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Association between the HLA-DQB1 Gene Polymorphism and Chronic Progression of Hepatitis B in Indonesia

Fatmawati Fatmawati[1], Ellyza Nasrul[2], Nasrul Zubir[3], Jamsari Jamsari[4], Bastian Nova[4], Aulia Janer[5]*

[1]Department of Biomedical Science, Faculty of Medicine, Andalas University, Padang, West Sumatra, Indonesia; [2]Department of Clinical Pathology, Faculty of Medicine, Andalas University, Limau Manis, Padang, West Sumatra, Indonesia; [3]Department of Internal Medicine, Faculty of Medicine, Andalas University, Limau Manis, Padang, West Sumatra, Indonesia; [4]Department of Agrotechnology, Faculty of Agriculture, Andalas University, Limau Manis, Padang, West Sumatra, Indonesia; [5]Department of Surgery, Faculty of Medicine, Riau University, Pekanbaru, Riau, Indonesia

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***Correspondence:** Aulia Janer, Department of Surgery, Faculty of Medicine, Riau University, Pekanbaru, Riau, Indonesia. E-mail: auliaaj89@gmail.com
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Abstract

BACKGROUND: The progression of hepatitis B is affected by the activity of T lymphocytes. Activation of T lymphocytes requires a primary signal originating from the presentation of antigen by HLA molecules to T cell receptors. HLA-DQB1 gene polymorphisms can affect the ability of HLA to bind and present viral antigens to T cells, thus affecting T cell activation and potentially associated with the progression of chronic hepatitis B.

AIM: We aimed to investigate the polymorphisms of the HLA-DQB1 gene and its influence on chronic hepatitis B progression of chronic hepatitis B patients in Indonesia.

METHODS: This cross-sectional research studied chronic hepatitis B patients at the Internal Medicine Department, Arifin Ahmad Hospital, Pekanbaru, from January 2018 to December 2018. Subjects were grouped into three categories: (1) Inactive chronic hepatitis B, (2) active chronic hepatitis B, and (3) end stage liver disease (ESLD) which consisted of patients with liver cirrhosis and hepatocellular carcinoma. Examination of the HLA-DQB1 gene polymorphism was performed with the SSP-PCR method and sequenced to verify the PCR results. Analysis results of $p < 0.05$ were considered statistically significant.

RESULTS: The most common allele in patients with chronic hepatitis B was the HLA-DQB1 0301. The HLA-DQB1 0301 allele found primarily in the inactive chronic hepatitis B group. The DQB1 0501 allele found to be more abundant in patients with active chronic hepatitis B. The HLA DQB1 0502 allele only found in patients with chronic hepatitis B with ESLD.

CONCLUSIONS: The HLA-DQB1 gene polymorphism is associated with the progression of chronic hepatitis B in chronic hepatitis B patients in Indonesia.

Introduction

T lymphocytes are activated during the immune response in hepatitis B virus (HBV) infection and are initiated by primary, secondary, and tertiary signals. The primary signal is generated from the attachment of the HBV antigen presented by the human leukocyte antigen (HLA) molecule to the T cell receptor (TCR/CD3) to form an antigen-receptor complex. The attachment causes the T cell to release the first signal that initiates T cell activation. The ability of HLA to present this antigen can be affected by functional and structural changes in HLA molecules caused by HLA polymorphism. Changes will affect the mechanism of binding of epitope antigens, indicating that the polymorphism of the HLA gene is one of the genetic factors in the host that can affect the sensitivity, degree, and progression of hepatitis B [1], [2].

HLA-DQ is the major isotype of HLA Class II and consists of α chain (encoded by the HLA-DQA1 gene) and β chain (encoded by the HLA-DQB1 gene). HLA-DQ plays an important role in the regulation of the immune response in HBV infection because it can influence the cellular and humoral immune response in chronic hepatitis B infection. If there is a change in the HLA-DQ molecule, it will affect the ability of viral antigen presentation so that it interferes with T cell activation in the cellular immune response [3].

Several HLA-DQ alleles have been identified and reported to be associated with acute and chronic HBV infection. Thio *et al.* in 1999 reported that the alleles DQA1*0501, DQB1*030, DQA1*0501, DQB1*0301, and DRB1*1102 were associated with African-American chronic HBV patients [4]. In this study, we investigated the polymorphisms of the HLA-DQB1 gene and its influence on chronic hepatitis B progression of chronic hepatitis B patients in Indonesia.

