Association between HLA-DRB1* polymorphisms and hepatitis B infection in a Brazilian population

Bruno de Melo Corrêa[1], Edmundo Pessoa de Almeida Lopes[2], Maria de Fátima Pessoa Militão de Albuquerque[3], Lúcia Dourado[4]

[1] MSc in Health Sciences, Universidade Federal de Pernambuco (UFPE), Recife, PE, Brazil
[2] Adjunct Professor, UFPE, Recife, PE, Brazil
[3] Researcher, Centro de Pesquisas Aggeu Magalhães/FIOCRUZ, Recife, PE, Brazil
[4] Physician, Hemocentro de Pernambuco, Recife, PE, Brazil

Summary

Objective: The aim of the present study was to determine the genotype association for alleles of class II human leukocyte antigens (HLA) in the DRB1* locus among blood donors at the Fundação Hemope (Brazil) infected by or immunized for the hepatitis B virus (HBV). **Methods:** A case-control study was performed, comprising a group of individuals infected by HBV and a control group of immunized individuals at a proportion of 1:4. Blood samples were taken for the HLA typing of the DRB1* locus. Univariate and multivariate analyses were performed for the assessment of associations between the categorical variables using the chi-squared test and Fisher's exact test. **Results:** A total of 320 blood donors were analyzed (241 males [75%] and 79 females [25%] with a mean age of 39 years). The case group consisted of 64 HBV-infected donors and the control group was composed of 256 HBV-immunized donors. The multivariate analysis stratified by gender revealed that the DRB1*09 allele was associated with infected male donors (p = 0.016) and the DRB1*08 allele was associated with infected donors aged 39 years or younger (p = 0.031). **Conclusion:** The results of the present study reveal that younger blood donors and male blood donors who respectively exhibit the DRB1*08 and DRB1*09 alleles are more susceptible to intensification of HBV infection.

Keywords: HLA antigens; major histocompatibility complex; hepatitis; HLA and HBV infection.

Resumo

Associação entre polimorfismos HLA-DRB1* e infecção por hepatite B em uma população brasileira

Objetivo: O objetivo do presente estudo foi determinar a associação genotípica dos alelos de classe II dos antígenos leucocitários humanos (HLA) presentes no locus DRB1* entre doadores de sangue da Fundação Hemope (Brasil), infectados pelo ou imunizados contra o vírus da hepatite B (HBV). **Métodos:** Estudo caso-controle foi realizado com um grupo de indivíduos infectados pelo HBV e um grupo controle composto de indivíduos imunizados na proporção de 1:4. Amostras de sangue foram coletadas para a tipagem HLA do locus DRB1*. Análises univariada e multivariada foram realizadas para a avaliação de associações entre as variáveis categóricas pelo teste do qui-quadrado e teste exato de Fisher. **Resultados:** Um total de 320 doadores de sangue foram analisados (241 homens [75%] e 79 do sexo feminino [25%], com idade média de 39 anos). O grupo de casos consistiu de 64 doadores infectados pelo HBV e o grupo controle foi composto de 256 doadores imunes ao HBV. A análise multivariada estratificada por sexo revelou que o alelo DRB1*09 foi associado com os doadores infectados do sexo masculino (p = 0,016) e do alelo DRB1*08 foi associado com os doadores infectados com idade entre 39 anos ou mais jovens (p = 0,031). **Conclusão:** Os resultados do presente estudo revelam que doadores de sangue mais jovens e doadores de sangue do sexo masculino que exibem, respectivamente, os alelos DRB1*08 e DRB1*09, são mais suscetíveis à cronificação da infecção pelo HBV.

Unitermos: Antígenos HLA; complexo maior de histocompatibilidade; hepatites; HLA; infecção por HBV.

Study conducted at Fundação de Hematologia e Hemoterapia de Pernambuco (Hemope) Recife, PE, Brazil

Submitted on: 01/16/2012
Approved on: 05/18/2012

Correspondence to:
Bruno de Melo Corrêa
Rua Simão Mendes, 92/201
Recife, PE, Brazil
CEP: 52050-110
Phone: +55 81 3032-5503
Fax: +55 81 3421-3387
brunomelocorrea@yahoo.com.br

Conflict of interest: None.

