



Association of HLA DRB1*15 Gene among Acute and Chronic Hepatitis B Infected Bangladeshi Patients

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Abstract

Background: Elucidating differences in HLA DRB1* genes distribution may be useful in understanding the molecular pathogenesis of viral hepatitis B. **Objective:** The aim of the study was to find out the HLA DRB1*15 gene susceptibility among acute and chronic Hepatitis B infected Bangladeshi patients. **Methodology:** This cross-sectional study was carried out in the Department of Virology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh during the period of June 2012 to June 2013 for a period of one year. Evaluation HLA DRB15*gene distribution was performed among acute hepatitis B and chronic hepatitis B infected (HBV) Bangladeshi patients. HLA DRB15*gene distribution was detected by conventional PCR followed by agarose gel electrophoresis. **Result:** A total of 60 serologically pre-diagnosed 30 acute hepatitis B and 30 chronic hepatitis B infected (HBV) Bangladeshi patients were evaluated. The study revealed a significant increase of DRB1*15 allele among chronic hepatitis B infected patients compared to acute hepatitis B (46.7% vs 13.3%; RR=5.8, X² test=7.2; P< 0.05). This is the first report to investigate HLA DRB1* gene associations among acute and chronic HBV infected Bangladeshi patients. **Conclusion:** In conclusion HLA DRB1*15 is more frequent in chronic hepatitis B infected Bangladeshi patients compared to acute hepatitis B. [*Bangladesh Journal of Infectious Diseases, June 2018;5(1):3-9*]

Keywords: Hepatitis B infection; Chronic hepatitis B infection; HLA, DRB1* allele; Agarose gel Electrophoresis; PCR

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Introduction

Worldwide, hepatitis B virus (HBV) infection is a major public health problem with significant morbidity and mortality¹. About one third of the world's population have been infected with the hepatitis B virus². Globally, over 2 billion people are infected with HBV and among them, about 660,000 die annually due to the consequences of this infection³. Of the estimated 50 million new cases of hepatitis B virus (HBV) infection diagnosed annually, 75% are in Asia where hepatitis B is the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma⁴. In the last two decades, public health interventions and implementation of universal vaccination programs have substantially reduced the incidence of HBV infections in many countries in this region⁵.

HBV infection is a dynamic process, and the outcome of HBV infection varies due to differences in host responses. Some people with chronic HBV infection remain asymptomatic even after many decades of infection with slow disease progression, whereas, others rapidly progress to cirrhosis and hepatocellular carcinoma. A strong genetic component like HLA gene expression seems to be a major driving force affecting the course of viral hepatitis⁶. Previous epidemiological investigation in humans suggests that there is a strong genetic component to affect the individual susceptibility to infectious pathogens, although to date, no single allele has been clearly associated with HBV persistence or disease severity⁷⁻⁹. However, the following reflects individual and ethnic differences in response to HBV infection: Infection with the same hepatitis B virus has been found to cause various clinical outcomes in patients (acute hepatitis B, chronic hepatitis B, liver cirrhosis, hepatocellular carcinoma), long-term follow-up studies indicate that some individuals in high-risk groups like spouses in hepatitis B infected families never develop the disease, this suggest the existence of an individual-specific resistance to HBV infection¹⁰⁻¹¹.

There is different incidence and infection rates among global ethnic groups. Hepatitis B virus infection is significantly endemic in Asia and Africa, and there is a significantly higher incidence of chronic hepatitis B infection among Chinese compared to Caucasians¹². In hospital, hepatitis B virus-infected individuals may display complete, partial or no response to interferon-alfa or lamivudine antiviral therapy alone or in combination. Around 85.0% of healthy subjects can produce an efficient protective anti-HBsAg antibody upon hepatitis B virus vaccination, while

the remaining fail. The above-mentioned data suggests that the knowledge of understanding human genetic factors may provide critical clues not only to the ethnic diversity of hepatitis B virus infection, but also to the issue of disparity in therapeutic response¹³.

