RESEARCH ARTICLE

CCR5 Polymorphism as a Protective Factor for Hepatocellular Carcinoma in Hepatitis B Virus-Infected Iranian Patients

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Abstract

The CC chemokine receptor 5 (CCR5) delta 32 allele results in a nonfunctional form of the chemokine receptor and has been implicated in a variety of immune-mediated diseases. CCR5Δ32 may also predispose one to chronic liver disease or be linked with resistance to HBV infection.This study was undertaken to investigate any association between CCR5 polymorphism with resistance to hepatitis B or susceptibility to HBV infection. A total of 812 Iranian individuals were enrolled into two groups: HBV infected cases (n=357), who were HBsAg-positive, and healthy controls (n=455). We assessed polymorphisms in the CCR5 gene using specific CCR5 oligonucleotide primers surrounding the breakpoint deletion. Genotype distributions of the HBV infected cases and healthy controls were determined and compared. The CCR5/CCR5 (WW) and CCR5/ CCR5Δ32 (W/D) genotypes were found in (98%) and (2%) of HBV infected cases, respectively. The CCR5 Δ32/ Δ32genotype was not found in HBV infected cases. Genotype distributions of CCR5 in healthy controls were W/W genotype in (87.3%), W/D genotype in (11.2%) and D/D genotype in (1.5%). Heterozygosity for CCR5/ CCR5Δ32 (W/D) in healthy controls was greater than in HBV infected cases (11.2% vs 2%, $p < 0.001$). W/D and D/D genotypes were more prominent in healthy controls than in HBV infected cases. This study provides evidence that the CCR5Δ32 polymorphism may have a protective effect in resistance to HBV infection at least in the Iranian population.

Keywords: CC chemokine receptor 5 - chemokine receptor - disease susceptibility - hepatitis B virus

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Introduction

Hepatitis B Virus (HBV) is one of the most important chronic liver diseases worldwide, particularly in various areas of Asia and Africa (Liang et al., 2013). HBV causes hepatitis B and leads to cirrhosis and hepatocellular carcinoma (Shaban et al., 2016; Sriprapun et al., 2016). Hepatocellular Carcinoma (HCC) is one of the most frequent of liver cancer and the third cancer related death worldwide (Wanich et al., 2016). It had been estimated that in Iran over 35.0% of the population have been exposed to HBV and approximately 3.0% are chronic carriers of HBV (Bahmani et al., 2010). The greatest number of individuals that are exposed to HBV infection recovered and developed protective antibodies; however, approximately 5% of adults remain chronically infected with HBV and are at risk for developing end-stage liver disease and hepatocellular carcinoma (Lu et al., 2015; Thio et al., 2008).

Hepatitis B resistance occurs more often in individuals who develop a broad and strong T-cell response rather than in those with a weak and narrowly-focused response (Rehermann et al., 1996), nevertheless the genetic basis for differences in adaptive immunity remain poorly understood. Several studies suggest that genetic polymorphisms are involved in imperviousness to persistent HBV infection or development of HCC (Cheong et al., 2006; Attar et al., 2015; Azar et al., 2016) Chemokines, a large family of leukocyte chemoattractants that act by binding to G-protein coupled receptors, have become recognized as increasingly important mediators of hepatic inflammation and injury (Murai et al., 1999). Chemokine–chemokine receptor interactions are likely to be important in chronic viral hepatitis, where T-cells are recruited to the liver parenchyma to mediate the clearance of hepatocytes infected with hepatitis virus (Ahn et al., 2006). CCR5 (chemokine receptor 5) is a CC chemokine receptor expressed by granulocytes, macrophages, immature dendritic cells, CD8+ lymphocytes, and Th1 lymphocytes, and it influences their migration and activation (Wong et al., 2003).