Introduction

The reason why allelic diversity in the genes of the major histocompatibility complex has been conserved throughout the evolution of the human species has not yet been clarified. Thus, the role of polymorphisms in these molecules in the determination of susceptibility or resistance to disease has merited attention[1]. The tracking of histocompatibility alleles in different ethnic groups from different geographic regions indicates that gene conversion and mutation are the main generating mechanisms of allelic diversity on the population level, and that sites of greater polymorphism are found in the regions of the α and β chains, which are in contact with antigenic peptides[2]. Allelic diversity is believed to be generated from interactions with pathogenic agents[3,4]. Indeed, studies on the characteristics of patients may contribute toward the understanding of the association between human leukocyte antigens (HLA) and susceptibility to infections, such as by the hepatitis B virus (HBV)[5,6].

The highly polymorphic HLA antigens are of key importance in the activation of the immune response against viruses through their enormous capacity of attracting and binding viral peptides. It is estimated that one cell has 100,000 to 300,000 class I or II HLA molecules on its surface[7]. As all HLA molecules expressed on the cell surface contain peptides, each cell can have thousands of peptides. The peptide-HLA bond is not as strong as the antigen-antibody bond, but has slow dissociation constants, lasting hours to days, which are sufficient to remain linked to the viral peptide until they are presented to the CD4+ T lymphocytes, culminate in the activation and amplification of the immune response[7].

The binding of particular peptides is a crucial phenomenon to the elimination of the aggressive agent[7]. Previously attributed only to the rejection of grafts, a number of studies have demonstrated associations between the development of autoimmune endocrine diseases and the genes of the histocompatibility system[8]. In recent years, there has been an intensification of studies on the HLA function in the presentation of viral antigens to CD4+ and CD8+ T lymphocytes, aiming to eliminate viral agents[7]. In Spain, Cotrina et al.[9] analyzed the HLA-DRB1 genotype in patients with acute and chronic hepatitis caused by HBV and found that the HLA-DRB1* 1301 and DRB1* 1302 alleles were associated with the resolution of the infection.

Considering the need to better understand the HLA profile of HBV infection in northeastern Brazil, the aim of the present study was to determine the genotype association for alleles of class II HLA in the DRB1* locus in blood donors at the Hematology and Hemotherapy Foundation of the state of Penambuco (Fundação Hemope, Brazil) infected and immunized by HBV.

Methods

A case-control study nested in a cohort of blood donors at the Fundação Hemope was performed between December 2008 and December 2009. The donors were analyzed consecutively based on demand. Those who tested positive for HBsAg or anti-HBc during routine donations were asked to perform confirmatory tests with another blood sample and were thus denominated return donors. On their return, all donors were asked to read and sign an informed consent. All personal data of interest to the study, such as age, gender, and donation date, were gathered during the initial donation and entered the database. This study was approved by the Human Research Ethics Committee of the Fundação Hemope.

Male and female blood donors between 18 and 65 years of age were included in the study. The case group was composed of donors positive for anti-HBc and HBsAg markers. The control group comprised individuals immunized for HBV who were positive for anti-HBc and anti-HBs markers. Donors positive for anti-HCV, anti-HIV, anti-HTLV, or Chagas disease, and those who failed to return for the confirmatory test were excluded from the study.

The determination of serum HBV markers was performed using immunoenzymatic assays for the qualitative detection of HBsAg (MUREX, HBsAg version 3, Abbott) and anti-HBc (ORTHO, HBc ELISA Test System), as well as for the quantitative detection of anti-HBs (AxSYM AUSAB, Abbott), following the manufacturers' instructions.

Besides the blood sample for donation, 5 mL of venous blood were collected–for subsequent HLA typing. The samples were sent to the Molecular Biology Laboratory at Hemope for the extraction of genomic DNA, which was frozen and stored at - 80º C until HLA DRB1* typing.

The polymerase chain reaction/specific oligonucleotide sequence (PCR-SSO) method was used with the RSSO2B1 from One Lambda. The analysis of the material obtained in the hybridization phase was performed in a Labscan 100 cytometer (LUMINEX). The interpretation of the results was performed with the appropriate software provided by One Lambda[10].

The database was constructed using the Statistical Package for the Social Sciences, version 13.1 for Windows (SPSS Inc. – Chicago, IL, 2005). The characteristics of the case and control groups (gender and age) were described in absolute and relative frequencies. Moreover, all 78 possible HLA-DRB1* types in pairs of alleles were described in absolute and relative frequencies for the case and control groups. Univariate and multivariate analyses were performed for the assessment of associations between the categorical variables using the chi-squared test and Fisher's exact test. The univariate analysis considered all 13 DRB1* alleles in a contingency table, determining the presence or absence of each allele in the case and control groups (Table 1).