Material and Methods

The population of this cross-sectional study was all chronic hepatitis B patients in the internal

medicine polyclinic and ward of the Arifin Ahmad Hospital Pekanbaru, Riau, regardless of their race and ethnicity. Treated and untreated patients were included in the study. Patients with chronic hepatitis B with HCV and/or HIV infection, autoimmune liver disease, and alcohol-induced liver disease were excluded from the study. A consecutive sampling technique was used and samples were grouped into three groups: Inactive chronic hepatitis B group, active chronic hepatitis B group, and end-stage liver disease (ESLD) group consisting of patients with liver cirrhosis and hepatocelullar carcinoma (HCC). All patients had given their informed consent and all research procedures were approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Riau University, Pekanbaru.

Operational definition of variable

Progression of chronic hepatitis B based on clinical symptoms was the clinical course of chronic hepatitis B consists of inactive chronic hepatitis B, active chronic hepatitis B, and end-stage liver disease (ESLD) consisting of chronic hepatitis with liver cirrhosis and hepatocellular carcinoma. ALT was measured using the ECLIA method Clinical chemistry analyzer Cobas 501 (Roche Germany), HBsAg, anti-HBcAg IgG, HBeAg, and anti-HBe, AFP by the ELFA Immunology analyzer Architect 1000SR (Abbot), ANA by ANA screen Euroimmune (Sweden), degree of liver fibrosis, and evaluation of HCC by Ultrasound (Phillip). Inactive chronic hepatitis B if: HBsAg-positive, HBeAg or anti-HBe-positive, Anti-HBc IgG-positive, and normal plasma ALT. Active Chronic hepatitis B if HBsAg-positive, HBeAg or anti-HBe-positive, anti-HBc IgG-positive, plasma ALT increased, and receiving hepatitis B therapy [5]. Patients with a diagnosis of liver cirrhosis or hepatocellular carcinoma-related Hepatitis B infection are grouped into the end-stage liver disease group.

The criteria for the diagnosis of liver cirrhosis are the following: HBsAg-positive, HBeAg or anti-HBe-positive, anti-HBc IgG-positive, and plasma ALT elevated or normal, ultrasound reveals regenerative nodules surrounded by fibrotic tissue with or without therapy. The criteria for the diagnosis of hepatocellular carcinoma are as follows: HBsAg-positive, HBeAg or anti-HBe-positive, anti-HBc IgG-positive, and plasma ALT elevated or normal, on ultrasound a focal lesion measuring >2 cm with arterial hypervascularization and AFP examination >400 ng/mL with or without therapy [6]. HLA-DQB1 gene polymorphism is genetic diversity or variation of the HLA-DQB1 gene which has various alleles and which is used in this study 14 alleles, measured by PCR-SSP.

Blood sampling and preparation

10 mL of venous blood was drawn by a trained phlebotomist. All specimens are clearly labeled and stored according to the type of examination to be performed. There are two types of blood, 3 mL separated for a serum for the examination of seromarkers or markers of the HBV virus such as HBsAg, HBeAg, anti-HBe, anti-HBc IgG, anti-HCV, anti-HIV examination ANA and serum ALT and 2 mL of EDTA blood for DNA examination were stored at −30°C.

Genomic DNA extraction

The examination for 14 alleles of the HLA-DQB1 gene was carried out using the SSP-PCR technique using primers from the previous studies [7]. Genomic DNA from peripheral blood was extracted from the buffy coat to obtain mononuclear cells using the Wizard® Genomic DNA purification kit (Promega, USA) according to the manufacturer's procedure. PCR for the HLA-DQB1 allele was performed using the sequence-specific primer (SSP)-PCR method. The primers used in this study are for the previous studies (Table 1). Allele identification was carried out based on the presence or absence of the PCR product formed after being observed through agarose gel electrophoresis.

