



## Role of HLA-DRB1\*13 Allele as a Recovery Factor Among Acute Hepatitis B than Chronic Hepatitis B Infected Patients: A Comparative Study from Bangladesh

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### Abstract

**Background:** The course of hepatitis B virus (HBV) infection is not only determined by variations in viral virulence but may be influenced by host immune response, where Human Leukocyte Antigen (HLA) plays an important role. **Objective:** The purpose of the present study was to explore whether HLA-DRB13\* allele of MHC gene had any influence in spontaneous recovery from HBV infection among Bangladeshi adults. **Method:** This cross sectional study was carried out at the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka Bangladesh. A total of 90 randomly selected hepatitis B virus infected adult patients, consisting of 30 acute HBV infections, 30 chronic HBV infection and 30 healthy controls were selected according to selection criteria for evaluation of HLA DRB1\*13 allele. Detection of HLA DRB1\*13 allele was done by conventional PCR followed by agarose gel electrophoresis. **Result:** The study revealed a significant increase of DRB1\*13 in acute hepatitis B (AHB vs HC-40% vs 6.7%, RR= 9.4; P value <0.05, AHB vs CHB=40% vs 10%; RR=2.27, P value <0.05) compared to chronic hepatitis B infected (HBV) patients and healthy controls (CHB vs HC-10% vs 6.7%, RR= 1.5, P>0.05). This is the first report on HLA DRB1\* gene associations among hepatitis B (HBV) infected Bangladeshi patients. **Conclusion:** The present study revealed that HLA DRB1\*13 was associated with protection against persistent HBV infection among acutely infected adult HBV patients in Bangladesh. [*Bangladesh Journal of Infectious Diseases, December 2019; 6(2):39-43*]

**Keywords:** Acute hepatitis B infection; Chronic Hepatitis B Infection; HLA, DRB1\* Gene; Agarose Gel Electrophoresis; PCR

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## Introduction

Population-specific distribution of HLA alleles is necessary both in population genetics and in HLA disease association studies. Anthropological studies show that the distribution of HLA alleles differ among different ethnic groups. The genetic background of Bangladeshi people appears to be a mixture of different populations, mainly Indo-Aryan, Austro-Asiatic, Dravidian, Mughal, Arab, Persian, Turkic and British<sup>1</sup>.

Host gene variants may influence the natural history of hepatitis B virus (HBV) infection. The human leukocyte antigen (HLA) system, the major histocompatibility complex (MHC) in humans, is one of the most important host factors correlated with the clinical course of HBV infection. Various genome-wide association studies (GWASs) have shown that certain HLA gene loci are strongly associated with not only persistent HBV infection but also spontaneous HBV clearance and seroconversion, disease progression, and the development of liver cirrhosis and HBV-related hepatocellular carcinoma (HCC) in hepatitis B infected patients, in various countries throughout the world.

Different HLA alleles have been demonstrated to play a role in hepatitis B virus infection but this relationship is not universal on the basis of the investigated population. A strong virus-specific CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocyte response to hepatitis B virus was associated with viral clearance. Patients with acute hepatitis B carrying HLA-DRB1\*13 had a more vigorous CD4<sup>+</sup> T cell response to hepatitis B virus core than patients not carrying HLA-DRB1\*13, suggesting that HLA-DRB1\*13 is associated with a self-limited course of HBV infection, and the beneficial effect of HLA-DR13 alleles on the outcome of hepatitis B infection could be explained by a more vigorous hepatitis B virus core-specific CD4<sup>+</sup> T cell response, which may be due to a more proficient antigen presentation by HLA-DRB1\*13 molecules themselves<sup>2</sup>.

HLA is a critical genetic factor that determines individual variations of immune response. The structure of HLA molecules and their roles in the control of immune response have been clearly elucidated<sup>3</sup>. There is many research has been performed about statistical associations between HLA and the host response against different diseases worldwide. In Bangladesh different research work has been found correlation of HLA with spondylo-arthropathies, natural rubella infection but little is known regarding HLA-

DRB\*1 prevalence and its relation with HBV infection<sup>4</sup>. To the best of our knowledge, the present study is the first study from Bangladesh regarding HLADRB\*1 gene association with HBV infection.

