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Prevalence of HLA-DQ Alleles and Haplotypes in Patients with Hepatitis B Infection

Behnaz Riazalhosseini[a]*, Zahurin Mohamed[b], Yamunah Devi Apalasamy[c], Hooi Sian Eng[d], Rosmawati Mohamed[e]

[a,b]*The Pharmacogenomics Laboratory, Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*

[c]*Social Security Research Centre, Faculty of Economics and Administration, University of Malaya, 50603 Kuala Lumpur, Malaysia*

[d]*Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*

[e]*Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*

[a]*Email: beh.riazi@gmail.com*

[b]*Email: zahurin@um.edu.my*, [c]*Email: yamunahdevi@um.edu.my*

[d]*Email: enghs1@um.edu.my*, [e]*Email: ros@ummc.edu.my*

Abstract

Objective: This study aimed to investigate whether HLA-DQA1 and HLA–DQB1 alleles and haplotypes are associated with progression of HBV infection to liver cirrhosis (LC) and hepatocellular carcinoma (HCC) in HBV infected patients. Methods: Genotyping of the HLA-DQ variants were carried out in 121 HBV patients (including 52 HBV without LC/HCC and 69 HBV with LC/HCC), using a polymerase chain reaction amplification with sequence specific oligonucleotide (PCR-SSO) technique and Luminex profiling system. Results: The two highest allele frequencies were seen for HLA-DQA1*01 and HLA-DQB1*03. A total of 66% of the HBV patients with LC/HCC and 62% of HBV patients without LC/HCC are carriers of HLA-DQA1*01 (p value= 0.908, OR= 0.95, CI= 0.41 - 2.10). As for HLA-DQB1*03, 73% of the HBV patients with LC/HCC and 60% of HBV patients without LC /HCC are carriers of this allele (p value= 0.320, OR= 1.56, CI= 0.65 - 3.72). In addition, 62% of HBV patients with LC/HCC (n=32) and 48% of HBV patients without LC/HCC (n=25) were carriers of haplotype DQA1*01-DQB1*05 (p value= 0.495, OR= 0.76, CI= 0.34 - 1.68). Conclusion: no association was seen between the HLA-DQA1 and the HLA–DQB1 alleles and haplotypes with HBV disease progression in the Malaysian population.

Keywords: HLA-DQ genotyping; HBV infection; PCR-SSO technique; liver cirrhosis; HCC.

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* Corresponding author.

1. Introduction

Hepatitis B virus (HBV) infection is a major public health problem affecting around two billion people globally [1]. Among individuals with persistent HBV infection, 10%-30% develop some form of liver disease such as liver cirrhosis (LC) and hepatocellular carcinoma (HCC) [2]. HCC is the third leading cause of cancer death in the world with more than 600,000 individuals affected [3] while Asian countries account for almost 78% of the HCC reported death globally [4]. Besides HBV infection and liver cirrhosis, other factors such as old age, male gender, ethnicity and host genetic factors such as the immune system are involved in predisposition to HCC [4]. Evidence has shown that host immune mechanisms influence the outcome of HBV infection [5] as well as persistence of the viral infection [6].

Human leukocyte antigen (HLA) is an important component of the immune response and plays a vital role against virus infection and tumor resistance. The HLA class II molecules are presented on antigen presenting cells including B cells, dendritic cells, thymic epithelial cells, macrophages and activated T cells. The recognition of the presented antigens to CD4+ T cells leads to the secretion of cytokines and differentiation of T cells [7]. The HLA molecules are located on the chromosome 6p21[8] and contain the highest genetic variations in the human genome which is associated with the function of the immune system [7, 8]. The polymorphism in HLA class II gene are known to affect the ability of antigen presentation as well as cytokines production [9].