The factors that determine the outcome of hepatitis B virus infection in an individual patient are poorly understood. Both virological like viral load, genotype, and genetic divergence due to viral gene mutations) and host immunological factors including the innate and adaptive immune responses against viral infection, which play important roles in modulating both the antiviral immune response and host susceptibility to hepatitis B virus infection may play important roles in determining the outcome^{11,14}. It is generally accepted that viral clearance or chronic viremia following HBV infection is determined by the host immune response against HBV in which human leukocyte antigens (HLA) play a central role. The progressions of antigen-presenting cells presenting viral antigens to B and T cells, B and T cells recognizing antigens, and B and T cells being reactivated are all restricted by HLA. It is, therefore, presumed that the HLA polymorphism possibly determines the pathogenesis and outcome of HBV infection¹⁵. 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. Most genetic studies involving hepatitis B virus susceptibility have focused on its correlations with HLA Class I and Class II. Different HLA Class II alleles are reported to be important in persistence or clearance of hepatitis B virus in various studies throughout the world¹⁶⁻¹⁷. However there are no such study from Bangladesh yet. The aim of this study was to detect specific HLA DRB1*15 gene distribution among acute and chronic hepatitis B infected (HBV) Bangladeshi patients.

Methodology

Study Subjects: This cross-sectional study was carried out in the Department of Virology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from June 2012 to June 2013 for a period of one (1) year. Blood samples were collected from clinically definite 30 acute hepatitis B infected patients who were HBsAg positive for less than 6 months with Anti-HBc IgM positive and 30 chronic hepatitis B infected patients who were HBsAg positive for more than 6 months with Anti-HBc IgM negative in the age of 18 to 55 years. A detailed evaluation of patient history,

identified clinical variables, disease severity, age at onset, initial clinical manifestations and informed consent were recorded for every patient in pre-designed data collection sheets. Samples were selected by non-probability purposive sampling method.

Primer and reagents: For PCR reaction, the primer (forward and reverse) of the HLA DRB1*15 gene and β actin gene (Housekeeping gene) were selected.

DNA extraction: Genomic DNA was extracted from peripheral blood by using classical phenol/chloroform DNA extraction method.

DNA quantitation: DNA concentration was measured in ng/ μ l by Thermo Nanodrop Spectrophotometer (2000C) 260 nm wave length.

PCR amplification: A PCR reaction volume 13 μ l was used containing :- 50 nanogram / microlite (ng/ μ 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* genes and rest molecular grade

water, then 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*15 gene 3% agarose gel was used, for detection of β actin (Housekeeping gene) 4% agarose gel was used. Agarose gel mixed with 100ml TBE (Tris, Boric acid, Ethylene-diamine-tetra-acetic acid) containing 6 μ l of ethidium bromide 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 band corresponding to 197 bps were considered positive for presence of HLA DRB1*15 gene. If the pooled DNA template result was negative following gel electrophoresis, the sample was considered negative for HLA DRB1* gene. Only the presence of the amplified product with correct size was interpreted as a test positive. The DNA bands were visualized using Wealtec Dolphin view Gel Imaging System (Wealtec Bioscience Co, Ltd., USA).

Table 1: The Following HLA DRB1*15 Oligonucleotide Primers & Beta actin Housekeeping Gene used

Gene product Primer sequences HLA DRB1*(Direction of Strand)	Fragment Size (Bases)	Primer Séquences
DRB1*15 (5')	197 bps	CCCGCTCGTCTTCCAGGAT
DRB1*15 (3')		TCCTGTGGCAGCCTAAGAG
Beta actin (5') (Housekeeping gene)	56 bps	CCAGCTCACCATGGATGATG
Beta actin(3')		ATGCCGGAGCCGTTGTC

Statistical Method: Allele frequencies of HLA-DRB1 were calculated by direct count. AF for the study group (Acute & Chronic hepatitis B) was compared using Chi-square test. Relative risk frequencies (RR) were calculated. Mann-Whitney U test was done. Statistical analysis was made using SPSS 17.0 software, and p value < 0.05 considered as statistical significance.

