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Original Article

Association between Hepatitis B Virus Infection Outcome and HLA-A and DRB1 Genotyping in North Part of Iran

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ABSTRACT

Background and Objective: **The outcome of hepatitis B virus (HBV) infection may be influenced by host factors like Human Leukocyte Antigen (HLA). We have investigated HLA-A and DRB1 alleles in patients with persistent hepatitis B infection compared to subjects who had spontaneously recovered from HBV infection. To complete the findings of this study we performed another survey in certain HLA alleles that were significantly related to the outcome of HBV infection. The current study aimed to determine association between HBV infection outcome and HLA-A and DRB1 genotyping in North part of Iran.**

Patients and Methods: **Ninety-four HBV infected patients were enrolled in this cross sectional study. First HLA-A and DRB1 alleles were analyzed by using low resolution PCR sequence-specific-primer (PCR-SSP) and then we used high resolution PCR-SSP method for subtyping HLA-A*33 and DRB1*13 alleles which were significantly related to the outcome of HBV infection.**

Results: **HLA-A*33 allele was significantly higher in persistent group than recovered group and sub typing showed HLA-A*3303 in 75% and HLA-A*3301 in 25% of cases. HLA-DRB1*13 allele was significantly lower in persistent group than in recovered group and its subtypes were DRB1*1301 in 66.7% and DRB1*1303 in 33.3% of subjects.**

Conclusion: **Host HLA polymorphism is an important factor to determining the outcome of HBV infection. HLA-A*3303 and DRB1*1301 were the predominant subtypes of HLA-A*33 and DRB1*13 alleles in Iranian HBV infected patients.**

Keywords: Hepatitis B, HLA Antigens, Polymerase Chain Reaction

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Introduction

Host and viral factors influence the clinical expression and behavior of hepatitis B infection (HBV). HLA is a critical genetic factor that determines individual variations of immune response. HLA genotype of an individual may influence the progression of HBV infection (1,2).

Many different HLA alleles have been demonstrated to play roles in HBV infection (3) but this relationship is not universal on the basis of the investigated population. HLA DRB1*13 and HLA DRB1*11/*12 are consistently associated with viral clearance and viral persistence of HBV infection respectively in major populations (4,5). In contrast, HLA DRB1*11/*12 alleles are associated with HBV clearance in Chinese (6,7) and HLA DRB1*13 is reported as a susceptibility gene for chronic HBV infection in Turkish populations (8).

Susceptibility to HBV infection and chronicity is attributed to HLA A*0206 allele in Taiwanese, B35 in Chinese(9), B18, B35, B40, Cw3 allele in Russian, A3 and B18 in Kazakhs(10) B8-Cw-7 haplotype in Senegalese(11), and HLA A*01-B*08-DRB1*03, B-44-Cw1601 and B*44-Cw*0501 haplotype in American Caucasians(12).

Yang data indicated that HLA-DRB1*03 and HLA-DRB1*07 were related to susceptibility to chronic HBV infection, and DRB1*15 was negatively related to persistence to chronic HBV infection among people in northwestern China (3).

We have investigated HLA-A and DRB1 alleles in patients with persistent hepatitis B infection compared to subjects who had spontaneously recovered from HBV infection. To complete the findings of this study we performed another survey in certain HLA alleles that were significantly related to the outcome of HBV infection. The current study aimed to determine the association between HBV infection outcome and HLA-A and DRB1 genotyping in North part of Iran.

Material and Methods

Study population

Ninety-four HBV infected patients [(55 males, 39 females with mean age: 32.9 ± 10.5 years (range 14-60 years)] who admitted to a hepatitis referral center in north part of Iran, from October 2006 to March 2007, were enrolled in this cross sectional study. The cases were homogeneous and there was not any ethnic diversity within them. The study groups consisted of 30 HBV recovered group (Hepatitis B surface antigen (HBsAg) negative, Hepatitis B core antibody (AntiHBc) positive, Hepatitis B surface antibody (AntiHBs) positive, recovered from HBV infection) ; 31 inactive healthy carrier group (HBsAg (+) for more than six months and sustained normal transaminase level); 33 chronic hepatitis B (HBsAg (+) for more than six months with elevated transaminase (≥2 times the upper limit of normal) and/or HBsAg persistence for more than six months with liver biopsy showing signs of chronic hepatitis B which confirmed by pathologist). Thirty three CHB patients and 31 inactive healthy carriers were collapsed in one group (persistent group) and compared with recovered group.

None of the subjects were positive for the Hepatitis C virus (HCV) and human immunodeficiency virus (HIV) antibody.

This project was approved by the Pasteur Institute of Iran Ethics Committee and informed consent was obtained from patients prior to their enrollment.

