Original article:

Susceptibility of Specific HLA DRB1*15 AlleleAmong Chronic Hepatitis B Infected Bangladeshi Patients

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Abstract

Background: The outcome of hepatitis B virus (HBV) infection may be influenced by host factors like Human Leukocyte Antigen (HLA).The expression of HLA genes in peripheral blood mononuclear cells (PBMCs) may reflect the molecular mechanism underlying different HBV infection outcomes. ***Objective:*** The purpose of the present study was to explore whether HLA DRB1*15 allele confer susceptibility to chronic hepatitis B infected Bangladeshi patients. ***Method:*** This cross sectional study was carried out in the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU) during July 2012 to June 2013 for a period of one year.Evaluation of HLA DRB1*15 allele distribution among 30 chronic hepatitis B infected (HBV) Bangladeshi patients compared them with 30 healthy individuals.HLA DRB1*15 allele distribution was detected by conventional PCR followed by agarose gel electrophoresis, using commercial low-resolution DRB1*15 allele polymerase chain reaction sequence specific priming kit. ***Result:*** A total of 30 chronic hepatitis B infected (HBV) Bangladeshi patients were evaluated together with 30 healthy controls. The study revealed a significant increase of DRB1*15 allele (46.7% vs 20%; RR= 3.5; X^2 = 7.2; P<0.05) compared to healthy controls. This is the first report on HLA DRB1*15 allele associations among chronic hepatitis B (HBV) infected Bangladeshi patients. ***Conclusion:*** The present study reveals that HLA DRB1*15 allele was more frequent in chronic hepatitis B infected Bangladeshi patients compared to healthy individuals. Thus, HLA DRB1*15 allele of HLA class II molecules significantly affect the outcome of hepatitis B infection.

Keywords: Chronic hepatitis B infection; HLA, DRB1*allele; Agarose Gel Electrophoresis; PCR.

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Introduction

Hepatitis B infection is a potentially severe disease accounting for over 400 million chronic hepatitis B infected patients and nearly 1.2 million deaths every year[1]. Among hepatiis B infected patients about 15–40% lead to chronicityare at a higher risk of developing liver cirrhosis and hepatocellular carcinoma (HCC) during their lifetime[2]. Bangladesh and most of the asia and also part of the Africa belong to the intermediate prevalence region of hepatitis B infection, where the lifetime risk of acquisition of hepatitis Binfection in between 20% to 60%, among themabout 40% of the world's population reside. Different studies indicate that hepatitis B virus is responsible for 31.25% cases of acute hepatitis, 76.3% cases of chronic hepatitis, 61.15% cases of cirrhosis of liver and 33.3% cases of hepatocellular carcinoma in Bangladesh[3-5].

There are various factors may be influence the progression of hepatitis B virus (HBV) infection including the viral genotype and the level of viremia, environmental factors, ethnicity etc but these factors alone do

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not account for the variability in hepatitis B infection outcome. Now a days increasing awareness that host factors are also important,involved inhepatitis B virus (HBV) infection outcome. A lot of evidences suggest that both cellular and humoral immune responses are required for viral elimination[6-8]. Human leukocyte antigen (HLA) variabilityinfluence theimmune responses. Variation of immune response is often associated with HLA polymorphism. Although, the actual mechanism is not yet fully understood, the reason behind for this difference in response to hepatitis B virus infection outcome is thought to be attributed to a complex web of inter-related factors such as host genetic, ethnicity, viral and environmental factors[9].

A lot of past epidemiological investigation in humans suggests that there is a strong genetic component like HLA affect the individual susceptibility to infectious pathogens, although now a days various studies suggest that, no single allele has been clearly associated with hepatitis B persistence, clearance or disease severity[10-12].

Patientsinfected with the same hepatitis B virus has been found to cause different clinical outcomes which includeacute hepatitis B, chronic hepatitis B, liver cirrhosis, hepatocellular carcinoma. Long-term follow-up of various studies of hepatitis B infected patients or healthy population indicate that some individuals are inspite of high-risk groups (e.g. spouses in hepatitis B-infected families) are never develop the disease, this means that the existence of an individual's specific resistance to hepatitis B virus infection[13-14]. Various genetic study regarding hepatitis B virus infections found that patients who have successfully recovered from acute hepatitis B develop strong HLA classes I and II restricted T cell immune response, whereas these immune responses are weak or absent in patients with chronic hepatitis B[15].