Table 1 – Association between the 78 possible combinations found among the HLA-DRB1* and HBV infection

HLA-DRB1*	Cases	Controls	X^2	p-value
DRB1*01				
Absence	49 (76.6%)	207 (80.9%)		
Presence	15 (23.4%)	49 (19.1%)	0.591	0.442
DRB1*03				
Absence	53 (82.8%)	209 (81.6%)		
Presence	11 (17.2%)	47 (18.4%)	0.047	0.828
DRB1*04				
Absence	52 (81.3%)	206 (80.5%)		
Presence	12 (18.8%)	50 (19.5%)	0.020	0.888
DRB1*07				
Absence	49 (76.6%)	205 (80.1%)		
Presence	15 (23.4%)	51 (19.9%)	0.387	0.534
DRB1*08				
Absence	49 (76.6%)	220 (85.9%)		
Presence	15 (23.4%)	36 (14.1%)	3.359	0.067
DRB1*09				
Absence	57 (89.1%)	243 (94.9%)		
Presence	07 (10.9%)	13 (5.1%)	3.0	0.083
DRB1*10				
Absence	63 (98.4%)	247 (96.5%)		
Presence	01 (1.6%)	09 (3.5%)	0.645	0.422
DRB1*11				
Absence	51 (79.7%)	195 (76.2%)		
Presence	13 (20.3%)	61 (23.8%)	0.356	0.551
DRB1*12				
Absence	63 (98.4%)	240 (93.8%)		
Presence	01 (1.6%)	16 (6.3%)	2.236	0.135
DRB1*13				
Absence	52 (81.3%)	199 (77.7%)		
Presence	12 (18.8%)	57 (22.3%)	0.374	0.541
DRB1*14				
Absence	58 (90.6%)	239 (93.4%)		
Presence	06 (9.4%)	17 (6.6%)	0.574	0.449
DRB1*15				
Absence	55 (85.9%)	199 (77.7%)		
Presence	09 (14.1%)	57 (22.3%)	2.105	0.147
DRB1*16				
Absence	56 (87.5%)	232 (90.6%)		
Presence	08 (12.5%)	24 (9.4%)	0.556	0.456

The multivariate analysis involved those variables that exhibited an association with the presence of HBV infection in the univariate analysis with a p-value ≤ 0.20. The multivariate logistic regression of associations between HBV infection and the DRB1* variables selected in the univariate analysis was stratified by gender and age. The magnitudes of these associations were estimated as odds ratios (OR), using 95% confidence intervals (CI). All p-values less than 0.05 in the multivariate analysis were indicative of statistical significance.

Results

Among the 320 blood donors, 241 (75%) were male and 79 (25%) were female. The mean age was 39 years, ranging from 18 to 65 years. The case group was composed of 64 donors infected by HBV (positive anti-HBc and HBsAg), and the control group was composed of 256 HBV-immunized donors (positive anti-HBc and anti-HBs). No demographic differences were observed between the two groups. The univariate analysis revealed that HBV infection was not significantly associated with gender (OR: 1.021; 95% CI: 0.542-1.924; p = 0.948) or age (OR: 0.983; 95% CI: 1.009- 5.94; p = 0.204). The most frequent combinations in the case group were DRB1*03*08 and DRB1*04*07 (6.3%). The DRB1*11*13 combination was more frequent in the control group (4.3%). However, these differences did not achieve statistical significance (p = 0.314).

The univariate analysis revealed that the following DRB1* alleles were associated to the infected donors (case group) at a level of significance of p ≤ 0.2: DRB1*08 (p = 0.067), DRB1*09 (p = 0.083), DRB1*12 (p = 0.135), and DRB1*15 (p = 0.147) (Table 1). The multivariate analysis stratified by gender revealed that the DRB1*09 was associated with infected donors of the male gender (OR: 4.20; 95% CI: 1.30 to 13.60; p = 0.016) (Table 2). The multivariate analysis stratified by age revealed that DRB1*08 was more frequent in infected donors aged 39 years or younger (OR: 2.54; 95% CI: 1.09 to 5.94; p = 0.031) (Table 3).

Discussion

The susceptibility to persistence or resolution of HBV infection depends on the immune response and may be explained by immunogenetic factors in the host[11-14]. Studies on the correlation between HLA and HBV infection have been performed for a number of years[12,15,16]. The traditional serological methods used in HLA typing in some investigations are imprecise and have become obsolete. In the present study, HLA-DRB1 alleles were investigated using PCR-SSO on blood donors infected by HBV and those immune to the virus in order to determine possible associations. 13 HLA-DRB1 alleles were detected, which agrees with the number reported by Ye-Gui Jiang et al.[17].

