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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, X^2 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 mortality[1]. About one third of the world's population have been infected with the hepatitis B virus[2]. Globally, over 2 billion people are infected with HBV and among them, about 660,000 die annually due to the consequences of this infection[3]. 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 carcinoma[4]. 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 region[5].

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 hepatitis[6]. 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 severity[7-9]. 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 infection[10-11].

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 Caucasians[12]. 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 response[13].

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 infection[15].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 world[16-17]. However there are no such study from Bangladesh yet. The aim of this study was to detect specific HLA DRB1*15gene 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-HBcIgM 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 fromperipheral blood by using classical phenol/chloroform DNA extraction method.

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

PCR amplification: A PCR reaction volume 13µl was used 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* genes and rest molecular grade water,then Low-resolution Single Specific Primer-Polymerase Chain Reaction (SSP-PCR) was performed with NYSTECHNIK Semiquantitative PCR matchine (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 ß actin (Housekeeping gene) 4% agarose gel was used. Agarose gel mixed with 100ml TBE (Tris, Boric acid, Ehylene-diamine-tetra-acetic acid) containing 6µl of ethidium bromide electrophoresed for170 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 forHLA 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 ± SD) 31.6 ± 8.84 year. The mean age of acute hepatitis B and Chronic hepatitis B were 32.9±10.06, 28.7 ± 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 ± 18.15 IU/L, 159.73 ± 25.15 IU/L respectively. The mean ALT level between inter-groups (acute hepatitis B andchronic 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± 29.67 and 95.10±81.54 respectively (range 64.23 ng/µl to 156.45 ng/µl (Table 2).