Incidence and infection rates of hepatitis B infection varies among global ethnic groups. Higher incidence of chronic hepatitis B infection among Chinese people compared to Caucasianswas found significantly. Hepatitis B virus infection is highly endemic in Asia and Africa. Almost around 85% of healthy subjects can produce an efficient protective anti-HBsAg antibody upon hepatitis B virus vaccination, while the remaining fail to response. Hepatitis B virus-infected individuals may display complete, partial or no response to interferon-alpha or lamivudine antiviral therapy alone or in a combination in hospital based management[16].

The above-mentioned data highlights that the knowledge of understanding human genetic factors may provide important critical clues not only to the ethnic diversity of hepatitis B virus infection, but also to the issue of disparity in therapeutic response[17]. However there are no such study from Bangladesh yet. The aim of this study was to detect specific HLA DRB1*15 alleledistribution among chronic hepatitis B infected (HBV) Bangladeshi patients and healthy individuals.

Materials and methods

Subjects

Blood samples from clinically and serologically define thirty chronic hepatitis B infectedpatients (Patients who were HBsAg positive for >6 months, Anti HBcIgM negative, aged 18-55 years) was collected along with laboratory work from July 2012 to June 2013at the Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU). 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. Thirty age and sex matched normal individuals (HBV markers negetive) studied for their HLA tissue typing during the same period as well as negative for HbsAg, anti HBc, and anti HBs were compared as controls.

***Primer and reagents*-**For PCR reaction, the primer (forward and reverse) of the HLA DRB1*15 allele and β actin gene (Housekeeping gene, forward and reverse) were selected as control **(Table1).**

Table1: The Following HLA DRB1*15Oligonucleotide Primers and Beta actin Housekeeping gene were used -

Primer(5'-3') HLA DRB1* allele	Amplicon Size(bps)	OligonucleotidPrimer sequences
DRB1*15 (5′)	197 bps	CCCGCTCGTCTTCCAGGAT
DRB1*15 (3′)		TCCTGTGGCAGCCTAAGAG
Beta actin(5′)	56 bps	CCAGCTCACCATGGATGATG
Beta actin(3′) (Housekeeping gene)		ATGCCGGAGCCGTTGTC

*Detection of DRB1*15 allele -*

DNA extraction-All non-coagulated ethylene-diamine-tetraacetic acid blood samples were stored at -20^{0}C before DNA extraction.Genomic DNA was extracted from peripheral blood by using classical phenol/chloroform DNA extraction method using

Red Cell Lysis Buffer containing sucrose, Tris, $Mgcl_2$, Triton x, Pk buffer, Pk solution etc.

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

Preparation of master mix: 13 µl reaction volume-containing :- 50 nanogram / microlite (ng/µl) 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* 15 allele and rest molecular grade water.

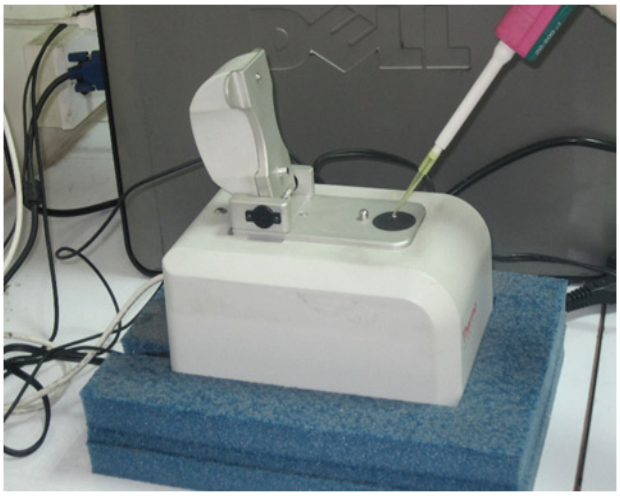

Figure: I DNA Quantitation by Thermo Nanodrop (2000C) Spectrophotometer.

Polymerase chain reaction (PCR)- The thermal cycler programmed for 47 amplification cycles, was performed consisting of initial denaturation at 94ºC for 4 minutes, then further denaturation at 94^0C for 45 seconds, annealing at 55ºC for 60 seconds, elongation at 72°C for 30 seconds and final extension at 72ºC for 5 minutes. After amplification amplicons were processed for gel documentation or kept at 4^0 C till tested (specific primers withlow-resolution Single Specific Primer-Polymerase Chain Reaction (SSP-PCR) with NYSTECHNIK Semi-quantitative PCR machine, Genome Diagnostic Pvt.Ltd, India).