Results

In this cross-sectional study, during one-year period, blood samples were collected from 30

acute hepatitis B and 30 chronic hepatitis B infected patients, aged ranged from 18 to 55 years with (mean \pm SD) 31.6 \pm 8.84 year. The mean age of acute hepatitis B and Chronic hepatitis B were 32.9 \pm 10.06, 28.7 \pm 6.55 years respectively. Male and female ratio was 1:1. The mean ALT level of acute hepatitis B and Chronic hepatitis B were 227.26 \pm 18.15 IU/L, 159.73 \pm 25.15 IU/L respectively. The mean ALT level between inter-groups (acute hepatitis B and chronic hepatitis B) was statistically significant (p<0.05). The mean DNA concentration of the acute hepatitis B and chronic hepatitis B infected patients were 69.13 \pm 29.67 and 95.10 \pm 81.54 respectively (range 64.23 ng/ μ l to 156.45 ng/ μ l (Table 2).

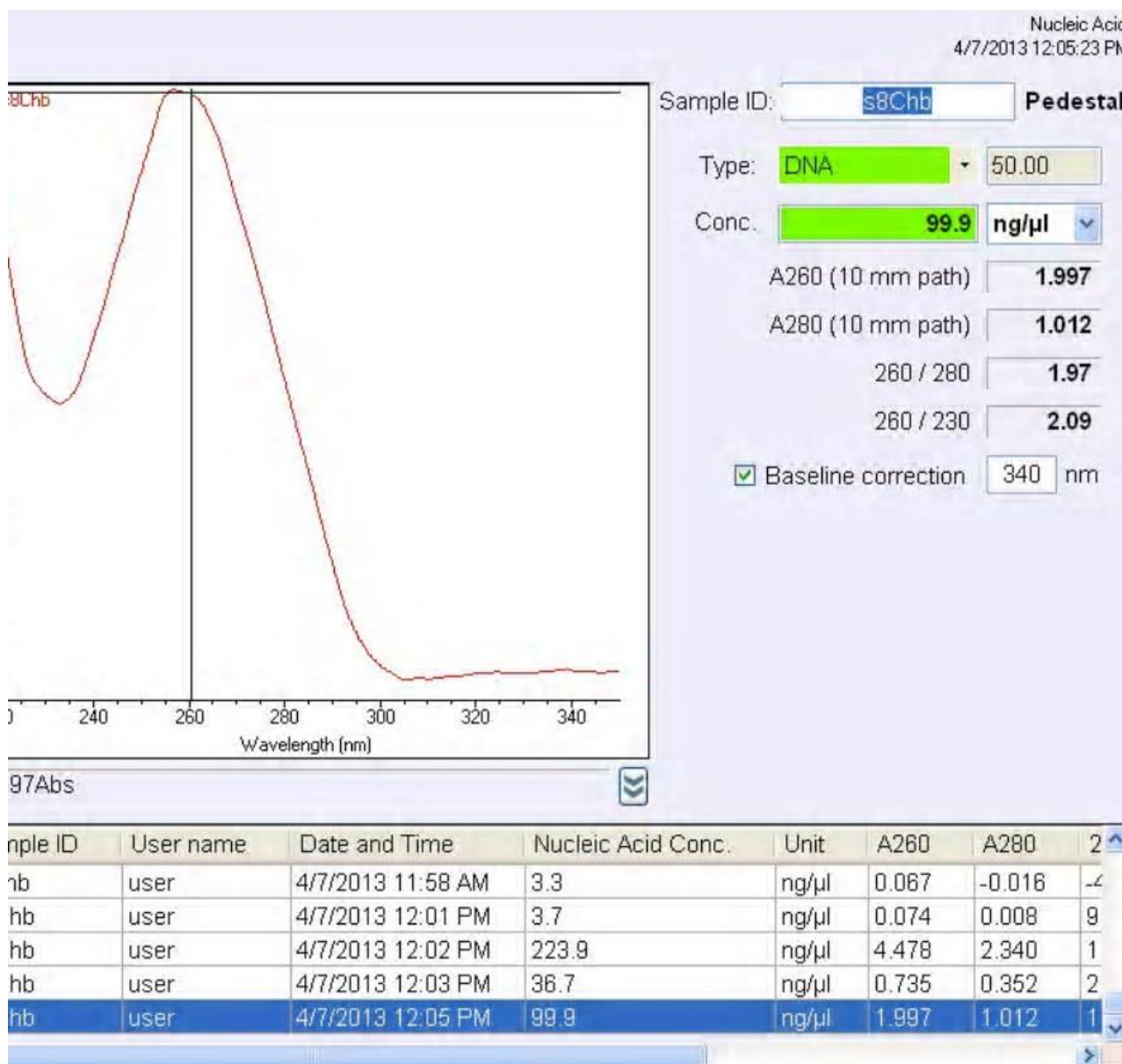


Figure I: Nanodrop DNA curve (Quantitation of DNA) in Thermo-nanodrop Spectrophotometer (2000C) 260 nm wave length

The comparison of HLA DRB*1 genes between acute hepatitis B and chronic hepatitis B groups revealed that the risk of frequency of DRB1*15 (46.7% vs 13.3%) was five times higher in chronic

hepatitis B than acute hepatitis B (RR=5.8; X² test=7.2 respectively, P< 0.05). The mean viral load of chronic hepatitis B patients was 6.62 ± 9.60 [log₁₀ (copies/ ml)] (Table 3).

Table 2: Clinical and Virological Characteristics of Individuals Enrolled in the Study

Variables	Acute Hepatitis B	Chronic Hepatitis B	P* value
Mean Age (Years)	32.9 ± 10.06	28.7 ± 6.55	-
Sex (F:M)	15 : 15	15 : 15	-
Mean ALT (IU/L) ± SD	227.26 ± 90.1	159.73 ± 46.8	P<0.05
Mean DNA Con(ng/μl)	69.13± 29.67	95.10 ±81.54	-