A 32-base-pair deletion in the CCR5 gene (CCR5Δ32) results in loss of a functional CCR5 protein, and this confers some protection against infection with HIV-1 (Dean et al., 1996; Samson et al., 1996). Moreover, CCR5 has been identified as a co-receptor for the human

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immunodeficiency virus-1 (HIV-1) (Shahbazi et al., 2009) CCR5Δ32 allele has been identified in 10 to 15% of Caucasians. Some studies showed that the protective effect of CCR5Δ32 in recovery from an HBV infection, For instance, a study in Caucasians of US showed that individuals who have at least one copy of the gene encoding a nonfunctional receptor (CCR5Δ32) are twice as likely to recover from hepatitis B (Thio et al., 2007). Other studies showed that CCR5Δ32heterozygosity was associated with susceptibility to HBV-related liver disease, for example a study in India indicated that CCR5 Wt/mt allele was more often present in patients with chronic hepatitis B than in healthy controls (Suneetha et al., 2006). The main aim of this study was to investigate the association between CCR5 polymorphism with resistance to HBV infection.

Matherial and Methods

Subjects

A total number of 812 Iranian were involved in this study, the study was designed in a cross sectional sampling case control pattern. During a (2011-2014) three year period of time, with more clear medical records in the Cellular & Molecular Research Center of Gorgan (MCMRC), Taleghani hospital was considered to collect samples. HBV infected cases (N=357), according to medical records patients with a HBAg positive test and PCR test for HBV DNA and also remaining positive were recruited at this study. Healthy individuals (N= 455) who referred to Blood Transfusion Organization of Gorgon during the same period of time and had negative HBs-Ag test without history of Renal, Endocrine, Autoimmune, Liver and Cardiovascular disorders were selected as control group (Age mean: 34.9±13.1). The study was achieved with approval of the Local Ethical Committee of Golestan University of Medical Sciences, and informed consent was obtained from all recruited individuals. None of the approached subjects refused to participate.

DNA Extraction and Genotyping

Genomic DNA was extracted from 10 ml of peripheral venous blood by a modified "phenol/chloroform" technique (Shahbazi et al., 2009) precipitated with ethanol and re-suspended in sterile distilled water and DNA concentrations were determined with a UV spectrophotometer at 260 nm (Techne, UK). The CCR5Δ32 polymorphism was evaluated by PCR amplifications using sequence specific primers (5′ CTTCATTACACCTGCAGCTCT 3′ and 5′ CACAGCCCTGTGCCTCTTCTTC 3′). For PCR amplification, a total volume of 25µL, containing 250 ng genomic DNA, 20 pmol of each primers, 300 µmol dNTPs mix (CinaGen, Iran), 1500 µmol MgCl2, 2.5µL10×PCR buffer (500 mM KCl and 200 mM Tris–HCl, pH: 8.4) and 2 units Taq DNA polymerase (Cina-gen, Iran) were used. PCR conditions were as following: denaturation at 94˚C for 5 minutes, 10 cycles of 15 s at 95 ċ, 50 s at 64 ċ, and 40 s at 72 ċ; 20 cycles of 20 s at 95 ċ, 50 s at 58 ċ, and 50 s at 72 ċ, followed by one cycle of final extension at 72˚C for 5 minutes. The PCR products were then electrophoresed on 2% agarose gel stained with ethidium bromide and visualized under ultraviolet (18.02 bp for the wild-type allele and 150 bp for the 32-bp-deletion allele).

Statistical analysis

Statistical analysis was carried out with the SPSS version 16. Categorical variables were evaluated by standard Chi-square or Fisher exact tests, allele and genotype frequencies were calculated and compared with non-parametric tests followed by Fisher' exact analysis using STATA v-8 (CA, US). A P value of <0.05 was considered significant.

Results

The study population included 455 males and 357 females with their age ranging from 15-57 years. The CCR5/CCR5 (W/W) and CCR5/ CCR5Δ32 (W/D) genotypes were found in 352 (98.6%) and 5 (1.4%) 0f HBV infected cases, respectively. The CCR5 Δ32/ Δ32 genotype was not found in HBV infected cases. Genotype distributions of CCR5 in healthy controls were W/W genotype in 397 (87.3%), W/D genotype in 51 (11.2%) and D/D genotype in 7 (1.5%) respectively under co-dominant genetic midel (Table 1). The CCR5/CCR5 (W/W) genotype was more present in HBV infected cases than in healthy controls (98.6% vs 87.3%, $p < 0.001$). Homozygosity for 32 bp deletion (D/D genotype) was observed in 1.5% (7) of healthy controls, and however, not observed in HBV infected cases. Heterozygosity for CCR5/ CCR5Δ32 (W/D) allele in healthy controls was more than in HBV infected cases (11.2% vs 1.4%, $p < 0.03$). W/D and D/D genotypes were more present in healthy controls than in HBV infected cases (Table 1).

