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ORIGINAL RESEARCH ARTICLE

Cytochrome P450 Genes (CYP2E1 and CYP1A1) Variants and Susceptibility to Chronic Hepatitis B Virus Infection

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Abstract Hepatitis B virus (HBV) infection is a worldwide health concern which is associated with significant morbidity and mortality. Both viral and host factors have a significant effect on infection, replication and pathogenesis of HBV. The aim of this study was to investigate the effect of *CYP2E1* and *CYP1A1* genetic variants on susceptibility to HBV. 143 individuals including 54 chronic HBV patients and 89 healthy controls were enrolled in the genotyping procedure. rs2031920 and rs3813867 at *CYP2E1* as well as rs4646421 and rs2198843 at *CYP1A1* loci were studied in all subjects using PCR–RFLP (restriction fragment length polymorphism) analysis. Both variants at *CYP2E1* locus were monomorphic in all studied subjects. Genotype frequency of rs4646421 was significantly different between chronic HBV patients and healthy blood donors ($P = 0.04$, OR 4.31; 95% CI 1.04–17.7). Furthermore, individuals carrying at least one C allele (CC or CT genotypes) for rs4646421 seemed to have a decrease risk of hepatitis in comparison with TT genotype ($P = 0.039$). Our results showed a relationship between rs4646421 TT genotype (rare genotype) and the risk for developing chronic HBV infection (four times higher). Further studies are needed to examine the role of CYP1A1 polymorphism in susceptibility to chronic HBV infection.

Keywords Hepatitis · Cytochrome P450 · CYP2E1 · CYP1A1 · RFLP

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Introduction

Hepatitis B virus (HBV) infection is an important global health problem which may lead to significant morbidity and mortality especially in developing countries [1]. The World Health Organization (WHO) has estimated that 2 billion people have been infected worldwide with HBV, among which more than 350 million are chronically infected [2] with the highest prevalence being observed in Southeast Asia, sub-Saharan Africa and Greenland [3]. The infection can cause acute and chronic liver diseases, including cirrhosis and hepatocellular carcinoma [4]. It has been well documented that both viral and host factors have a significant effect on infection, replication and pathogenesis of HBV [5–7]. The host factors for HBV pathogenesis include environmental factors (alcohol and aflotoxin) and genetic factors. Therefore, some host genes may act as strong contributors to disease outcome

[8]. Genes controlling drugs and metabolism such as cytochrome P450 (*CYP 450*) may exert an important effect on disease development. Cytochrome P4502E1 (CYP2E1) enzyme is a member of the CYP 450 superfamily which is important for the metabolic activation of many toxic materials [9, 10]. Also, many drugs or small molecules are inducers of CYP P450 enzymes [11]. CYP2E1 which is primarily expressed in the liver, represents a major CYP isoform in the liver [12]. The *CYP2E1* gene could play an important role in human susceptibility to liver cancer [13]. *CYP2E1* has several variants and its functional variants are associated with increased or decreased susceptibility to many cancer types, including esophageal, lung, colorectal and nasopharyngeal carcinoma [14, 15]. The CYP1A1 enzyme is essential for the catalysis of the first step of polycyclic aromatic hydrocarbons (PAHs) metabolism, and therefore has been considered as a primary candidate in hepatocellular carcinoma (HCC) susceptibility [16]. Two genetic variants of *CYP1A1* have been reported to be associated with an increased risk of HCC in smokers, but the association was not replicated in subsequent studies [13]. The aim of this study was to investigate for the first time the association between *CYP2E1* and *CYP1A1* variants with susceptibility to HBV infection in a population of North Iran.

Materials and Methods

Subjects

This study included 54 patients (38 male, 16 female) aged between 18 and 68 years who were referred to Razi hospital in Ghaemshahr, Iran, and 89 healthy controls that were referred to Valiasr hospital in Ghaemshahr, Iran. Participants gave their consent to be included in the study. The diagnosis of acute or chronic HBV infection was based on serology for the hepatitis B surface antigen (HBs Ag) and the presence of HBV DNA which was investigated by real-time PCR assay. Demographic and serological features of the HBV infected patients are summarized in Table 1.

DNA Extraction

Genomic DNA of HBV infected patients was extracted from 200 µl of whole blood using the QIAamp DNA Blood Mini Kits (Qiagen Inc., USA) according to the manufacturer's instructions. Healthy controls' genomic DNA was extracted from whole blood using salting out method as previously described [17].