Detection of PCR products- Visualization and Interpretation of results-

The amplified PCR products were detected by agarose gel electrophoresis. For detection of DRB1*15allele 3% agarose gel was used, for detection of ß actin (housekeeping gene) 4% agarose gel was used. Agarose gel mixed with 100 ml TBE (Tris, Boric acid, Ehylenediaminetetraacetic acid) containing 6µl 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 ofHLA DRB1*15 allele and specific DNA band corresponding to 56 bp band were considered positive band for presence of ß actin -gene used as housekeeping gene. If the pooled DNA template result was negative following gel electrophoresis, the sample was considered negative forHLA DRB1* allele. 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).

Ethical clearance: This study was approved by ethics committee of Bangabandhu Sheikh Mujib Medical University (BSMMU).

Results

In this cross sectional study, during one-year period, blood samples were collected from 30 chronic hepatitis B patients and 30 healthy individuals.

In Table I The age of the study population ranged from 18 to 55 years with (mean ± SD) 31.6 ± 8.84 years. The mean age of chronic hepatitis B patients, and healthy controls were 28.7 ± 6.55, 33.2 ± 9.9 years respectively. The male female ratio was 1:1. The mean ALT level of chronic hepatitis B patients was 159.73 ± 25.15 IU/L.

Table II The HLADRB1* typing revealed that DRB1*15 (46.7% vs 20%; Relative Risk test (RR)= 3.5, Chi - Square Test(X^2) = 7.2, P* value <0.05) was increased among the chronic hepatitis B patients when compared to controls.The mean viral load of chronic hepatitis B patients was 6.62 ± 9.60 [log10 (copies/ ml].

Statistical Method-

Allele frequency (AF) of HLA-DRB1* were calculated by direct count. Allele frequency (AF) for the study group (chronic hepatitis B & control group) was compared using Chi-square test. Statistical analysis was made using SPSS 17.0 software, and p value < 0.05 considered as statistically significance.

Table I: Clinical Characteristics Of Individuals Enrolled In The Study.

Variables (n=30)	Chronic hepatitis B	Healthy controls (n=30)	P* value
Age (Years) mean ± SD	28.7 ± 6.55	33.2 ± 9.9	…….
Sex (F:M)	15 : 15	15 : 15	…….
ALT (IU/L) mean ± SD	159.73 ± 46.8	ND	…….

ND indicate not done

Table II:Distribution of HLA DRB1*15 Allele among Chronic Hepatitis B (CHB) and Healthy Controls.

HLA DRB1*15allele (n= 30)	Chronic hepatits B (n= 30)	Healthy controls (RR) test	Relative Risk	Chi - Square Test (X^2 test)	P* value
DRB1*15	14 (46.7%)	06 (20%)	3.5	7.2	<0.05
Viral load [log10 (copies/ ml)] mean ± SD	6.62 ± 9.60	ND	…….	……..	

Discussion

Clinical expression and behavior of chronic hepatitis Bundoubtedly influence by various factors likehost genetic factor and viral factors, environmental factors, ethnicity etc. HLA is a most importantcritical genetic factor that determines individual variations of immune response. HLA gene play significant roleto the host response against hepatitis B infection. In individuals with different HLA alleles may differ in susceptibility or resistance to diseases and associations between HLA polymorphism and susceptibility or resistance to diseases have been identified in various studies throughout the world[18.] 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 worldwide[19-20]. In Bangladesh different reseach work has been found association of HLA class II alleles with spondyloarthropathies, natural rubella infection but little is known about of HLA- DRB*1 allele susceptibility or resistance with hepatitis B virus infection[21.]As far I know, the present study is the

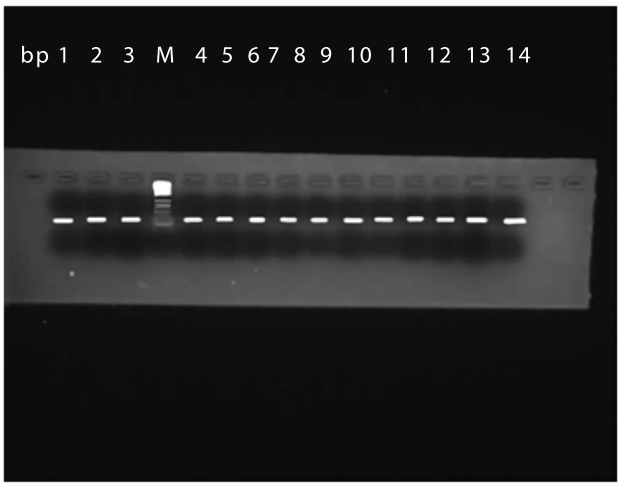

Figure: IIIn Agarose Gel Electrophoresis Positive Band of HLA

DRB1*15 Allele in Chronic Hepatitis B infected Bangladeshi Patients Lane M-100bp ladder, lanes 1-14 (Arrow marks 197bp).