Genotype distribution of CCR5 according to gender showed that the W/W genotype was more present in males than in females (94.2% vs 87.9%, $p = 0.05$) then and there W/D (11% vs 4.9%, $p = 0.03$) and D/D (1.1% vs 0.9%, $p = 0.6$) genotypes were more present in females than in males.

Discussion

The present study provides evidence that CCR5Δ32 allele may be associated with resistance to HBV infection in the Iranian population. To study the influence of the CCR5Δ32 on resistance to hepatitis B or susceptibility to HBV infection we have identified the CCR5Δ32 polymorphism in 257 HBV infected patients and 455 healthy controls from the Iranian population. Our results demonstrate that the CCR5Δ32 allele was more frequent in healthy controls than in HBV infected.

A study on CCR5 in Caucasians of US revealed that There is one copy of thegeneencoding a nonfunctional receptor (CCR5Δ32) will increase the probability of recovery of patients with hepatitis B, and the protective effect appears to be codominant (Thio et al., 2007) This data suggested that the CCR5Δ32 has a protective effectin resistance to HBV infection, provides genetic

Table 1. Frequency of the CCR5-Delta32 Allele and Genotypes Among Patients (N=357.0) and Controls (N=455.0) Under Co-Dominant, Dominant, Recessive and Over-Dominant Model

Model	Alleles and Genotype	Controls	HBV patients	OR (95% CI)	P-value
Codominant	W/W	397 (87.3%)	352 (98.6%)	1.0	-
	W/D	51 (11.2%)	5 (1.4%)	0.5 (0.3-1.0)	0.03
	D/D	7 (1.5%)	0 (0.0%)	7.4 (3.3-18.8)	<0.001
	W	845 (92.8%)	709 (99.3%)	1.0	-
	D	65 (7.2%)	5 (0.7%)	2.3 (1.6-3.3)	<0.001
Dominant	D/D	7 (1.5%)	0 (0.0%)	0.0 (0.0-0.7)	0.02
	W/D-W/W	448 (98.5%)	357 (100.0%)	1.0	
Recessive	D/D-W/D	58 (12.7%)	5 (1.4%)	1.0	<0.001
	W/W	397 (87.3%)	352 (98.6%)	10.3 (4.1-33.2)	
Overdominant	W/W- D/D	404 (88.8)	352 (98.6%)	8.9 (3.5-28.8)	<0.001
	W/D	51 (11.2%)	5 (1.4%)	1	

epidemiological evidence for a role of CCR5 in the immune response to HBV, and suggests a potential therapeutic treatment for patients persistently infected with HBV.

Based on Zhou and et al results from study of ccr5 −/− mouse models, there are potential explanations for these observations. Some studies shown that The CCR5-Δ32/+ genotype results in markedly diminished levels of CCR5 on the cell surface and low expression of CCR5 correlates with reduced infection of T cells in vitro.

CCR5- deficient mice have increased CD4+ and CD8+ T-cell responses to a variety of antigens and to a dendritic cell vaccine (Nansen et al., 2002; Ng-Cashin et al., 2003). Such findings suggest that CCR5 may behave as a negative regulator of T cells in an immune response. CCR5-mediated attenuation of the immune response may increase the risk of HBV persistence. Second, based on a concanavalin A (ConA)-induced fulminant hepatitis murine model, which is a model of T-cell-mediated hepatitis, CCR5 deficiency prevents hepatic natural killer T (NKT) cell apoptosis and upregulates NKT cell function (Ajueboret al., 2005) a phenomena that would favor recovery from HBV infection since NK and NKT cells are important in controlling HBV replication in HBV transgenic mouse models (Kakimi et al., 2000). Thus, CCR5 deficiency appears to impact the response to a variety of infections in ways that are not necessarily easy to predict, possibly due to differences in pathogenic mechanisms employed by different infectious organisms.