Serological testing

Hepatitis B surface antigen (HBsAg), antibodies to HBsAg (AntiHBs) and Hepatitis B core Antigen (AntiHBc) were tested by ELISA assay using commercial Kit (Hepanostika, bioMerieux, Boxtel, Netherlands). Samples were also tested for hepatitis C antibody (anti-HCV) by ELISA. The commercial ELISA kit which used for anti-HCV was (Bio-Rad Laboratories, Segrate, Italy). Recombinant immunoblot assay (RIBA Innogenetics, Ghent, Belgium) was employed to confirm anti-HCV reactivity. HIV-antibody status was determined by ELISA (MP Biomedicals, Illkirch, France); with positive tests confirmed by the Western blot assay (Diaplus, San Francisco, USA).

HLA allele typing

Genomic DNA was extracted from peripheral blood using Miller and Dykes method (13).

HLA alleles were determined by a low resolution DNA typing method, a PCR sequence-specific-primer (PCR-SSP) technique, according to the manufacturer's instruction (HLA-ABDR SSP kit; Biotest, Dreieich, Germany). The typing results were analyzed with the software supplied by Biotest. Then HLA subtyping was performed by high resolution PCR-SSP method (Olerup SSP AB, Saltsjöbaden, Sweden), according to the manufacturer's instruction, for determination of the predominant allelic subtypes of HLA-A*33 (n=12) and DRB1*13 (n=11).

Statistical Analysis

The frequency of HLA alleles were calculated by direct counting. Data were analyzed by the Chi-square test and Fisher's exact test using the SPSS 13.0 data analysis software package; P values <0.05 were considered statistically significant. The odds ratio (OR), which reflects the likelihood of a subject carrying a specific allele, and the 95% confidence interval (95 %CI) were calculated.

Results

The study groups consisted of 30 HBV recovered group (17 males, 13 females with mean age: 32.2 ± 9.6); 31 Inactive healthy carrier group (18 males, 13 females with mean age: 28.1± 9.2) and 33 Chronic hepatitis B patients (20 males, 13 females with mean age: 38 ± 10.5).

The frequency of A*33 allele in persistent group was higher than in recovered group (9.37% vs. 0%, P<0.008) and sub typing showed HLA-A*3303 and HLA-A*3301 in 75% (allele frequency: 7.3) and 25% (allele frequency: 2.34) of persistent HBV infected cases, respectively. The frequency of DRB1*13 allele was lower in persistent group than in recovered group (3.13% vs. 11.67%, P < 0.03, OR = 0.22, 95%CI 0.06-0.82), and HLA- DRB1*1301 and HLA-DRB1*1303 were found in 66.7%(allele frequency: 4.89) and 33.3% (allele frequency: 3.45) of cases respectively. HLA-A*3303 and DRB1*1301 were the predominant subtypes of HLA-A*33 and DRB1*13 at high resolution PCR-SSP method.

No significant differences of other HLA-A and DRB1 alleles' distribution were found between the two groups.

Discussion

Our results suggested that HLA-A*33 including HLA-A*3303 and A*3301 both predispose patients to HBV persistency. Evidence for chronicity effect of the A*3303 subtype is stronger than for A*3301, probably due to the higher over all frequency of the former in our study subjects.

It has been reported that the HLA polymorphism correlates with the outcome of HBV infection, but this relationship is not universal on the basis of the investigated population. In Caucasia (5,12) and Korea (14), for example, HLA-DRB1*1301-02 has been found to be associated with acute self-limited hepatitis B. In Taiwanese, HLA-DRB1*0406 is associated with recovery from HBV infection in Han Chinese, and so is HLA-B*4001 in Aborigines (9).

Recently, a large comprehensive study on white patients demonstrated an association between HLA class I alleles, particularly A*0301, and viral clearance (12). Another analysis showed that B*08 and B*44 in white (3) and B*35 in Latin American populations are associated with HBV persistence (15).

HLA-A*3303 and DRB1*0701 were associated with HBV chronicity among Koreans people (16). DRB1*1301, was less frequent in children with persistent infection than in those with transient infection (17).

Cotrina *et al.* (18) analyzed the HLA-DRB1 genotype in a series of patients with chronic hepatitis B and acute hepatitis B, which further confirmed that HLA-DRB1*1301 and -DRB1*1302 alleles were associated with the clearance of HBV infection and protected people against chronic hepatitis B. HLA DRB1*1302 and A*0301 were associated with clearance of hepatitis B (12).

This study showed that HLA-A*33 was related with susceptibility to persistence of hepatitis B infection, and HLA-DRB1*13 was related with protection against persistency of hepatitis B infection. In our investigation the frequency of DRB1*1301 allele was lower in persistent group than in recovered group which was in agreement with previous findings (5;17;18). Our results indicated that the frequency of HLA-A*3303 allele was higher in persistent group than recovered group which was consistent with Hwang *et al.* (16) results.

Conclusion

Host HLA polymorphism is an important factor to determining the outcome of HBV infection. HLA-A*3303 and DRB1*1301 were the predominant allelic subtypes related to HBV infection outcomes in our study.

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The authors declare that there is no Conflict of Interests.

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