Although the presence of a particular HLA allele alone is not sufficient to define the immune status against HBV, it has been demonstrated that in certain groups, such as males or elder individuals, some genotypes are more prevalent in chronically infected individuals. In the present

Table 2 – Multivariate logistic regression of association between HBV infection and variables of the DRB1* locus (*08,*09,*12,*15) stratified by gender

Sex	Cases	Controls	OR	IC 95%	p-value
Female					
DRB1*08					
Absence	12 (75%)	54 (85.7%)	1.0		
Presence	04 (25%)	09 (14.3%)	1.94	0.48-7.76	0.348
DRB1*09					
Absence	15 (93.8%)	57 (90.5%)	1.0		
Presence	01 (6.3%)	06 (9.5%)	0.62	0.06-5.81	0.682
DRB1*12					
Absence	16 (100%)	61 (96.8%)	1.0		
Presence	00 (00%)	02 (3,2%)	0.00	0.00-0.00	0.999
DRB1*15					
Absence	13 (81.3%)	51 (81%)	1.0		
Presence	03 (18.8%)	12 (19%)	1.05	0.24-4.60	0.941
Male					
DRB1*08					
Absence	37 (77.1%)	166 (86%)	1.0		
Presence	11 (22.9%)	27 (14%)	1.80	0.80-4.06	0.153
DRB1*09					
Absence	42 (87.5%)	186 (96.4%)	1.0		
Presence	06 (12.5%)	07 (3.6%)	4.20	1.30-13.60	0.016
DRB1*12					
Absence	47 (97.9%)	179 (92.7%)	1.0		
Presence	01 (2.1%)	14 (7.3%)	0.30	0.03-2.43	0.264
DRB1*15					
Absence	42 (87.5%)	148 (76.7%)	1.0		
Presence	06 (12.5%)	45 (23.3%)	0.46	0.18-1.18	0.110

study, HBV infection in men and individuals under 39 years of age was associated with the HLA-DRB1*09 and DRB1*08 alleles, respectively.

It should be stressed that differences in allelic frequencies may occur between different populations, which does not allow for the generalization of the present results. Thus, studies involving specific populations, especially mixed-raced, such as that found in Brazil, are needed to determine whether the HLA associations are the same described in the literature, and whether there are new associations[9]. There are different relationships between polymorphic HLA genes and HBV infection in different populations, which implies that different HLA molecules could exhibit different HBV epitopes in order to induce an efficacious immune response[6,18]. Indeed, a study conducted in China[19] reported that the DRB1*06, DRB1*08 and DRB1*16 alleles may be related to the intensification of HBV alleles in comparison to other HLA-DRB1 alleles. The HLA-DRB1* locus is an important factor associated with the evolution of HBV infection and should be the subject of further studies on the pathogenesis of the chronicity of infection by this agent[6,20].

The present study revealed limited power for the detection of associations with low-frequency alleles, and the associations found cannot be applied to all populations. The study of ethnically diverse populations and greater knowledge of the association between the HLA system and HBV infection will allow for the design of strategies for the identification of risk groups. In the near future, the type of clinical manifestation of the virus may be predictable, which will allow for orientation of the use of medications based on the pharmacogenomic profile of patients as well as the establishment of measures for preventing the occurrence of HBV infection.

Table 3 – Multivariate logistic regression of association between hepatitis B and DRB1* locus variables (*08,*09,*12,*15) stratified by age

Age category	Cases	Controls	OR	IC 95%	p-value
Up to 39 years					
DRB1*08					
Absence	32 (72.7%)	128 (87.7%)	1.0		
Presence	12 (27.3%)	18 (12.3%)	2.54	1.09-5.94	0.031
DRB1*09					
Absence	39 (88.6%)	137 (93.8%)	1.0		
Presence	05 (11.4%)	09 (6.2%)	2.05	0.63-6.65	0.230
DRB1*12					
Absence	43 (97.7%)	136 (93.2%)	1.0		
Presence	01 (2,3%)	10 (6.8%)	0.37	0.04-3.11	0.367
DRB1*15					
Absence	37 (84.1%)	113 (77.4%)	1.0		
Presence	07 (15.9%)	33 (22.6%)	0.70	0.28-1.76	0.453
40 years or more					
DRB1*08					
Absence	17 (85%)	92 (83.6%)	1.0		
Presence	03 (15%)	18 (16.4%)	0.79	0.20-3.06	0.733
DRB1*09					
Absence	18 (90%)	106 (96.4%)	1.0		
Presence	02 (10%)	04 (3.6%)	2.62	0.43-15.96	0.293
DRB1*12					
Absence	20 (100%)	104 (94.5%)	1.0		
Presence	00 (00%)	06 (5.5%)	0.00	0.00-0.00	0.999
DRB1*15					
Absence	18 (90%)	86 (78.2%)	1.0		
Presence	02 (10%)	24 (21.8%)	0.38	0.08-1.79	0.221