Table 1: Human leukocyte antigen-DQB1 primers

Allele	HLA-DQB1	Spesific primers	Products (bp)
DQB1*0201	5'	5' GTGCGTATTGTGAGCAGAAG 3'	205
	3'	5' GCAAGGTCGTGCGGAGCT 3'	
DQB1*0201/0302	5'	5' GACGGAGCGCGTGCGTCT 3'	129
	3'	5' CTGTTCCAGTACTCGGCGG 3'	
DQB1*0501	5'	5' CGGAGCGCGTGCGGGG 3'	128
	3'	5' GCTGTTCCAGTACTCGGCAA3'	
DQB1*0301	5'	5' GACGGAGCGCGTGCGTTA 3'	122
	3'	5' AGTACTCGGCGTCAGGCG 3'	
DQB1*0302/0303	5'	5' GACGGAGCGCGTGCGTCT 3'	122
	3'	5' AGTACTCGGCGTCAGGCG 3'	
DQB1*0303	5'	5' GACGGAGCGCGTGCGTCT 3'	129
DQB1*0401	5'	5' CACCAACGGGACCGAGCT 3'	200
	3'	5' GGTAGTTGTGTCTGCATACG 3'	
DQB1*0402	5'	5' CACCAACGGGACCGAGCG3'	200
	3'	5' GGTAGTTGTGTCTGCATACG 3'	
DQB1*0502	5'	5' TGCGGGGTGTGACCAGAC 3'	117
	3'	5' TGTTCCAGTACTCGGCGCT 3'	
DQB1*0503	5'	5' TGCGGGGTGTGACCAGAC 3'	87
	3'	5' GCGGCGTCACCGCCCGA 3'	
DQB1*0601	5'	5' GCCATGTGCTACTTCACCAAT 3'	198
	3'	5' CACCGTGTCCAACTCCGCT 3'	
DQB1*0602	5'	5' CGTGCGTCTTGTGACCAGAT 3'	121
	3'	5' GCTGTTCCAGTACTCGGCAT 3'	
DQB1*0603	5'	5' GGAGCGCGTGCGTCTTGTA 3'	127
	3'	5' GCTGTTCCAGTACTCGGCAT 3'	
DQB1*0604	5'	5' CGTGTACCAGTTTAAGGGCA 3'	254
	3'	5' GCAGGATCCCGCGGTACC 3'	
	3'	5' CTGTTCCAGTACTCGGCGT 3'	

HLA: Human leukocyte antigen.

Data analysis

Statistical analysis was performed using appropriate computer programs. The difference was considered statistically significant if $p < 0.05$.

Results

Characteristics of the research subjects

The characteristics of the research subjects are shown in Table 2.

Table 2: Characteristics of research subjects

Variables	*n*	Percentage
Sex		
Male	29	62.2
Female	17	37.8
Total	45	100
Age		
18–40	18	40
41–60	23	51.1
> 60	4	8.9
Total	45	100
ALT (IU/ml)		
Inactive chronic	23 ± 4.10	
Active chronic	40 ± 4.22	
ESLD	60 ± 21.20	
HBV DNA (IU/ml)		
Chronic inactive	<20	
Chronic active	$1.82 \times 10^5 \pm (1.45–5.10 \times 10^5)$	
ESLD	$1.45 \times 10^6 \pm (1.6–4.5 \times 10^6)$	
HBeAg positive	27	60

ALT: Alanine aminotransferase, ESLD: End stage liver disease, HBeAg: Hepatitis B e antigen, HBV: Hepatitis B virus.

Relationship of HLA-DQB1 gene polymorphism with chronic hepatitis B progression

The bands of HLA-DQB1 PCR products were visualized using GelDoc BioRad (Figure 1). The PCR product showed 122 bp of DQB1*0301 and 198 bp of DQB1*0601. Up to 20 µl of HLA-DQB1 PCR products were sent for sequencing to 1st BASE Malaysia. The sequencing method used is the Sanger method. Sequencing data were analyzed with Geneious bioinformatics software. Sequencing data for each sample from the forward primer is combined (contig) with the sequencing data from the reverse primer.

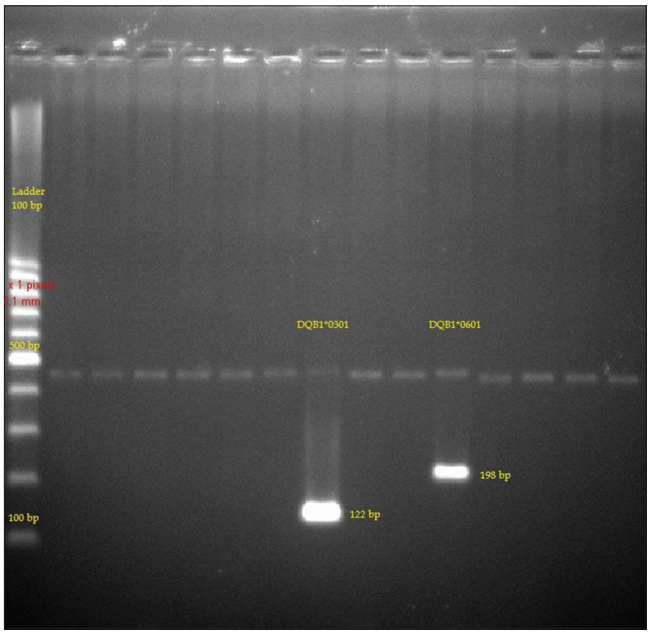

Figure 1: Electrophoresis of HLA-DQB1 allele amplification

Furthermore, the data for the transmission are BLAST on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST results provide information about the HLA-DQB1 allele. Sample 07 is one of the positive samples amplified in DQB1 PCR with the DQB1*0301 primer. Therefore, the sample PCR product was sent to 1st BASE through Genetics Science, Malaysia, for sequencing.