Bangladesh is a country in the intermediate prevalence zone of hepatitis B virus and the estimated prevalence of chronic HBV carrier among the general population and different high risk groups like intravenous drug users, professional blood donor ranges from 0.8% to 6.2%<sup>5-7</sup>. Although there have been a good number of publications describing the association of HLA antigens and outcome of HBV infection from different population and ethnic groups worldwide, no such published date is available from Bangladeshi HBV infected people. In order to address this issue, this study was performed to determine the influence of HLA DRB1\*13 allele in the outcome of HBV infection among Bangladeshi people.

## Methodology

**Study Subjects:** This cross sectional study was carried out among HBV infected adult patients attending the in-patients and out-patient's departments of Hepatology and Gastroenterology of Bangabandhu Sheikh Mujib Medical University (BSMMU), a tertiary care hospital in Dhaka city. Participants were selected by non-probability purposive sampling method from three different groups: 30 acute hepatitis B infected patients (HBsAg positive for <6 months, Anti HBcIgM positive and anti-HBs negative) and 30 chronic hepatitis B infected patients (HBsAg positive for >6 months, Anti HBc total positive Anti-HBc IgM negative and anti-HBs negative) and 30 healthy individuals of different ages and genders according to the CDC guidelines for the interpretation of hepatitis B serological test results.<sup>8</sup> Their age ranged from 18 to 55 years with mean age 32.9±10.06, 28.7±6.55, 33.2±9.9 years for acute hepatitis B, Chronic hepatitis B, and healthy controls respectively. The male female ratio was 1:1.). The mean ALT level of AHB patients was 227.26±18.15 IU/L and the mean ALT level of chronic hepatitis B patients was 159.73±25.15 IU/L. A detailed evaluation of patient history, identified clinical variables, disease severity, age at onset, initial clinical manifestations were recorded in pre-designed data collection sheets and their informed written consent were taken. Venous blood samples were collected from selected patients for virological tests and HLA typing and all laboratory tests were performed at the Department of Virology, BSMMU. **Primer and reagents:** For PCR reaction, the primer (forward and reverse) of

the HLA DRB1\*13 gene and  $\beta$  actin gene (Housekeeping gene) were selected. **DNA extraction:** Genomic DNA was extracted from peripheral blood by using classical phenol or chloroform DNA extraction method. **DNA quantitation:** DNA concentration was measured in ng/ $\mu$ l by Thermo Nanodrop Spectrophotometer (2000C) 260 nm wave length (Figure I). **PCR amplification:** A PCR reaction volume of 13 $\mu$ l was used containing 50 nanogram/microliter (ng/ $\mu$ l) of DNA, 0.1 microliter Taq polymerase, 1.25 microliter 10X PCR buffer, 0.25 microliter dNTPs, 0.5 microliter each, primers (forward primer 0.5 microliter and reverse primer 0.5 microliter) of the HLA DRB1\*13 gene and rest molecular grade water. Low-resolution Single Specific Primer-Polymerase Chain Reaction (SSP-PCR) was performed with NYSTECHNIK Semiquantitative PCR machine (Genome Diagnostic Pvt.Ltd, India). **Detection of PCR products:** The amplified PCR products were detected by agarose gel electrophoresis. For detection of DRB1\*13 gene

3% agarose gel was used, and for detection of  $\beta$  actin (Housekeeping gene) 4% agarose gel was used. Agarose gel mixed with 100 ml TBE (Tris, Boric acid, Ethylene-diamine-tetra-acetic acid) containing 6 $\mu$ l of ethidium bromide was electrophoresed for 170 Volt for 35 minutes. DNA bands were identified according to their molecular size by comparing with 100 bp DNA ladder. 100 bp. DNA size standard (Bio-Rad, USA) was used as marker to measure the molecular size of the amplified products. Samples showing the presence of specific DNA bands corresponding to 197 bps were considered positive for presence of HLA DRB1\*13 gene. If the pooled DNA template result was negative following gel electrophoresis, the sample was considered negative for HLA DRB1\* gene. The presence of amplified product with correct size was only interpreted as a test positive. The DNA bands were visualized using Wealtec Dolphin View Gel Imaging System (Wealtec Bioscience Co, Ltd., USA).