Recent studies have shown the associations between HLA class II alleles with susceptibility to HBV infection as well as vaccine responsiveness and clearance of the HBV virus[10, 11]. To date, several studies have been conducted to investigate the association between HLA class II alleles and hepatitis B infection outcomes. Most of the findings are related to HLA-DR and HLA-DP alleles. For instance, it has been reported that HLA-DRB1*07 is associated with HBV persistence in various Asian populations [12, 13]. Moreover, it has been shown that HLA- DRB1*1301 is associated with clearance of HBV among different populations [14, 15]. Recently a study revealed association between a new protective allele named HLA-DPB1*02:01 as well as a new risk allele HLA-DPB1*09:01 with HBV disease in the Asian population[16]. However, there are a few studies regarding the association between HLA-DQ alleles and consequences of HBV infection. Thus, this study aims to investigate the possible association between HLA-DQ alleles and progression of HBV infection in the Malaysian population.

2. Materials and Methods

2.1 Subjects

A total of 121 HBV chronically infected patients (76 males and 45 females) were enrolled in this study. Of them, 52 were diagnosed with chronic hepatitis B (CHB) without liver cirrhosis / HCC and 69 CHB with liver cirrhosis / HCC. The diagnosis of chronic hepatitis B was confirmed by persistence of HBsAg in excess of six months. Presence of liver cirrhosis amongst the chronic hepatitis B infected patients were confirmed by liver biopsies, clinical, biochemical or radiological indication of cirrhosis[17] . Diagnoses of HCC were based on the radiological evidence of liver mass with arterial hypervascularity and washout in the venous-delayed phase on

dynamic imaging and/or by liver histology [18]. All patients were negative for other potential causes of liver disease such as Wilson's disease, hepatitis C infection, primary biliary cirrhosis, autoimmune hepatitis, hemochromatosis and alpha-1 anti-trypsin deficiency. Written informed consent was obtained from all participants. Ethnicities of the subjects were confirmed by self-report. The study protocol, MEC Ref. No: 938.42, was approved by the medical ethics committee of the University Malaya Medical Centre (UMMC).

2.2 Sample collection

Venous blood from all patients were collected in EDTA tubes. The genomic DNA was extracted from buffy coat using the GeneAll@ ExgeneTM DNA purification kit (Dongnam-ro, Songpa-gu, Seoul, South Korea) according to the manufacturer's instructions.

2.3 HLA-DQA1 / -DQB1 genotyping

A low resolution (2-digit) genotyping was used to determine the HLA-DQA1 and HLA-DQB1 genotypes in CHB patients with and without cirrhosis/HCC. LAB Type polymerase chain reaction amplification (PCR) with sequence specific oligonucleotide (PCR-SSO) probes technique together with HLA DQA1/DQB1 One Lambda kit (Canoga Park, California, USA) and a Luminex profiling system (xMAP; Luminex, Austin, TX) were used for genotyping according to the manufacturer's protocol.

The samples were used in final DNA concentration of 20 ng/µl and was considered of good quality if absorbance of 260/280 <1.80 and >1.65. Briefly, the target DNA was amplified with biotinylated specific primers for the purpose of detection by R-Phycoerythrin conjugated Streptavidin (SAPE).

The amplified DNA was then denatured, neutralized and hybridized with the oligonucleotide probes which are labelled with SAPE and finally detected by Luminex 200 system.

The HLA alleles were recognized by HLA visual 1.0 software via referring to HLA typing pattern data for -DQA1 and -DQB1 provided by manufacturer.

2.4 Statistical analysis

Allele frequencies of HLA-DQA1 and -DQB1 were calculated using the direct counting method. A chi-square test was done to examine deviation from Hardy-Weinberg equilibrium using SPSS version 16.0. Logistic regression was applied to conclude the association between HLA-DQA1 and -DQB1 with disease progression in CHB patients.

The Linkage Disequilibrium of two locus haplotype was calculated using the SPSS version 16.0. Odd ratios (ORs) with 95% confidence intervals (CI) were calculated after adjustment for confounding factors such as sex and age. The data were presented as percentage or mean ± standard deviation.