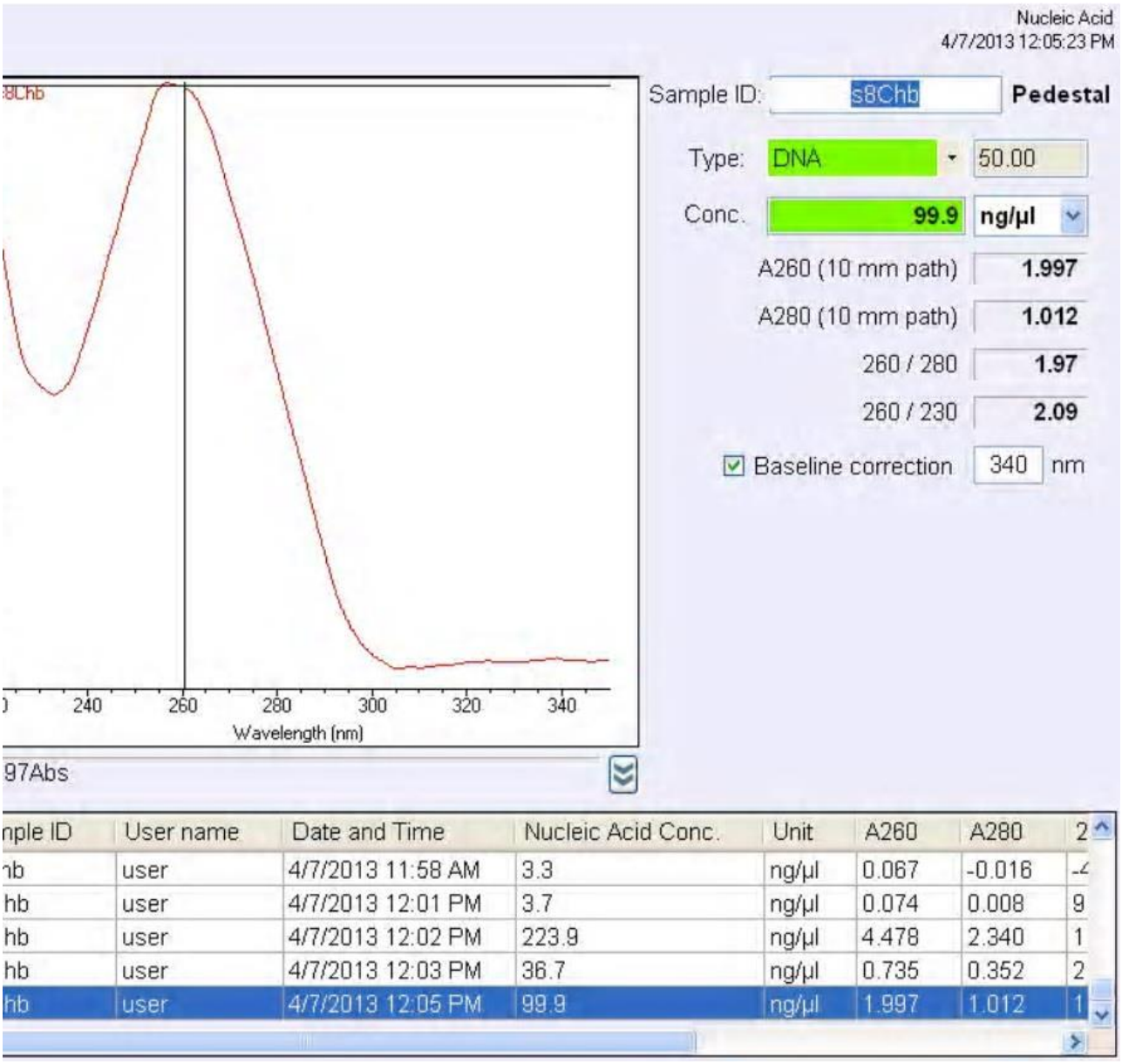

Figure I: Nanodrop DNA curve (Quantitation of DNA) in Thermo-nanodrop Spectrophotometer (2000C) 260 nm wave length

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; X^2 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 [log10(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 factors[6]. Virological factors include viral load, viral genotype and mutations in the viral genome.

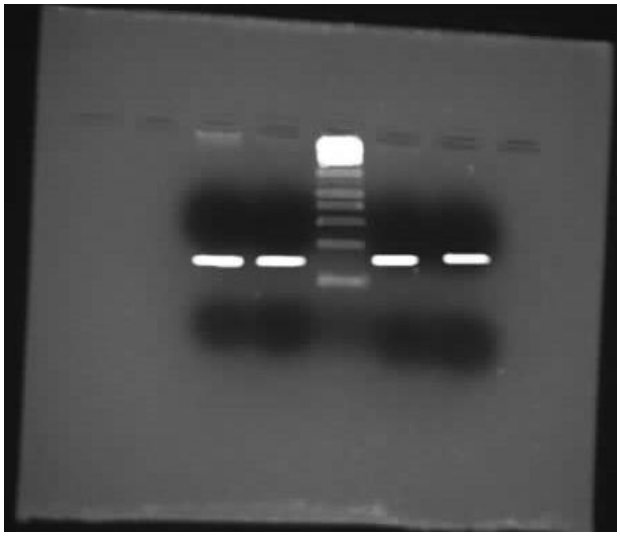

Figure II: Electrophoresis of HLA DRB1*15 genes in acute hepatitis B infected patient after amplification by PCR/SSP

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 populations[20-21].

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 world[18-19]. 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 B[26-27]. Opposite result showing in previous studies where observed that HLA DRB1*03 was associated with persistent hepatitis B infection among Chinese and Caucasians[28-30].

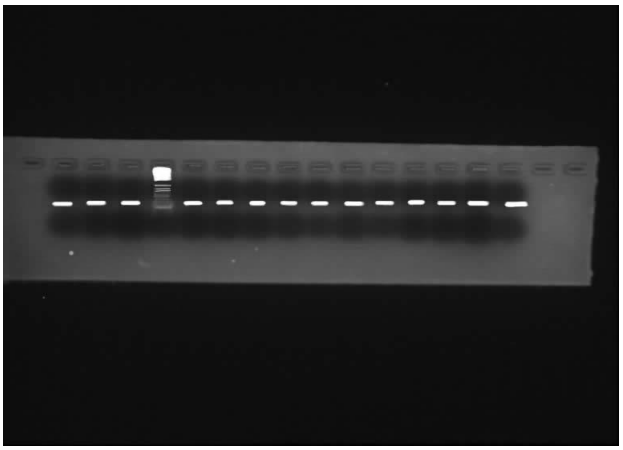

Figure III: Electrophoresis (Agarose gel) of HLA DRB1*15 gene after amplification by PCR/SSP in Chronic hepatitis B infected patients

