Original Article

HLA Class II Genotyping in Chronic Hepatitis B Infection

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Abstract
Objectives: To analyze the role of HLA genotypes in persistence of chronic hepatitis B in Western India.

Methods: HLA genotyping for class II-DR was done in 26 subjects having chronic hepatitis B infection (HBsAg positive) and in 100 healthy controls. Statistics were done using Halden’s modification of Woolf’s formula.

Result: Significant association of chronic hepatitis B infection was found for class II-DR antigens - DRB1*15XX (57.6 vs. 25%) and DRB1*11XX (23 vs. 4%). DRB1*13XX (0 vs. 19%) was negatively associated with chronic hepatitis B infection.

Conclusion: HLA phenotype, which varies with different regions, is one of the factors in persistence of hepatitis B infection. Our study supports negative association of DRB1*13XX to persistence of HBV. Also there may be role of DRB1*11XX and DRB1*15XX in persistence of HBV and development of chronic HBV hepatitis.

**INTRODUCTION**

Hepatitis B viral (HBV) infection is a major health challenge worldwide. Over 350 billion people in the world are carrier of HBV, of whom more than 150,000 die annually of HBV-related liver disease- chronic hepatitis, cirrhosis and hepatocellular carcinoma.1 Prevalence of HBV worldwide is falling due to availability of effective vaccination, safe blood practice and AIDS campaign against sexual promiscuity and needle sharing.2 In Southeast Asian countries, HBV carriage rates are 10-20% with major route of transmission being vertical/percutaneous, whereas age of infection being perinatal/early childhood.3 In India, HBV prevalence in general population ranges from 1.1 to 12.2% with estimated 40 million infected people.4

Outcome of HBV infection mainly depends on the host immune response, but also influenced by capability of the HBV to escape defense mechanisms by integration into hosts’ hepatocyte genome.4 The host immune response seems to be function of age of the infection. In adults, chronic infection develops in 1-10% of infected individuals, whereas in children chance of chronicity is more than 90%.5 Major histocompatibility complex plays a pivotal role in host immunity and in turn, clearance of virus-infected hepatocytes. For eliminating the virus, CD8+ cytotoxic T lymphocytes must recognize the combination of the viral epitope and HLA class I antigen co-expressed on hepatocytes. HLA class II molecules on antigen presenting cells present extracellularly derived antigens like virus peptides to CD4+ helper T cells to stimulate cytokine release generating both humoral and cellular immune response to clear the virus.6

Majority of host genetic studies worldwide have concentrated on HLA associations. No studies have identified association between HLA class I alleles and viral persistence. In various studies throughout the world, different HLA class II alleles are reported to be important in persistence or clearance of HBV.6

In this study, role of HLA class II alleles is analyzed in persistence of hepatitis B virus leading to chronic hepatitis B infection in western Indian population.

**MATERIAL AND METHODS**

This prospective study was carried out from January 2002 to July 2002 at Bombay Hospital and Medical Research Center, Mumbai. The study group comprised of total of 26 consecutive patients of current chronic hepatitis B infection, which was defined as persistence of HBsAg for more than six months. In the second group - control group, total of 100 consecutive normal healthy people without evidence of past or present evidence of HBV infection (negative for total

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antiHBe, HBsAg and antiHBs) undergoing HLA genotyping study as a donor in renal transplantation program at our centre.

In both the groups, HLA class II genotype analysis - HLA DRB1 was carried out by following technique. Theogenic DNA was extracted using commercially procured DNA extraction kit (Qiagen kit). HLA-DRB1 low resolution typing was followed using the commercial DRB1 PCR kit protocol (Dynal, Oslo).

Genotype-disease association was analyzed by using Halden’s modification of the Woolf’s formula. P value of < 0.001 was statistically highly significant, < 0.05 significant and > 0.05 not significant.

**RESULTS**

In chronic hepatitis B group, total of 26 patients were included with mean age of 45 years (range of 21-55 years) and male to female ratio of 2.7:1. In the control group, total of 100 patients was included with mean age of 41 years (range of 18-62 years) and sex ratio M:F of 2:1.

On comparing HLA class II-HLA DRB1 alleles in both the groups, HLA DRB1*11XX (23% vs. 4%, p value < 0.05) and HLA DRB1*15XX (57.6% vs. 25%, p value < 0.05) showed association with chronicity of HBV infection. HLA DRB1*13XX (0 vs. 19%, p value < 0.05) was negatively associated with chronicity of HBV infection. Other HLA class II - DRB1 alleles were neither positively nor negatively associated with chronicity of infection.