Conversely, Pothakamuri VS and his colleagues in India suggested that CCR5Δ32heterozygosity was associated with susceptibility to HBV-related liver disease. They found that the CCR5 Wt/mt (W/D) genotype was more often present in patients with chronic hepatitis B than in healthy controls. They found that CCR5Δ32 heterozygosity was associated with susceptibility to HBV-related liver disease (Suneetha et al., 2006). In this study, No association was seen between susceptibility to HBV infection and CCR5Δ32 polymorphism and CCR5Δ32 was more frequent in healthy controls than in HBV infected.

However, CCR5Δ32 were not observed in Southeast Asian countries such as Korea and China. Sang and colleagues in a study on the association of genetic variation in CCR5 and its ligand, RANTES with the clearance of hepatitis B in Korea reported that CCR5Δ32 homozygosity or heterozygosity was not found in Korean population. The association between the CCR5Δ32 polymorphism and HBV clearance could not be confirmed because of a total absence of the CCR5Δ32 polymorphism (Ahn et al., 2006). Also, Kui Tan and colleagues in a study on the association of CCR5Δ32 polymorphism with HBV infection in China reported that no CCR5Δ32 allele is detected (Tancredoet al., 2009) Kazemi A M from Iran reported that none of the Occult hepatitis B infection (OBI) patients had δ32 mutation in the CCR5 chemokine receptor whereas 2 (2%) of controls had heterozigotic form of this mutation (Kazemi et al 2009) Khorramdelazad and colleagues in North-East of Iran have shown no significant difference between HBV infection and healthy individuals (Khorramdelazad et al., 2013). The disagreement of our results might be as a result of the genetic background differences of our population and also the size of the sample. The sample size of our study is larger than that of their and that could result in these differences.

In conclusion, CCR5Δ32 polymorphism may have a protective effectin resistant to HBV infection in Iranian population. Even though CCR5Δ32 is associated with resistant to HBV infection, the majority of people who recovered from infection did not have this deletion. This is expected since resistant to hepatitis B is certainly polygenic; accordingly CCR5Δ32 is one of the several genes involved in HBV pathogenesis. Diverse findings about the effect of CCR5delta32 development and resistance of HBV might be due to differences in the genetic background of different populations. Hence, genetic interactions have been described in many disorders. There is no doubt regarding the importance of chemokine receptors and their ligands in HBV. However, more studies are needed to reveal the exact role of chemokine network and its association with other factors in the pathogenesis of HBV infection.

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Reference

Ahn SH, Kim do Y, Chang HY, et al. (2006) Association of genetic variations in CCR5 and its ligand, RANTES with clearance of hepatitis B virus in Korea. *J Med Virol*, **78**, 1564-71.

Ajuebor MN, Aspinall AI, Zhou F, et al (2005). Lack of chemokine receptor CCR5 promotes murine fulminant liver failure by preventing the apoptosis of activated CD1d-restricted NKT cells. *J Immunol*,**174**, 8027-37.

Attar M, Azar SS, Shahbazi M. (2015) Interleukin-6-174 promoter polymorphism and susceptibility to hepatitis B virus infection as a risk factor for hepatocellular carcinoma in Iran. *Asian Pac J Cancer Prev*, **17**, 2395-9.

Azar SS, Mansoori M, Attar M, Shahbazi M. (2016) Tumor necrosis factor alpha-308 G/A single nucleotide polymorphism and susceptibility to hepatocellular carcinoma via hepatitis B infection. *Asian Pac J Cancer Prev*, **17**, 3381-4.

Bahmani MK, Khosravi A, Mobasser A, Ghezelsofla E. (2010) Seroprevalence of hepatitis B virus infection and vaccination compliance among health care workers in Fars Province, Iran. *Arch Clin Infect Dis*, **5**, 45-50.

Cheong JY, Cho SW, Chung SG, et al.(2006) Genetic polymorphism of interferon-gamma, interferon-gamma receptor, and interferon regulatory factor-1 genes in patients with hepatitis B virus infection. *Biochem Genet*, **44**, 246-55.

Dean M, Carrington M, Winkler C, et al. (1996) Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia growth and development study, multicenter AIDS cohort study, multicenter hemophilia cohort study, san francisco city cohort, ALIVE study. *Science*, **273**, 1856-62.