A study from India observed that HLA DRB1*03 was associated with self limited course of acute hepatitis B[31]. Another study among South Indian

population, HLA-DRB1*0701 was strongly associated with hepatitis B virus chronicity[32] while in a study from Korea, HLA-DRB1*0301, HLADQA1*0501 and HLADQB1*0301 were closely correlated with susceptibility to chronic hepatitis B[33]. In a study from Qatar[34], HLA DRB1*07 was associated with persistence of hepatitis B virus infections. A study from China[35], 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 studies[36] 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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References

1. Lee WM. Hepatitis B virus infection. N Engl J Med 1997;337:1733–45
2. Wu H, Yim C, Chan A, Ho M, Healthcote J. Socio cultural factors that potentially affect the Institution of prevention and treatment strategies for hepatitis B in Chinese Canadians. Can J gastroenterol 2009;23:31-36
3. CDC. Recommendations for Identification and public health management of persons with Chronic Hepatitis B Virus Infection. National Notifiable Diseases Surveillance System, 2008.
4. Merican I, Guan R, Amarapuka D, Alexander M, Chutaputti A, Chien RN, Et al. Chronic hepatitis B virus infection in Asian countries. J Gastroenterol Hepatol 2000;15:1356-1361
5. Laurentius A, Lesmana N, Leung W, Mahachai V, Phiet PH, Suh DJ, Yao G, Zhuang H. Hepatitis B:overview of the burden of disease in the Asia-Pacific region . Liver Int 2006;26:3-10
6. Thursz M. Genetic susceptibility in chronic viral hepatitis. Antiviral Res. 2001;52:113-116
7. Kwiatkowski D. Genetic dissection of the molecular pathogenesis of severe infection. Intensive Care Med 2000;26
8. Weatherall D, Clegg J, Kwiatkowski D The role of genomics in studying genetic susceptibility to infectious disease. Genome Research. 1997;7:967-973
9. Powell EE, Edwards-Smith CJ, Hay JL, Clouston AD, Crawford DH, Shorthouse C, Purdie DM, Jonsson JR. Host genetic factors influence disease progression in chronic hepatitis C. 2000;31: 828-833
10. Gu CH, Luo KX. Hepatitis B: Basic biology and clinical science. Second edition. Beijing, People Medical Publishing House: 2001; 1-6
11. Luo KX. Hepatitis B: Basic biology and clinical science. Second edition. Beijing, People's Medical Publishing House:2001;56-70
12. Hoffmann SC, Stanley EM, Cox ED, Di Mercurio BS, Koziol DE, Harlan DM. Ethnicity greatly influences cytokine gene polymorphism distribution. Am J Transplant 2002;2:560-567
13. Knolle PA, Kremp S, Hohler T, Krummenauer F, Schirmacher P, Gerken G. Viral and host factors in the prediction of response to interferon-alpha therapy in chronic hepatitis C after long-term follow-up. J Viral Hepat 1998;5:399-406
14. Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol 1995;13:29-60
15. Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. Annu Re Immunol 2001;19:65-91
16. Zeng Z, Guan L, An P, Sun S. A population-based study to investigate host genetic factors associated with hepatitis B infection and pathogenesis in the Chinese population. BMC Infect Dis 2008;8:1
17. HanYN, Yang JL, Zheng SG, Q Tang Q, Zhu W. Relationship of human leukocyte antigen class II genes with the susceptibility to hepatitis B virus infection and the response to interferon in HBV-infected patients. Gastroenterol 2005;11:5721-5724
18. Verdon R, Pol S, Landis P. Absence of association between HLA antigens and chronicity of viral hepatitis in hamodialyzed patients. J Hepat 1994;21:388-393
19. Mota AH, Fainboim H, Terg R, Fainboim L. Association of chronic hepatitis and HLA B 35 in patients with hepatitis B virus. Tissue Antigens 1987;30:238-240
20. Jung MC and Pape GR. Immunology of hepatitis B infection. Lancet Infect Dis 2002;2:43-50
21. Frodsham A. Host genetics and the outcome of hepatitis B viral infection Transplant Immunology 2005;14:183 – 186
22. Han KH, Kim H and Cheng HY. Immunogenetics of hepatitis B virus infection. Gastroenterol Hepatol. 2002;17 (Suppl 3):S 329-S332
23. Zampino R, Lobello S, Chiaramonte M, Venturi-Pasini C, Dumpis U, Thursz M Karayannis P. Intrafamilial transmission of hepatitis B virus in Italy: Phylogenetic sequence analysis and amino-acid variation of core gene. Hepatol 2002;36:248-253