**Table 1: HLA DRB1\*13 oligonucleotide primers & Beta actin Housekeeping gene**

Gene Product HLA DRB1*(Direction of strand)	Fragment size (bases)	Primer séquences
DRB1*13(3')	130 bps	CCGCGCCTGCTCCAGGAT
DRB1*13(5')		TACTTCCATAACCAGGAGGAGA
Beta actin (5') (Housekeeping gene)	56bps	CCAGCTCACCATGGATGATG
Beta actin(3')		ATGCCGGAGCCGTTGTC

**Statistical Analysis:** Allele frequency of HLA-DRB1 gene was calculated by direct count. Allele Frequency (AF) among study groups (Acute & Chronic hepatitis B) were compared using Chi-square ( $X^2$ ) test and their Relative risk frequencies (RR) were calculated, Mann-Whitney U test was done for observation of mean ALT level between inter-groups (chronic hepatitis B and acute hepatitis B) which was statistically significant. Statistical analysis was made using SPSS 17.0 software, and p value <0.05 considered as statistical significance.

## Results

The comparison of HLA DRB1\*13 genes between acute hepatitis B and chronic hepatitis B groups revealed that the risk of frequency of DRB1\*13 (AHB vs HC 40% vs 6.7%, RR= 9.4; P value <0.05 & CHB vs HC 10% vs 6.7%, RR= 1.5; P>0.05 (Table 3).

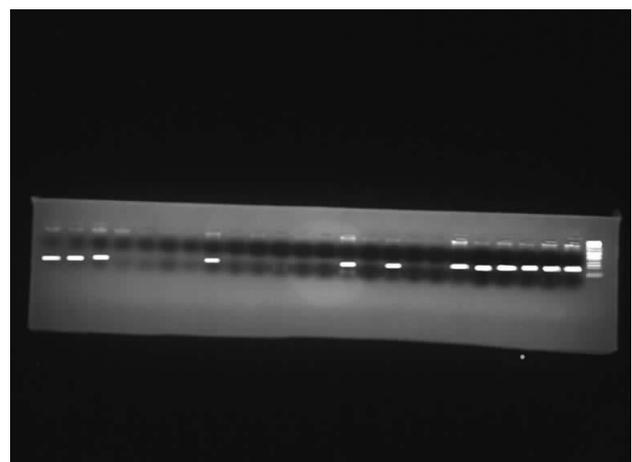


Figure I: Positive bands of HLA DRB1\*13 gene in agarose gel electrophoresis of Acute Hepatitis B Infected patients after amplification by PCR

**Table 2: Comparison of HLA DRB1\* 13 allele of HLA DRB1 genes between Acute Hepatitis B and Healthy Controls**

HLA DRB1 *13 Allele	Acute Hep B	Healthy Control	RR	P value
Positive	12(40%)	2(6.7%)	9.4	0.05
Negative	18(60%)	28(93%)		

Relative Risk (RR) test and Chi-Square Test ( $X^2$  test) were done.  $P < 0.05$  indicates statistical significance. P\* value indicates for AHB vs HC; Acute Hep B=Acute Hepatitis B

**Table 3: Comparison of HLA DRB1\* 13 allele of HLA DRB1 genes between Chronic Hepatitis B and Healthy Control**

HLA DRB1 *13 Allele	Chronic Hep B	Healthy Control	RR	P* value
Positive	3(10%)	2(6.7%)	1.5	0.05
Negative	27(90%)	28(93%)		

Relative Risk (RR) test and Chi-Square Test ( $X^2$  test) were done;  $P < 0.05$  indicates statistical significance; P\* value indicates CHB vs HC; Chronic Hep B= Chronic Hepatitis B

AHB vs CHB=40% vs 10%; RR=2.27, P value  $< 0.05$ ) was higher among acute hepatitis B than chronic hepatitis B (Table 4).

**Table 4: Comparison of HLA DRB1\*13 allele of HLA DRB1 genes between Acute Hepatitis B and Chronic Hepatitis**

HLA DRB1 *13 Allele	Hepatitis B		RR	P* value
	Acute	Chronic		
Positive	12(40%)	3(10%)	2.27	$< 0.05$
Negative	18(60%)	27(90%)		

Relative Risk (RR) test and Chi - Square Test ( $X^2$  test) were done;  $P < 0.05$  indicates statistical significance; P\* value indicates AHB vs CHB

## Discussion

Undoubtedly both host and viral factors influence the clinical expression and behavior of hepatitis B infection. Attempts to explain the clinical expression and behavior of hepatitis B infection by viral factors have shown the importance of viral genotypes and viraemia level for the clinical presentation. However, there remain large inconsistencies, and it is very likely that immune response to hepatitis B virus (HBV) by the host can modify disease outcome<sup>3,9,10</sup>.