Conclusions

Based on the results of the present study, preventive measures are suggested for infection by HBV, especially for young individuals of the male gender in Northeastern Brazil with the HLA-DRB1*08 or DRB1*09 profile, respectively. Studies with other samples should be performed in order to confirm these results or to reveal other genetic determinants associated with susceptibility or resistance to infection by HBV.

References

1. Powis SH, Geraghty DE. What is the MHC? Immunol Today. 1995;16:466-8.
2. Riley E, Olerup O. HLA polymorphisms and evolution. Immunol Today. 1992;13:333-5.
3. Clayton J, Gee H. Major histocompatibility complex: the evolutionary angle. Nature. 1993;365:111-2.
4. Parham P. HLA, anthropology, and transplantation. Transpl Proc. 1993;25:159-61.
5. Singh R, Kaul R, Kaul A, Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. World J Gastroenterol. 2007;13:1770-87.
6. Xingku L, Wei L, Hongyan W, Xi J, Shaohong F, Yuguang S, Zhen L, Shuyun Z, Shufen Y. The influence of HLA alleles and HBV subgenotypes on the outcomes of HBV infections in Northeast China. Virus Res. 2012;163:328-3.
7. Klein J, Sato A. The HLA system: first of two parts. N Engl J Med. 2000;343:702-9.
8. Thorsby E. Invited anniversary review: HLA associated diseases. Hum Immunology 1987;53:1-11.
9. Cotrina M, Buti M, Jardi R, Campins M, Esteban R, Guardia J. Study of HLA-II antigens in chronic hepatitis C and B and in acute hepatitis B. Gastroenterol Hepatol. 1997;20:115-8.
10. Visentainer JEL, Sell AM, Tsuneto LT, et al. Metodologia de genotipagem HLA SSO-Luminex no Laboratório de Imunogenética da Universidade Estadual de Maringá. Rev Bras Hematol Hemoter. 2005;1:61-5.
11. Wang JS, Zhu QR. Interruption of the transmission of hepatitis B virus from mother to babies. Zhonghua Gan Zang Bing Za Zhi. 2002;10:308-10.
12. Thio CL, Thomas DL, Karacki P, Gao X, Marti D, Kaslow RA, et al. Comprehensive analysis of class I and class II HLA antigens and chronic hepatitis B virus infection. J Virol. 2003;77:12083-7.
13. Thursz MR. Host genetic factors influencing the outcome of hepatitis. J Viral Hepat. 1997;4:215-20.
14. Diepolder HM, Jung MC, Keller E, Schraut W, Gerlach JT, Grüner N, et al. A vigorous virus- specific CD4+ T cell response may contribute to the association of HLA-DR13 with viral clearance in hepatitis B. Clin Exp Immunol. 1998;113:244-51.
15. Meng XQ, Chen HG, Ma YL, Liu KZ. Influence of HLA class II molecules on the outcome of hepatitis B virus infection in population of Zhejiang Province in China. Hepatobiliary Pancreat Dis Int. 2003;2:230-3.

16. Ahn SH, Han KH, Park JY, Lee CK, Kang SW, Chon CY, et al. Association between hepatitis B virus infection and HLA-DR type in Korea. Hepatology. 2000;31:1371-3.
17. Jiang YG, Wang YM, Liu TH, Lee CK, Kang SW, Chon CY, et al. Association between HLA class II gene and susceptibility or resistance to chronic hepatitis B. World J Gastroenterol. 2003;9:2221-5.
18. Han YN, Yang JL, Zheng SG, 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. World J Gastroenterol. 2005;11:5721-4.
19. Xu Y, Yu J, Zhong Y, Song HB, Lui HH, Jia LL, et al. Association between the frequency of class II HLA antigens and the susceptibility to intrauterine infection of hepatitis B virus. Int J Biol Sci. 2008;4:111-5.
20. Wu YF, Wang LY, Lee TD, Lin HH, Hu CT, Cheng M, et al. HLA phenotypes and outcomes of hepatitis B virus infection in Taiwan. J Med Virol. 2004;72:17-25.