Liang T, Chen E, Tang H (2013). Hepatitis B virus gene mutations and hepatocarcinogenesis. *Asian Pac J Cancer Prev*, **14**, 4509-13.

Lu Y, Bao J, Deng Y, et al (2015). Role of IL-18 gene promoter polymorphisms, serum IL-18 levels, and risk of hepatitis B virus-related liver disease in the Guangxi Zhuang population: a retrospective case-control study. *Asian Pac J Cancer Prev*, **16**, 6019-26.

Kakimi K, Guidotti LG, Koezuka Y, Chisari FV.(2000) Natural killer T cell activation inhibits hepatitis B virus replication in vivo. *J Exp Med*, **192**, 921-30.

Kazemi Arababadi M, Pourfathollah AA, et al.(2009) Detection of the CCR5-d32 Mutation in Patients Infected with Occult Hepatitis B. *Zahedan J Res Med Sci*, **11**, 94-8.

Khorramdelazad H, Hakimizadeh E, Hassanshahi G, et al.(2013) CCR5 Delta 32 mutation is not prevalent in Iranians with chronic HBV infection. *J Med Virol*, **85**, 964-8.

Murai M , Yoneyama H, Harada A, et al. (1999) Active participation of CCR5(+)CD8(+) T lymphocytes in the pathogenesis of liver injury in graft-versus-host disease. *J Clin Invest*, **104**, 49-57.

Nansen A, Christensen JP, Andreasen SO, et al. (2002)The role of CC chemokine receptor 5 in antiviral immunity. *Blood*, **99**, 1237-45.

Ng-Cashin J, Kuhns JJ, Burkett SE, et al.(2003) Host absence of CCR5 potentiates dendritic cell vaccination. *J Immunol*, **170**, 4201-8.

Rehermann B, Lau D, Hoofnagle JH, Chisari FV. (1996) Cytotoxic T lymphocyte responsiveness after resolution of chronic hepatitis B virus infection. *J Clin Invest*, **97**, 1655-65.

Samson M, Libert F, Doranz BJ, et al.(1996) Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature*, **382**, 722-5.

Shaban N, Salem H, ElsadanyM, et al (2016). Distribution of glutathione S-transferase omega gene polymorphism with different stages of HBV infection including hepatocellular carcinoma in the egyptian population. *Asian Pac J Cancer Prev*, **17**, 2145-50.

Shahbazi M, Ebadi H, Fathi D, et al.(2009) CCR5-delta 32 allele is associated with the risk of developing multiple sclerosis in the Iranian population. *Cell Mol Neurobiol*, *29*, 1205-9.

Sriprapun M, Chuaypen N, Khlaiphuengsin A, et al. (2016) Association of PINX1 but not TEP1 polymorphisms with progression to hepatocellular carcinoma in Thai patients with chronic hepatitis B virus infection. *Asian Pac J Cancer Prev*, **17**, 2019-25.

Suneetha PV, Sarin SK, Goyal A, et al (2006)Association between vitamin D receptor, CCR5, TNF-alpha and TNF-beta gene polymorphisms and HBV infection and severity of liver disease. *J Hepatol,* **44**, 856-63.

Tancredo as, pleasant, He Yan, et al. (2009)Association of CCR5-Δ32 polymorphism with HBV infection in special population of Guizhou Province. *Shi Jie Hua Ren Xiao Hua Za Zhi*, **17**, 2317-9.

Thio CL, Astemborski J, Bashirova A, et al.(2007) Genetic protection against hepatitis B virus conferred by CCR5Delta32: Evidence that CCR5 contributes to viral persistence. *J Virol*, **81**, 441-5.

Thio CL, Astemborski J, Thomas R, et al.(2008) Interaction between RANTES promoter variant and CCR5Δ32 favors recovery from hepatitis B. *J mmunol*, **181**, 7944-7.

Wanich N, Vilaichone R, Chotivitayatarakorn P, Siramolpiwat S (2016). High prevalence of hepatocellular carcinoma in patients with chronic hepatitis B infection in Thailand. *Asian Pac J Cancer Prev*, **17**, 2857-60.

Wong MM, Fish EN.(2003) Chemokines: attractive mediators of the immune response. *Semin Immunol*, **15**, 5-14.