A p-value less than 0.05 was considered to be statistically significant. The significance levels for α was set

according to the number of observed alleles at each locus. For HLA-DQA1 and -DQB1 alleles, the number of observed alleles was 5 for each of them. Thus, the significant levels for HLA-DQA1 and -DQB1 alleles were set at α=0.05/5 after Bonferroni correction.

3. Results

Demographic information of the 121 study subjects is presented in Table 1. Participants were made up of 44 who were of the Malay ethnic group and 77 were Chinese. Data from these two ethnicities were pooled to reveal the presence of five HLA-DQA1 alleles (-DQA1*01, -DQA1*02, -DQA1*03, -DQA1*05 and -DQA1*06) and five HLA-DQB1 alleles (-DQB1*02, -DQB1*03, -DQB1*04, -DQB1*05 and -DQB1*06).

Table 1: Demographic data of participants for HLA typing.

	HBV infected without LC / HCC (n= 52)	HBV infected with LC / HCC (n= 69)	p value
Age	55.23 ± 10.76	65.84 ± 9.21	0.001
Ethnicity			0.239
Malay	22 (42.3%)	22 (31.8%)	
Chinese	30 (57.7%)	47 (68.2%)	
Indian	-	-	
Gender			0.313
Male	30 (57.7%)	46 (66.7%)	
Female	22 (42.3%)	23 (33.3%)	

LC: liver cirrhosis; HCC: hepatocellular Carcinoma

P <0.05 was considered significant

The allele frequencies of HLA-DQA1 and HLA-DQB1 and their associations with progression of HBV disease are presented in Table 2. The two highest allele frequencies are seen with HLA-DQA1*01 and HLA-DQB1*03. A total of 66% of the HBV patients with cirrhosis / HCC (n=46) and 62% of the HBV patients without cirrhosis / HCC (n=32) are carriers of HLA-DQA1*01 (p value= 0.908, OR= 0.95, CI= 0.41 - 2.10).

In addition, 73% of HBV patients with cirrhosis / HCC (n=50) and 60% of HBV patients without cirrhosis / HCC (n=31) are carriers of HLA-DQB1*03 (p value= 0.320, OR= 1.56, CI= 0.65 - 3.72). Furthermore, 17% of HBV patients with cirrhosis / HCC (n=12) and 8% of HBV patients without cirrhosis / HCC (n=4) are carriers of HLA-DQA1*02 (p value= 0.167, OR= 2.49, CI= 0.68 - 8.05).

Whereas 4% of HBV patients with cirrhosis / HCC (n=3) and 12% of HBV patients without cirrhosis / HCC

(n=6) were carries HLA-DQB1*04 (p value= 0.455, OR= 0.55, CI= 0.12 - 2.61). There were no significant association between any of the HLA alleles studied with progression of CHB to cirrhosis/ HCC.

The DQA1-DQB1 haplotypes frequencies and their associations with progression of HBV disease are presented in Table 3. Twenty-five HLADQA1-DQB1 haplotypes were identified among CHB patients with and without cirrhosis/HCC.

In this study, 62% of HBV patients with cirrhosis/HCC (n=32) and 48% of HBV patients without cirrhosis/HCC (n=25) were carriers of haplotype DQA1*01-DQB1*05 (p value= 0.495, OR= 0.76, CI= 0.34 - 1.68). None of the haplotypes were significantly associated with progression of HBV infection.

Table 2: The allele frequencies and association of HLA-DQA1/-DQB1 with disease progression in CHB patients with and without cirrhosis/HCC.

Allele	HBV infected without cirrhosis/HCC (n= 52)		HBV infected with cirrhosis/HCC (n= 69)		(X^2)	OR (95%)	p Value
DQA1	Count	%	Count	%			
01	32	**62%**	46	**66%**	0.560	0.95 (0.41 - 2.10)	0.908
02	4	8%	12	17%	0.119	2.49 (0.68- 8.05)	0.167
03	22	42%	24	35%	0.399	0.88 (0.39 - 2.01)	0.773
05	12	23%	20	29%	0.466	1.58 (0.64 - 3.87)	0.319
06	13	25%	24	35%	0.248	1.18 (0.49 - 2.85)	0.709
DQB1							
02	9	17%	21	30%	0.096	2.28 (0.87 - 6.01)	0.098
03	31	**60%**	50	**73%**	0.137	1.56 (0.65 - 3.72)	0.320
04	6	12%	3	4%	0.136	0.55 (0.12 - 2.61)	0.455
05	25	48%	31	45%	0.731	0.74 (0.33 - 1.63)	0.449
06	10	19%	18	26%	0.376	1.21 (0.47 - 3.05)	0.695