The following is the sequence of nucleotide bases of Sample 07 which has been contig with Geneious bioinformatics software. Next, the nucleotide base sequences are aligned with the database on the NCBI or basic local alignment sequence tools (BLAST). The BLAST results of Sample 07 HLA-DQB1*0301 showed that Sample 07 has similarities to the HLA-DQB1 gene allele *0301 Homo sapiens. This has confirmed the results of the PCR method using the DQB1*0301 allele-specific primer.

The frequency distribution of each allele in hepatitis B (Table 3) and the relationship of each allele to the progression of chronic hepatitis B (Table 4) showed that six alleles were not found in patients with chronic hepatitis B, that are DQB1*0602, DQB1*0603, DQB1*0604, DQB1*0303, DQB1*0401, and DQB1*0402.

Table 3: Allele frequency distribution of human leukocyte antigen-DQB1

Alelle	*n*	Total	Percentage
DQB1*0201	4	45	8.9
DQB1*0201/0302	10	45	22.2
DQB1*0301	29	45	64.4
DQB1*0302/0303	3	45	6.7
DQB1*0303	0	45	0
DQB1*0401	0	45	0
DQB1*0402	0	45	0
DQB1*0501	9	45	36.0
DQB1*0502	14	45	31.1
DQB1*0503	2	45	4.4
DQB1*0601	11	45	24.4
DQB1*0602	0	45	0
DQB1*0603	0	45	0
DQB1*0604	0	45	0

The most common allele found in all patients was HLA DQB1*0301 (64%), DQB1*0502 (31.1%), and DQB1*0601 (24.4), and DQB1*0201/0302 (22.2%). The HLA-DQB1 alleles that were significantly associated with the progression of chronic hepatitis B were the DQB1*0501 allele (p = 0.000), and the DQB1*0502 allele (p = 0.000), and the DQB1*0301 allele (p = 0.015). The DQB1*0301 allele was most commonly found in patients with inactive chronic hepatitis that is 48%. The DQB1*0501 allele was found mainly in the group of patients with active chronic hepatitis B, which was 89%. The allele DQB1*0502 was only found in patients with ESLD, which was 100%. None of the other alleles had a significant association with the progression of chronic hepatitis B.

Discussion

Characteristics of subjects

In this study, at total of 45 subjects were found with a male patient predominance (62.2%). The mean age of patients in this study was 44 years, with the youngest age being 27 years and the oldest being 67 years old. The largest patient age group is in the 41–60 year group. The exact molecular mechanism underlying the increased risk of disease progression in men is unknown, but this may be related to the

Table 4: Association of human leukocyte antigen-DQB1 allele with the progressivity of chronic hepatitis B

Alelle	HBV clinical				
	Chronic inactive, *n* (%)	Chronic active, *n* (%)	ESLD, *n* (%)	Total, *n* (%)	p
DQB1*0201	2 (50)	2 (50)	0	4 (100)	0.334
DQB1*0201/0302	6 (60)	3 (30)	1 (10)	10 (100)	0.087
DQB1*0301	14 (48)	8 (28)	7 (24)	29 (100)	0.015
DQB1*0302/0303	1 (33)	2 (67)	0	3 (100)	0.343
DQB1*0501	0	8 (89)	1 (11)	9 (100)	0.000
DQB1*0502	0	0	14 (100)	14 (100)	0.000
DQB1*0503	1 (50)	0	1 (50)	2 (100)	0.593
DQB1*0601	3 (28)	4 (36)	4 (36)	11 (100)	0.887

HBV: Hepatitis B virus, ESLD: End stage liver disease.

antifibrogenic effect of estrogen. The progression of chronic hepatitis B to SH and HCC in postmenopausal males and females is suspected to occur due to low estradiol production [8].

Relationship of HLA-DQB1 gene polymorphism with chronic hepatitis B progression

The results showed that the alleles DQB1*0602, *0603, *0604, *0303, *0401, and *0402 were not found in patients with chronic hepatitis B in Indonesia. This indicates that the six alleles are not associated with chronic hepatitis B infection in Indonesia. This is in contrast to the report by Karra *et al.* in 2018, the study found that several HLA-DQB1 alleles in patients with chronic hepatitis B in India were associated with the progression of chronic hepatitis B. The DQB1*0402 allele is a risk allele for the incidence of chronic hepatitis B and the HLA-DQB1*0601 allele is a protective allele against the incidence of chronic hepatitis B [9].