Mann-Whitney U test was done; P< 0.05 indicates statistical significance

Table 3: Distribution of HLA DRB1*genes among Acute & chronic hepatitis B (CHB) with mean viral load of Chronic hepatitis B (CHB)-

HLA DRB1 *genes	Acute hepatitis B	Chronic hepatitis B	Relative Risk	Chi-Square	P value*
DRB1*15	04 (13.3%)	14 (46.7%)	5.8	7.2	<0.05
Mean Viral load [log ₁₀ (copies/ml)]	ND	6.62 ± 9.60	-	-	-

Relative Risk (RR) test and Chi-Square Test (X^2 test) were done. mean ± SD =mean viral load of chronic hepatitis B, ND indicates not done

Discussion

Most of the reports of human genes associated with HBV infection have currently focused on HLA associations. The factors that determine the outcome of chronic hepatitis B infection in individuals' patients are poorly understood. These may be classified into three categories, virological factors, immunological factors and host genetic factors⁶. Virological factors include viral load, viral genotype and mutations in the viral genome.



Figure II: Electrophoresis of HLA DRB1*15 genes in acute hepatitis B infected patient after amplification by PCR/SSP

The HLA genotype has been thought to be an important genetic factor for the predication of the susceptibility of individuals to hepatitis B infection and prognosis of disease in certain populations²⁰⁻²¹.

The HLA genotype has been thought to be an important genetic factor for the predication of the susceptibility of individuals to hepatitis B infection and prognosis of disease in certain populations^{6,20-21}. The association of individuals to hepatitis B infection and disease progression varies since multiple factors such as geography and ethnicity, affect this association^{13,22-24}.