Table 1 Characteristics of the HBV infected patients

	HBV infected patients (n = 54)
Age range (years)	16–68
Gender [n (%)]	
Female	38 (70)
Male	16 (30)
Serum HBs Ag	
Positive [n (%)]	54 (100)
Negative [n (%)]	0 (0)
HBV DNA	
Positive [n (%)]	54 (100)
Negative [n (%)]	0 (0)

PCR–RFLP Analysis of *CYP2E1* and *CYP1A1* Variants

After DNA extraction, polymerase chain reaction (PCR) based restriction fragment length polymorphism (RFLP) was used to examine the genetic variants (rs3813867 and rs2031920) of *CYP2E1*, and (rs4646421and rs2198843) *CYP1A1* as previously described [17]. Briefly, the PCR was carried out using the specific primers for each variant followed by digestion with appropriate restriction enzymes. For *CYP2E1* variants (rs3813867 and rs2031920), the PCR products were digested with RsaI and PstI, and classified into three types according to the digestion pattern, as follows: a predominant homozygous (RsaI+/PstI−), a heterozygous (RsaI+/PstI+), and a rare homozygous (RsaI−/PstI+) genotype (Fig. 1).

The PCR products of *CYP1A1* variants (rs4646421 and rs2198843) were subjected to digestion by NspI or PstI restriction enzymes, respectively. The expected sizes of the products after digestion with NspI were 417 bp for the G allele (no cutting), 231 and 138 bp for the C allele (Fig. 2c). Digestion with PstI showed 105 bp and 299 bp fragments for the C allele, while the G allele remained uncut with 404 bp size (Fig. 2d).

Results

Patients Characterization

Chronic HBV infection was defined as individuals who tested positive for HBs Ag and HBV DNA for more than 6 months. Among the HBV infected patients all individuals showed positive level of HBs Ag and HBV DNA, whereas all healthy controls were negative for HBs Ag and HBV DNA.

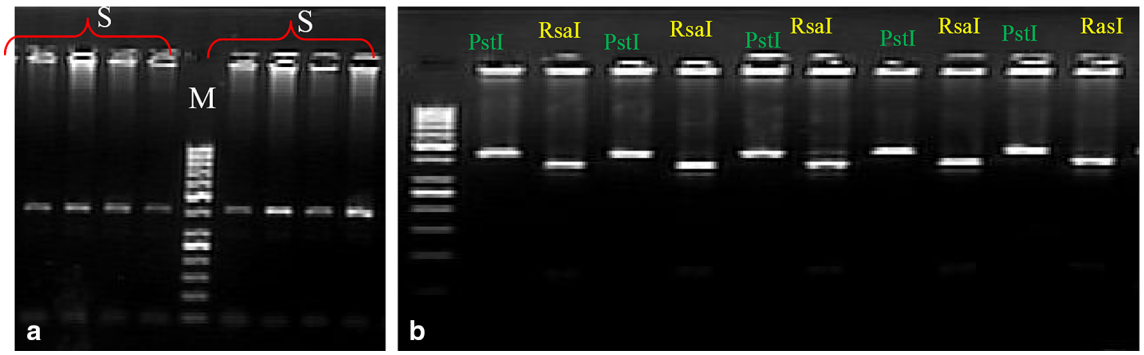

Fig. 1 RFLP analysis of *CYP2E1* gene. **a** Undigested PCR products (413 bp); **b** PCR products digested with RsaI and PstI. Lanes M: Molecular Weight Marker (50-bp ladder); lanes S: samples