In this study, the most common allele found in all patients was HLA-DQB1*0301 (64%). This result is similar to the report from Doganay in 2014 which found the most common HLA-DQB1 allele found in patients with chronic hepatitis B was the DQB1*0301 allele (48.2%) [7].

Changes in HLA can occur due to polymorphisms of the HLA gene that cause a decrease in the number of HLA molecules due to a decrease in the synthesis of HLA molecules or a decrease in the ability of HLA molecules to bind and present viral antigen peptide molecules to T cells. The results of this study also showed that several HLA-DQB1 alleles were associated with the progression of chronic hepatitis B, which are HLA-DQB1*0301, DQB1*0501, and DQB1*0502 [10], [11].

Many studies have reported a strong association between HLA Class II alleles and HBV infection, although the mechanism underlying this association is unclear. Among HLA Class II molecules, DQ molecules are unique and have many variations of amino acid residues at the helix antigen-binding site [3]. Patients who have the HLA-DQB1*0301 allele can develop chronic hepatitis B due to the inability of this molecule to bind and present antigens effectively. It is well-known that HLA molecules regulate host APCs and elicit specific cytotoxic T-cell responses. However, further investigation should be carried out to confirm this hypothesis.

In this study, the highest frequency distribution of HLA-DQB1*0501 was found in patients with active chronic hepatitis B. This indicates that the DQB1*0501 allele may be a risk allele for the progression of hepatitis B to chronically active. In this study, the frequency distribution of the HLA DQB1*0501 allele was higher in chronically active patients than in patients with chronic inactive hepatitis B and end-stage liver disease. The results of this study are the same as the 2014 Doganay study in Turkey, which found that the HLA-DQB1*0501 allele was found more frequently in patients with chronic active hepatitis than in chronic inactive patients [7]. The results of this study indicate the possibility that HLA DQB1*0501 is a ESLD-resistant gene.

The HLA-DQB1*0502 allele is only found in patients with chronic hepatitis B with ESLD, so the possibility that a patient has this allele will increase the likelihood that a patient develops chronic hepatitis B with ESLD. Riazalhosseini's study on hepatitis B patients also found that 62% of patients with liver cirrhosis and HCC had the DQB1*05 allele, but the study reported that there was no significant relationship between the HLA-DQB1 allele and the progression of chronic hepatitis B in the Malaysian population [12].

Xin *et al.* in 2011examined the association of several HLA-DQB1 alleles with the incidence of HCC, and reported that two allele families, namely, DQB1*02 and DQB1*03, were significantly associated with the risk of HCC. From the 13 specific HLA-DQB1 alleles, two of them, namely, HLA-DQB1*0502 and HLA-DQB1*0602 were significantly associated with the risk of HCC [13]. It is well known that human tumor cells express various antigens, depending on the etiology and pathogenesis of the disease. The relationship of specific HLA alleles with sensitivity or resistance to a malignant tumor shows the direct involvement of HLA molecules as antigen presenters with the incidence of HCC [13]. The HLA-DQB1 gene polymorphism affects the immune response to HBV by affecting the structure and quantity of HLA Class II molecules. Several previous studies reported the association of the HLA Class II allele with end-stage liver disease such as cirrhosis and HCC in patients with chronic hepatitis B [13].

This study found a difference in the frequency distribution of the HLA-DQB1 allele that varies at each stage of chronic hepatitis B disease suggesting that this gene polymorphism influences disease activity and may also influence the incidence of cirrhosis and

hepatocellular carcinoma. Whether the incidence of cirrhosis or HCC in patients with chronic hepatitis B in Indonesia is related to the HLA DQB1 allele, and whether the underlying mechanism is the same as previously thought, still requires further research and investigation.

Conclusions

There is an association between the HLADQB1 polymorphism and the progression of chronic hepatitis disease in Indonesian patients.

Authors' Contribution

Fatmawati, Ellyza Nasrul, Nasrul Zubir, and Jamsari participated in research conception, data acquisition, and interpretation of results. Fatmawati, Bastian Nova, and Aulia Janer were involved in manuscript preparation, presentation of figures and tables, and publishing preparation. All authors discussed the results and commented on the manuscript.

Ethical Approval

Local ethical approval was obtained.

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