Most genetic studies involving hepatitis B virus susceptibility have focused on its correlations with HLA Class I and Class II. Different HLA Class II alleles are reported to be important in persistence or clearance of hepatitis B virus in various studies throughout the world¹⁸⁻¹⁹. However, there is no such study from Bangladesh yet. The expression of selected HLA DRB1*alleles may reflect the molecular mechanism underlying the outcomes of chronic HBV infections. In the present study, the frequency of HLA DRB1*15 gene among acute hepatitis B and chronic hepatitis B revealed that HLA DRB1*15 was significantly higher among chronic hepatitis B (46.7%) compared to acute hepatitis B infected (13.3%) patients suggesting that HLA DRB1*15 may be associated with increased risk of infection and progression of hepatitis B infection. Similar results were reported from India, where HLA DRB1*15 was positively associated with chronic hepatitis B²⁶⁻²⁷. Opposite result showing in previous studies where observed that HLA DRB1*03 was associated with persistent hepatitis B infection among Chinese and Caucasians²⁸⁻³⁰.

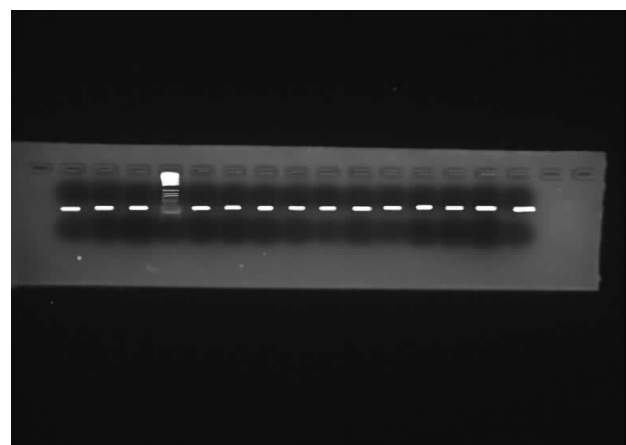


Figure III: Electrophoresis (Agarose gel) of HLA DRB1*15 gene after amplification by PCR/SSP in Chronic hepatitis B infected patients

A study from India observed that HLA DRB1*03 was associated with self limited course of acute hepatitis B³¹. Another study among South Indian

population, HLA-DRB1*0701 was strongly associated with hepatitis B virus chronicity³² while in a study from Korea, HLA-DRB1*0301, HLA-DQA1*0501 and HLA-DQB1*0301 were closely correlated with susceptibility to chronic hepatitis B³³. In a study from Qatar³⁴, HLA DRB1*07 was associated with persistence of hepatitis B virus infections. A study from China³⁵, suggested that the susceptibility to chronic hepatitis B was strongly associated with HLA-DRB1*09, HLA-DRB1*0301, HLA-DRB1*10 allele, while HLA-DRB1*03 genes were associated with persistence of hepatitis B infection in Caucasians. Previous studies³⁶ proved that there is a complexity of genetic susceptibility or resistance to hepatitis B infection in different populations in different ethnic group in different countries.

Conclusion

The present study reveals that HLA DRB1*15 is more frequent in chronic hepatitis B infected Bangladeshi patients compared to acute hepatitis B. These results support that HLA-DRB1*gene may influence the susceptibility to chronic hepatitis B infection. Thus, HLA class II molecules may affect the outcome of hepatitis B infection. Thus far, world over studies have shown inconsistent associations with regard to the effects of host genetic factors on HBV clearance and persistence. This ambiguity could be due to a complex interaction between the virus and host multiple alleles; and/or the ethnic differences in the studied population groups; and/or association with a gene in linkage disequilibrium with an HLA allele. Further, since genetic interactions are complex it is unlikely that a single allelic variants responsible for HBV resistance or susceptibility. Future studies have 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.

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