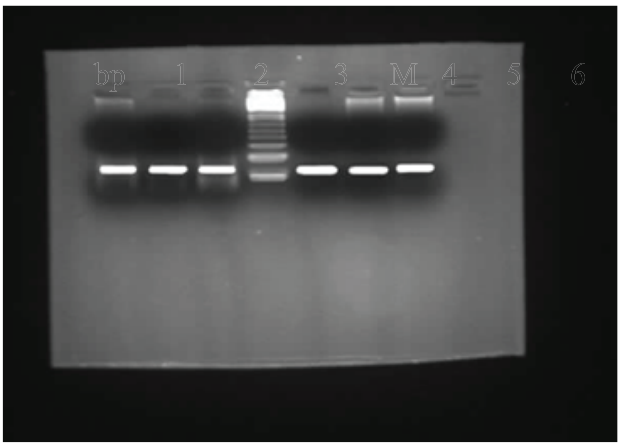

Figure:III. In Agarose Gel Electrophoresis, Positive Band of HLA DRB1*15 Allele in Healthy Controls. Lane M-100bp ladder, lanes 1-14 (Arrow marks 197bp).

first comparativestudy from Bangladesh regarding HLADRB*15 allele association with chronic hepatitis B infection with healthy controls till now.

In the present study, the frequency of HLA DRB1*15 allele among chronic hepatitis B infectedpatient with healthy control groups revealed that HLA DRB1*15 allele was significantly higher among chronic hepatitis B infected patient (46.7%) compared to healthy control (20%) 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 allele was positively associated with chronic hepatitis B infection [22-23.]

Association of HLA DRB1* allele with chronic hepatitis B infection showed opposite result from

other countries as for example, among South Indian population, HLA-DRB1*0701 was strongly associated with hepatitis B virus chronicity[24], while in a study from Korea, HLA-DRB1*0301, HLADQA1*0501 and HLADQB1*0301 were closely correlated with susceptibility to chronic hepatitis B[25].In a study from Qatar, HLA DRB1*07 was associated with persistence of hepatitis B virus infections[26]. A study from China, suggested that the susceptibility to chronic hepatitis B was strongly associated with HLA-DRB1*09, HLA-DRB1*0301, HLA-DRB1*10 allele[27], while HLA-DRB1*03 genes were associated with persistence of hepatitis B infection in caucasians[28.] Other studies from China reported that HLA DRB1*11/*12 alleles are associated with HBV clearance[29-31.]The inconsistent association of HLA DRB1*alleles with progression of hepatitis B infection in different region may be attributed to varying in ethnicity, geographical and environmental factors.

Conclusion

Although previous some studies have shown inconsistent associations with regard to the effects of host genetic factors on hepatitis B virus clearance and persistence due to ethnic differences in the studied population groups; and/or association with a gene in linkage disequilibrium with an HLA alleles. The results of the present study conclude that HLA DRB1*15 was more frequent among chronic hepatitis B infected Bangladeshi patients compared to healthy controls.Earlier various research works throughout the world prooved that host HLA class DRB1*alleles is an important genetic factor determining the outcome of hepatitis B infections, which may give some new insight to the study of molecular pathogenesis of hepatitis B.

Conflict of interest: None declared

Authors' contribution:

Data gathering and idea owner of this study: Akhter R, Afzalunnessa , Tabassum S, Hossen M

Study design: Akhter R

Data gathering: Akhter R, Afzalunnessa , Tabassum S, Hossen M

Writing and submitting manuscript: Akhter R

Editing and approval of final draft: Akhter R, Afzalunnessa , Tabassum S, Hossen M**Financial support:** The work was supported by authority. **Ethical Approval:**Research work was approved by the Institutional Ethical Review Committee (ERC) of BSMMU. **Acknowledgement:** We are thankful to the study subjects for their active, sincere and voluntary participation. The authors are also grateful to the Department of Hepatology and Virology BSMMU Shahbag Dhaka for all kind of support.

Conflict of interest: We do not have any potential conflict of interest.

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