**Table 1 : Frequencies of HLA class II alleles in persistent HBV infection and in control groups**

<table>
<thead>
<tr>
<th>HLA class II Alleles</th>
<th>Chronic hepatitis B (N=26)</th>
<th>Control group (N=100)</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*01XX</td>
<td>7.6%</td>
<td>2.5%</td>
<td>No</td>
</tr>
<tr>
<td>DRB1*03XX</td>
<td>19.2%</td>
<td>10.0%</td>
<td>No</td>
</tr>
<tr>
<td>DRB1*04XX</td>
<td>1.5%</td>
<td>3.0%</td>
<td>No</td>
</tr>
<tr>
<td>DRB1*07XX</td>
<td>19.2%</td>
<td>9.0%</td>
<td>No</td>
</tr>
<tr>
<td>DRB1*09XX</td>
<td>0.0%</td>
<td>2.0%</td>
<td>No</td>
</tr>
<tr>
<td>DRB1*10XX</td>
<td>0.0%</td>
<td>8.0%</td>
<td>No</td>
</tr>
<tr>
<td>DRB1*11XX</td>
<td>23.0%</td>
<td>4.0%</td>
<td>Positive</td>
</tr>
<tr>
<td>DRB1*12XX</td>
<td>3.8%</td>
<td>2.0%</td>
<td>No</td>
</tr>
<tr>
<td>DRB1*13XX</td>
<td>0.0%</td>
<td>19.0%</td>
<td>Negative</td>
</tr>
<tr>
<td>DRB1*14XX</td>
<td>11.5%</td>
<td>12.0%</td>
<td>No</td>
</tr>
<tr>
<td>DRB1*15XX</td>
<td>57.6%</td>
<td>25.0%</td>
<td>Positive</td>
</tr>
<tr>
<td>DRB1*16XX</td>
<td>0.0%</td>
<td>4.0%</td>
<td>No</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In our study, HLA DRB1*11XX and HLA DRB1*15XX were positively whereas HLA DRB1*13XX negatively associated with HBV persistence. In the world literature, different HLA alleles associations are described for persistence as well as clearance of HBV.

Negative association of DRB1*13XX alleles was demonstrated in two studies in the past. In Gambian children and adults, DRB1*1302 was demonstrated to be associated with virus clearance while comparing groups who cleared HBV and another group who experienced persistent infection. In that study class II haplotype DRB1*1302-DRB3*0301-DQA1*0102-DQB1*0501 appeared to be protective. In another study from Germany, while comparing persistent infection with no exposure or with clearance, DRB1*1301-2 was associated with HBV clearance. DRB1*13XX has a protective role in preventing vertical transmission of HIV, in preventing cervical cancer in HPV infection and also in resistance to severe complicated falciparum malaria. This protective effect of DRB1*13XX appears to be sustained in all different ethnic groups including our study. DRB1*13XX may be more efficient in presenting immunodominant epitopes from HBs antigen to CD4+ T cells. Anchor residue for DRB1*1301-2 might be allowing predilection for allelic specific epitopes within HBV proteins.

Other study stating negative association with persistent HBV infection while comparing to mixed- exposed-> cleared or unexposed group came from Qatar, implicated antigen was DR2 (DRB1*15-16XX). In a Japanese study, DR1 and DRw13 were associated with elimination of virus.

DRB1*1102 was previously demonstrated to be associated with persistence of HBV when compared with clearance in a study from Baltimore on African-American drug-users. In this study, three locus haplotype DQA1*0501-DRB1*0301-DRB1*1102 was significantly associated with persistence. In a study in Qatarians, DR7 was demonstrated being significant for persistence. In a study from Taiwan, DR3 was found to be significant for persistence when compared with healthy controls. In our study, DRB1*11XX and DRB1*15XX were found significant for persistence of HBV, though small number studied and compared to controls who were unexposed.

In an Italian study, none of the alleles were significantly associated with either persistence or clearance of HBV. Also, in a study on hemodialysed patients of Caucasian or African origin, Verdon et al failed to demonstrate any allelic significance. In a Taiwanese study no alleles were significantly associated with persistence of HBV.

Thus, one particular allele has not been identified for the persistence of the HBV. This can be a result of many factors. There may be a complex interaction between multiple alleles. Different ethnic groups may have different genetic background and so variations in HLA alleles. A possibility of a gene being in linkage disequilibrium with HLA alleles cannot be ruled out. Other genes may also be important like - a) Tumour necrosis factor (TNF)-α in MHC class III - 238 promoter variant is associated with persistence, b) Mannose binding protein (MBP) codon 52 and codon S4 polymorphism may be important in some ethnic groups; and c) Vitamin D receptor gene with tt genotype in one polymorphism was associated with HBV clearance. Also variations in literature can be related to inaccurate, different and/or low-resolution HLA typing methods; to small sample size and to difference in control groups.

Despite small sample size, our study supports negative association of DRB1*13XX to persistence of HBV. Also there may be positive role of DRB1*11XX and DRB1*15XX for
persistence of HBV and development of chronic HBV infection.

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References

Announcement

TAPICON - 2003

III Annual Conference of API, Tripura State Branch (TAPICON-2003) will be held on 12th and 13th September 2003 at 'Hotel Royal Guest Home', Agartala, Tripura.

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