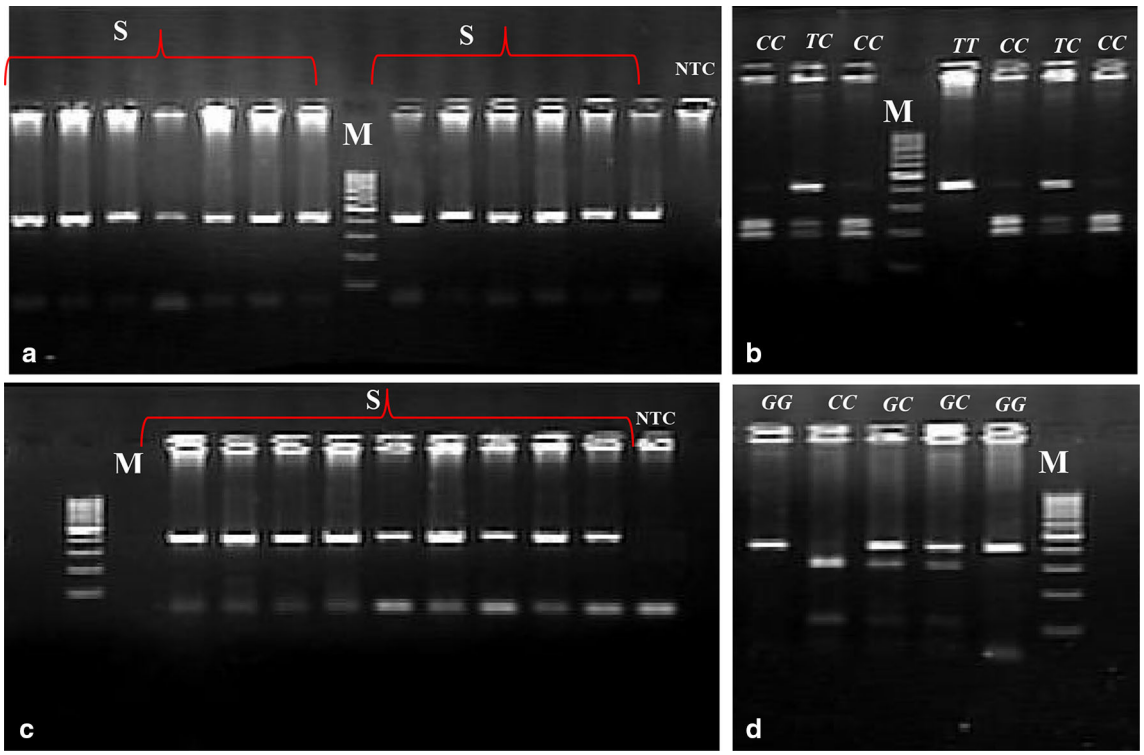

Fig. 2 Electrophoresis pattern of PCR–RFLP for detection of *CYP1A1* variants. **a**, **c** Undigested PCR products for rs4646421 and rs2198843, respectively; **b**, **d** PCR products digested with NspI and PstI, respectively. Lanes M: Molecular Weight Marker (100-bp ladder); lanes S: samples

Alleles and Genotypes Frequencies of rs2031920 and rs3813867 at *CYP2E1* Locus

Genotype frequencies of both single nucleotide polymorphisms (SNPs) were monomorphic in HBV infected patients and healthy controls (predominant homozygous RsaI+/PstI− genotype). Therefore, there was no difference in genotype frequencies between HBV infected patients and healthy controls.

Alleles and Genotypes Frequencies of rs4646421 and rs2198843 at *CYP1A1* Locus

The distributions of the genotypes and alleles of rs4646421 and rs2198843 are summarized in Table 2. The frequency of C and T alleles for rs4646421 were 0.82 and 0.17, respectively among the healthy controls, compared to 0.76 and 0.24, respectively in HBV infected patients. As shown in Table 2, 61.1% of patients and 68.5% of

Table 2 Distribution of cytochrome P450 1A1 genotypes and alleles frequencies in HBV infected patients compared to healthy controls for rs4646421 and rs2198843

SNP	Genotype/allele	Healthy controls n	HBV infected patients n	OR (95% CI)	*P* value
rs4646421	CC	61 (68.5%)	33 (61.1%)	1	
	CT	25 (28.09%)	14 (25.9%)	1.03 (0.47–2.2)	0.93
	TT	3 (3.3%)	7 (12.9%)	4.31 (1.04–17.7)	0.04
	C	0.82	0.74		
	T	0.17	0.26		
rs2198843	GG	47 (52. 8%)	26 (48.18%)	1	
	GC	32 (35.9%)	23 (42.59%)	1.29 (0.63–2.6)	0.47
	CC	10 (11.2%)	5 (9.25%)	1.1 (0.34–3.5)	0.86
	G	0.704	0.768		
	C	0.296	0.232		

Table 3 Association between [rs3813867; rs2031920] haplotypes of *CYP1A1*

Haplotype	Normal n[a]	Hepatitis n[a]	OR (95% CI)	*P* value
CG	109 (58.6%)	61 (61%)	1.00	
CC	24 (12.9%)	6 (6%)	0.4467 (0.17–1.15)	0.0957
TG	3 (1.62%)	6 (6%)	3.5738 (0.86–14.79)	0.0789
TC	10 (5.38%)	9 (9%)	1.6082 (0.61–4.17)	0.3288