In present study, association of HLA DRB1\*13 gene and outcome of acute hepatitis B infection showed a significant increase of DRB1\*13 in acute hepatitis B (AHB vs HC-40% vs 6.7%, RR= 9.4,  $X^2$  test = 14.4, P value  $< 0.05$ , AHB vs CHB 40% vs 10%; RR=2.27,  $X^2$  test= 0.11, P value  $< 0.05$  CHB VS HC-10% vs 6.7%, RR= 1.5,  $X^2$  test= 0.11,

$P > 0.05$ .) compared to chronic hepatitis B infected (HBV) patients and healthy controls. A study from India reported that Indian patients supported negative associations of DRB1\*13 to persistence of hepatitis B infection suggesting that patient with DRB1\*13 can mount a more vigorous CD4 positive T cell response<sup>11</sup>. Another study from India observed that DRB1\*13, DRB1\*14 and DRB1\*04 were negatively associated with hepatitis B virus persistence<sup>12</sup>. In contrast, HLA DRB1\*11/\*12 alleles were reported to be associated with hepatitis B virus clearance among Chinese population while HLA DRB1\*13 was reported as a susceptibility gene for chronic HBV infection among the Turkish populations<sup>9,10,11</sup>. In Caucasia and south Korea, HLA-DRB1\*1301-02 was found to be associated with acute self-limited hepatitis B<sup>12</sup>. In a study from Iran association of HLADRB1\*13 alleles with outcome of acute hepatitis B infection observed similar result with our study where the frequency of the DRB1\*13 allele was higher among the HBV recovered group<sup>13</sup>. A study from Gambia, investigating a large cohort of paediatric patients identified the association of the HLA class II allele DRB1\*1302 with a self-limiting course of acute hepatitis B<sup>14</sup>. However, the effect of DRB1\*1302 among Gambian adults seemed to clear hepatitis B virus (HBV) infection<sup>15</sup>. A study from Germany confirmed that DRB1\*13 was associated with self-limiting course of hepatitis B infection<sup>16</sup>. Investigators from Spain, analyzed the HLA-DRB1\* genes 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 hepatitis B virus infection and protected people against chronic hepatitis B<sup>17</sup>.

All these studies demonstrated the importance of HLA DRB1\*13 in clearance of hepatitis B virus infection. The distributive frequency of DRB1\*13 in the oriental people is quite low (5.7%) than among white (10.5%) and black people (7.5%). Among the Chinese population of North China, the distribution of DRB1\*13 is only 1.8% while in South China it is 1.9%. The lower distributive frequency of HLA DR13 may be one of the reasons why oriental people are more susceptible to chronic hepatitis B<sup>18</sup>. The beneficial effect of HLA DRB1\*13 allele with the clearance of hepatitis B virus infection may be the result of more proficient presenting immunodominant epitopes from HBs antigen to CD4+ T cells resulting in a more vigorous hepatitis B core specific CD4 T cell response<sup>19</sup>. In contrast, positive association of DRB1\*02 with hepatitis B clearance was reported in a study from Qatar<sup>20</sup>.

This finding suggests that there may be different relationship between HLA gene polymorphism with hepatitis B infection in different racial populations, implying that various HLA molecules may present different hepatitis B virus epitopes to induce effective immune responses<sup>21</sup>. Thus, HLA class II molecules may affect the outcome of hepatitis B infection.

## Conclusion

World over studies have shown inconsistent associations with regard to the effects of host genetic factors on HBV clearance and persistence due to complex interactions between the virus and multiple alleles, host ethnic differences in the studied population groups, and/or association with a gene in linkage disequilibrium with an HLA allele. However, many factors such as ethnic background, geographic variance and overall environmental factors should be considered before making a definite conclusion. Further, since genetic interactions are complex, it is unlikely that a single allelic variant responsible for HBV resistance or susceptibility. The present study demonstrated that HLA DRB1\*13 was more frequent among acute hepatitis B than chronic hepatitis B infected Bangladeshi patients. Previously various studies proved that host HLA class II gene is an important factor determining the outcome of HBV infections, which may give some new insight to the study of molecular pathogenesis of hepatitis B. Future extensive studies to investigate whether one of these HLA allele polymorphisms or a yet unidentified immune-regulatory gene is possibly associated with a more successful immune response against HBV infection may be promising.

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