X^2: Pearson Chi Square p value and OR were calculated in presence versus absence of each allele. Association of HLA-DQA1 and –DQB1 alleles with HBV disease progression assessed by logistic regression analysis adjusted for gender and age.

Table 3: The associations of DQA1-DQB1 haplotypes with disease progression in CHB patients with and without cirrhosis/HCC.

Haplotype		HBV infected without cirrhosis/HCC (n= 52)		HBV infected with cirrhosis/HCC (n= 69)		(X^2)	OR (95%)	p Value
HLA-DQA	HLA-DQB1	Count	(%)	Count	(%)			
01	02	4	(7.7)	10	(14.5)	0.247	1.70 (0.45 - 6.50)	0.437
01	03	13	(25)	28	(40.6)	0.073	1.38 (0.58 - 3.30)	0.469
01	04	2	(3.8)	1	(1.5)	0.401	0.37 (0.03 - 5.37)	0.464
01	05	25	(48.1)	32	(61.5)	0.853	0.76 (0.34 - 1.68)	0.495
01	06	10	(19)	18	(26)	0.376	1.21 (0.48 - 3.05)	0.695
02	02	4	(7.7)	11	(15.9)	0.173	2.02 (0.55 - 7.40)	0.289
02	03	1	(1.9)	8	(11.6)	0.045	4.64 (0.86 - 6.37)	0.066
02	04	0	0	1	(1.5)	-	-	NA
02	05	2	(3.8)	2	(2.9)	0.773	0.503 (0.06 - 4.12)	0.522
02	06	0	0	1	(1.5)	-	-	NA
03	02	3	(5.8)	5	(7.2)	0.746	1.69 (0.32 - 6.97)	0.536
03	03	19	(36.5)	23	(33.3)	0.714	1.05 (0.45 - 2.43)	0.916
03	04	6	(11.5)	3	(4.3)	0.136	0.55 (0.12 - 2.61)	0.455
03	05	3	(5.8)	3	(4.3)	0.721	0.75 (0.13 - 4.25)	0.745
03	06	1	(1.9)	9	(13)	-	-	NA
05	02	5	(9.6)	11	(15.9)	0.309	2.39 (0.69 - 6.21)	0.168
05	03	9	(17.3)	12	(17.4)	0.99	1.14 (0.41 -3.21)	0.804
05	04	2	(3.8)	0	0	-	-	NA
05	05	4	(7.7)	9	(13)	0.347	1.81 (0.48 - 4.82)	0.384
05	06	4	(7.7)	3	(4.3)	0.435	0.53 (0.11 - 2.49)	0.419
06	02	1	(1.9)	5	(7.2)	0.182	5.30 (0.47 - 5.77)	0.177
06	03	13	(25)	24	(34.8)	0.248	1.18 (0.49 - 2.85)	0.709
06	04	2	(3.8)	1	(1.5)	0.401	0.53 (0.04 - 6.52)	0.619
06	05	5	(9.6)	9	(13)	0.559	0.81 (0.22 - 2.88)	0.738
06	06	0	0	2	(2.9)	-	-	NA

X2: Pearson Chi Square p value and OR were calculated in presence versus absence of each allele. Association of HLA-DQA1 and –DQB1 alleles with HBV disease progression assessed by logistic regression analysis adjusted for gender and age. NA: not applicable.

