 11). With regarded to the evidences reported in studies using laboratory animals especially transgenic mice it is now well known that the expression of cytokines can reduce HBV replication without effect on hepatocytes (12). Recently it has been emphasized the importance of IFN-γ in the clearance of chronic HBV infection, because studies has well shown which in self-limited infections during acute infection Th1 cells can produce a large amount of IFN-γ and this cytokine is dominant, On the other hand the cell clones obtained from chronic persons has shown which cytokine production by Th2 are dominant (13, 14).

Changes in the binding site of transcription factors may influence the performance of a promoter and symptoms of a disease (15). Polymorphism in the promoter of INFGR1 was associated with several of diseases (16). In 2009 Isomi Naka and colleagues examined the effect of -56T/C SNP in the promoter of (INFGR1) and its association with malaria at the Thai ethnic people but was not observed significant difference between this genotype and disease (17). Has been studied association between several single nucleotide polymorphisms within promoter of INFGR1 and chronic HBV infection, there was found that from the several polymorphisms -56T/C (SNP) is related to infection (18). Kardom and

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colleagues have been studied association between two SNPs located in promoter of INFGR1 and tuberculosis infection, they could identified -56T/C and -611G/A SNPs in promoter of gene, the results of their study showed that the -56 T/C SNP was associated with chronic infection(19).

It seems that, host genetic background can play an important role in controlling HBV infection and its clinical consequences, As mentioned response to INF-γ is through a receptor dimer which is comprised of two chains, INFGR1which plays a role in binding to ligand (INF-γ) and INFGR2 which is involved in signaling pathway. Any defect in INFGR function can be effective on the eradication of infectious diseases, especially viral infections such as chronic HBV infection. The bases on important role of INF-γ and also critical role of INFGR1 in signaling for eradication of HBV infection we examined an important variation within promoter of INFGR1 gene, According our results it seems that genetic variations in promoter of INFGR1 may affect the clinical course of HBV Infection.

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