24. Carman WF, Thursz M, Hadziyannis S, McIntyre G, Colman K, Gioustoz A, et al. Hepatitis Be antigen negative chronic active hepatitis: hepatitis B core mutations occur predominantly is known antigenic determinants. Viral Hepat 1995;2:77-84
25. Verdon R, Pol S, Landis P. Absence of association between HLA antigens and chronicity of viral hepatitis in hamodialyzed patients. J Hepat 1994;21:388-393
26. Amarapurpar DN, Patel ND, Kankorkar SR. HLA class II genotyping in chronic hepatitis B infection J Assoc Physicians India 2003;51:2003;779-81
27. Kankonkar S, Shankarkumar U. HLA DRB1 Alleles in Chronic Hepatitis B Infected Patients. Int J Hum Genet Institute Immunohaematology 2008;8(4):331-334
28. Jiang YG, Wang YM, Liu TH, Liu J. Association between HLA class II gene and susceptibility or resistance to chronic hepatitis B. World J Gastroenterol 2003;9(10): 2221-2225
29. Wang FS, Xing LH, Liu MX, Zhu CL, Liu HG, Wang HF, Lei ZY. Dysfunction of peripheral blood dendritic cells from patients with chronic hepatitis B virus infection. World J Gastroenterol. 2001;7:537–541
30. Yang G, Liu J, Han S, Xie H, Du R, Yan Y, Xu D, Fan D. Association between hepatitis B virus infection and HLA –DRB1 genotyping in Shaanxi Han patients in northwestern China. Tissue Antigens 2007;69:170-5
31. Chen DF, Kliem V, Endres W, Brunkhorst R, Tillmann HL, Koch KM. Relationship between human leukocyte antigen determinants and courses of hepatitis B virus infection in Caucasian patients with end-stage renal disease. Scand J Gastroenterol 1996;31(12):1211–5
32. Ramezani A, Arezoo A, K Ebrahim, B Mohammad, E Ali, V Ali.Association between Hepatitis B Virus Infection Outcome and HLA-A DRB1 Genotyping in North Part Of Iran.Iranian Journal of Pathology 2009;4 (2):71-74
33. Fletcher GJ, Samuel P, Christdas J, Gnanamony M, Ismail AM, Anantharam R, et al. Association of HLA and TNF polymorphisms with the outcome of HBV infection in the South Indian population. Genes Immun. 2011;12:552–558
34. Hwang SH, Sohn YH, Oh HB, Hwang CY, Lee SH, Shin ES, et al. Human leukocyte antigen alleles and haplotypes associated with chronicity of hepatitis B virus infection in Koreans. Arch Pathol Lab Med. 2007;131(1):117-21
35. Almarri A, Batchelor JR. HLA and hepatitis B infection. Lancet. 1994;344 (8931):1194-1195
36. Shen JJ, Ji Y, Gu XL, Huang RJ, Sun YP. The association of HLA-DRB1*10 with chronic hepatitis B in Chinese patients. Zhonghua Weishengwuxue He Mianyixue Zazhi 1999;19:58-59