[a] For 17.9% of the analyzed healthy controls and 24% of HBV infected patients, haplotype assignment was not possible due to heterozygosity in both loci

controls had the CC genotype. The distribution of CT and TT genotypes in HBV infected patients was 25.9 and 12.9%, respectively compared to 28.09 and 3.3% in healthy controls. Therefore, TT genotype was significantly more prevalent in HBV infected patients [$P = 0.04$, OR 4.31 (1.04–17.7)] in comparison with healthy controls. The G and C allele frequencies for rs2198843 were found to be 0.704 and 0.296, respectively among healthy controls, compared to 0.76 and 0.24, respectively among HBV infected patients. The homozygous wild type genotype (GG) frequency was 52.8% in normal and 48.18% in HBV infected patients whereas the heterozygous genotype (GC) frequency was 35.9% in healthy controls and 42.59% in HBV infected patients. Moreover, 11.2% of healthy controls carried the mutant homozygous allele (CC genotype) while this genotype was found in 9.25% of the HBV infected patients. However, there was no statistical difference in genotype frequencies among the two groups. Genotype frequencies of both variants were in Hardy–Weinberg equilibrium. Haplotype data for *CYP1A1* locus is shown in Table 3. Four haplotypes were identified in HBV infected patients as well as healthy controls. The haplotype [C; G] was predominant in HBV infected patients (61%) and healthy controls (58.6%). In this study, [T; G] haplotype was rare in both studied groups.

Discussion

Several studies showed that hepatitis replication is highly dependent on host cell factors [18–21]. Although the role of *CYP*s genetic variants is clear in dealing with development of HCC, there is limited data about their association with susceptibility to chronic hepatitis B infection. A number of studies demonstrated that the hepatic CYPs are important in pathogenesis of several liver diseases. Li et al. [22] showed that chronic HBV infection down-regulates the expression of hepatic *CYP3A4*. Furthermore, Iizuka et al. [23] showed that the expression level of 28 CYPs were significantly changed in HBV- and/or HCV-infected livers compared with normal livers. However, there is no information about the impact of *CYP2E1* and *CYP1A1* genetic variants on HBV pathogenesis. In this study, polymorphisms in *CYP2E1* and *CYP1A1* genes were studied in 89 healthy controls and 54 HBV infected patients. According to the results of the present study, genotypes and alleles frequency analyzes for rs2031920 and rs3813867 showed that both HBV infected patients and healthy controls had only homozygous wild type genotype, indicating that these variants are monomorphic in the studied population. These data are in line with another study performed in Iran [24]. The genotype distribution in Iranian population is similar to Indian, Turkish, and some European populations such as German, British and French

[25–29], but is different from Japanese and Chinese, and also from Italians [30–32]. Some studies showed that *CYP2E1* variants were associated with HCC risk. Tian et al. [13] in a meta-analysis showed that the c2 allele of *CYP2E1* may be a protective factor for HCC among East Asians, especially among China populations. Allele frequency study for rs2198843 showed that allelic distribution was approximately similar in both healthy controls and HBV infected patients. There was no significant difference in genotypes and allele frequencies among the HBV infected patients and healthy controls. In the present study, genotype analysis for rs4646421 showed that the frequency of TT genotype was higher in HBV infected patients compared with healthy controls. A significant association was observed between healthy controls and HBV infected patients ($P = 0.04$), and hepatitis development risk was approximately four times greater in individuals with TT genotype than individuals with CC genotype. Nakai et al. [33] showed that the development of early stages of chronic hepatitis C infection was associated with drug metabolism enzymes such as CYP1A2, CYP2E1 and CYP3A4. Makpol et al. [34] indicated a significant relationship between *CYP1A1* genetic variants and HCC risk in Malaysian population. In another study, Yu et al. [35] showed that *CYP1A1* variants play an important role in hepatocarcinogenic effect of PAHs. Li et al. [16] demonstrated that rs2198843 and rs4646421 variants carriers have an increased HCC risk. In conclusion, individuals who have had TT genotype at rs4646421 locus were more susceptible to HBV infection development. It can also be concluded that the prevalence of HBV infection is higher in subjects with [T; G] and [T; C] haplotypes than in subjects with [C; G] and [C; C] haplotypes.

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