4. Discussion and Conclusion

Evidence has shown the association of HLA Class II molecules with susceptibility or persistence of infectious disease [7] such as hepatitis B infection [19]. HLA-DQ is heterodimeric molecule consisting of two polypeptides alpha and beta chains which are encoded by HLA-DQA1 and HLA-DQB1 respectively. HLA-DQ is highly polymorphic particularly in exon 2 which codes for antigen binding sites. Thus, high variations in this region lead to the existence of a number of alleles which are associated with outcome of HBV infection [20]. It has been shown that the presence of particular HLA class II alleles influence the effectiveness of the immune response to infection [21]. The highly polymorphic HLA genes are main factors in the triggering of the immune response against hepatitis B infection through their enormous ability of attracting and binding of hepatitis B viral peptides.

With regard to HBV infection, HLA as a vital element of immune system has been known to influence the outcomes of disease. Several studies indicated the association of HLA–DQA1 and –DQB1 alleles with HBV

infection outcomes in different populations. For instance, Jiang and his colleagues have conducted a study in Chinese Han population among three groups of patients, namely, those with chronic hepatitis B, with acute hepatitis B and on healthy controls subjects. Results of the study showed that HLA–DQB1*0301 and HLA–DQA1*0501 are significantly associated with predisposition to chronic hepatitis B while HLA–DQA1*0301 is closely linked to resistance to chronicity of HBV infection [22]. In another study conducted in African Americans by Thio and his colleagues it was demonstrated that two HLA alleles, HLA–DQB1*0301 and HLA–DQA1*0501 as well as their corresponding haplotypes are associated with HBV persistence [23]. In a study by Mbarek and his colleagues that was conducted on CHB patients and on non-HBV controls, it was revealed that HLA-DQA1*0102–DQB1*0604 and HLA-DQA1*0101–DQB1*0501 are protective haplotypes while HLA-DQA1*0102–DQB1*0303 and HLA-DQA1*0301–DQB1*0601 are risk haplotypes in the Japanese population [19]. Furthermore, some studies from China have revealed that the HLA-DQB1*03:03 and DQB1*05:03 are associated with HBV viral persistence [24, 25]. In two other studies from Turkey, it was shown that DQ3 allele was related to predisposition to chronic hepatitis B infection [26]. Furthermore, DQB1*05:01 allele is more common in chronic active HBV than in inactive patients indicating that the allele is associated with chronic HBV persistence [27]. However, the majority of these studies focused on clearance and persistence of HBV infection and there are only a few reports on the effect of HLA-DQ alleles on HBV progression to cirrhosis and HCC. For instance, HLA-DQA1*0104 was identified as a protective allele in progression to liver cirrhosis [28]. A recent meta-analysis has investigated the association between HLA-DQB1 alleles and HCC, and it was reported that HLA-DQB1*02 and HLA-DQB1*0502 are risk factors for HCC occurrence whereas DQB1*03 and HLA-DQB1*0602 have protective effects on HCC [29].

Our study is the first to demonstrate the prevalence of HLA-DQA1 and -DQB1 alleles and haplotypes in HBV infected patients in the Malaysian population. We found that 66% of HBV patients with cirrhosis / HCC and 62% of HBV patients without cirrhosis / HCC are carriers of HLA-DQA1*01. In addition, 73% of HBV patients with cirrhosis / HCC and 60% of HBV patients without cirrhosis / HCC are carries of HLA-DQB1*03. We could not find any association between HLA-DQA1 and HLA–DQB1 allele / haplotype and HBV disease progression in the Malaysian population. However, the relationship between HLA polymorphism, ethnicity and immune response is still unclear, hence further investigation is needed to provide more insight into the role of HLA-DQA1 and –DQB1 in the pathogenesis of cirrhosis and HCC.

Understanding the role of HLA-DQ in immune response in defense of HBV infection can lead to effective design of novel immune therapies. Given the inconclusive result of previous studies, further association studies with much larger sample size with regard to progression of HBV to cirrhosis and HCC, and HLA-DQ alleles are required to provide strategies for the management and prevention of chronic HBV infection.

5. Limitation of the study

A larger sample size and additional ethnically diverse samples are required to confirm these findings.

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