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Full Length Research Paper

Association between HLA-DRB1 alleles and susceptibility to chronic hepatitis B in patients of Basrah province, Iraq

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Hepatitis B virus (HBV) is one of the major diseases of mankind and is a serious global public health problem. The major histocompatibility complex (MHC) is a dense complex of genes with immunological and non-immunological functions and is present in all vertebrates. In humans, it is known as human leukocyte antigen (HLA). The genes encoding these molecules are the most polymorphic in the human genome and are ideal candidates for the investigation of association with HBV outcomes. This study was aimed to identify the influence of HLA-DRB1 alleles and susceptibility to chronic hepatitis B in patients of Basrah province. The blood samples were collected from Iraqi patients and HLA typing was carried out by using molecular methods (line probe assay). The present study indicate that alleles DRB1*3 (P=0.001), sub-allele DRB1*030101 (P=0.007) and DRB1*11 (P=0.014) were more frequent in patients and had higher significant than in the control group, and these alleles had highly elevated odds ratio (OR , and , respectively with positive association. Therefore, these antigens will be considered as liable to the HBV infection. Whereas alleles DRB1*01 (P=0.006), DRB1*7 (P=0.015), sub-allele DRB1*070101 (P=0.015), DRB1*01 (P=0.006) and DRB1*13 (P=0.013) were more frequent in control group and had higher significance than in the patients, hence, these antigens will be considered as protective to this disease. Our results indicate that alleles of DRB1*3 and DRB1*11 are associated with HBV as chronic disease. These findings support the theory that HLA class II–restricted helper T cell plays an important role in HBV as chronic disease.

Key words: Hepatitis B virus (HBV), human leukocyte antigen (HLA), allele.

INTRODUCTION

Hepatitis B virus (HBV) is one of the major diseases of mankind and is a serious global public health problem. Of the two billion people who have been infected with the HBV, more than 350 million have chronic infections whereby 500,000 to 1.2 million deaths per year are caused by chronic hepatitis, cirrhosis and hepatocellular carcinoma (Lavanchy, 2004). The major histocompatibility complex (MHC) is a dense complex of genes with immunological and non-immunological functions and is present in all vertebrates. In humans, it is known as human leukocyte antigens (HLAs) (Marsh, 2008). The immune response is coordinated by the HLA class I and class II molecules, which present as foreign antigens to $CD8^+$ cytolytic T cells and $CD4^+$ helper T cells, respectively (Diepolder et al., 1998). The genes encoding these molecules are the most polymorphic in the human

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Abbreviations: HBV, Hepatitis B virus; **MHC,** major histocompatibility complex; **HLA,** human leukocyte antigen.

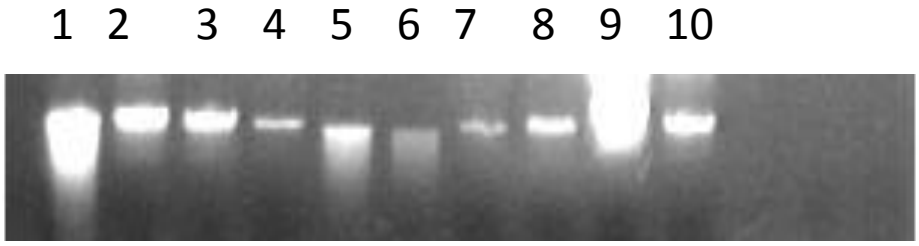

Figure 1. Electrophoresis of genomic DNA extracted from the whole blood. Lanes 1 to 10, Sample. Whole blood (WBC) were isolated by centrifugation, followed by DNA extraction according to the instruction by the commercial kit Wizard, genomic DNA purification kit, Protégé, USA, genomic DNA was extraction

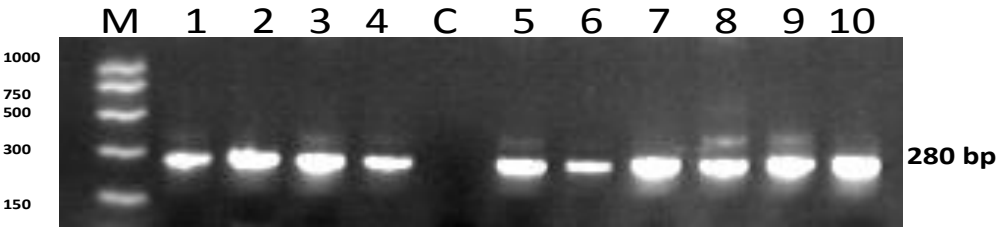

Figure 2. Electrophoresis of amplified biotinylated DNA material. Lanes 1 to 10, Samples. Lane M, PCR marker; Lane C, control. HLA-DRB1 alleles were amplified according to the instruction by amplification kit (INNO-LiPA HLA-DRB1 Amp plus) and was available for standardized preparation of biotinylated amplified material. This amplification kit was based on the polymerase chain reaction (PCR), and then the amplified products were electrophoresed in 2% agarose gel. The amplicon appeared as a single band with a length of 280 bp by using the PCR marker and were co-electrophoresed to verify the size of the amplified.

genome and are ideal candidates for the investigation of association with HBV outcomes (Najafizadeh and Sarkar 2008). Variations in immune response are often associated with polymorphism of the MHC (Thursz et al., 1995). HLA may be involved in the chronicity of HBV infection and plays an important role in immunological reaction to it (Thio et al., 1999).

MATERIALS AND METHODS

HLA typing was carried out by a line probe assay according to INNO-LiPA HLA-DRB1 PLUS kit; Innogenetics, Ghent, Belgium, for *in vitro* use, and designed for the molecular typing of HLA-DRB1 allele at the allele group level (DRB1*01 to DRB1*16), for 50 patients with chronic HBV infection and 50 healthy control group according to their serological profiles. All samples were tested for hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb), hepatitis B e antigen (HBeAg), hepatitis B e antibody (HBeAb), total anti-HBc and IgM Anti-HBc; determination was done by using enzyme-linked immunosorbent assay (ELISA) according to DRG kit, USA.

According to the instruction by the commercial kit Wizard, genomic DNA purification kit, Protégé, USA, genomic DNA was extracted from the whole blood for 50 patients and 50 persons as controls. Briefly, human genomic DNA extraction included cell lysis, nuclei lysis, protein precipitation for white blood cells and then finally DNA rehydration. Human genomic DNA was electrophoresed in 1% agarose gels and stained with ethidium bromide; then followed by amplification of exon 2 of the HLA-DRB1 allele. With the INNO-LiPA HLA-DRB1 PLUS kit, an amplification kit (INNO-LiPA HLA-DRB1 Amp PLUS) was available for standardized preparation of biotinylated amplified material. This amplification kit was based on the polymerase chain reaction (PCR). Briefly, human genomic DNA (at a concentration of 10 ng, and "260 nm/280 nm" of 1.7 to 2) was amplified according to INNO-LiPA HLA-DRB1 Amp PLUS kit using PCR reaction mix and condition.

The amplified products were electrophoresed in 2% agarose gel. The amplicon appeared as a single band with a length of 280 bp by using the PCR marker and were co--electrophoresed to verify the size of the amplified. Hybridization and stringent wash with 37 probes immobilized on one INNO-LiPA HLA-DRB1 PLUS strip (56°C). All positive reactivity probes on the INNO-LiPA HLA-DRB1 PLUS strips were identified by using the plastic reading card, then the DRB1 types were detected by using a version of the LiPA interpretation software.

The frequency of HLA alleles were calculated by direct counting. Data were analyzed by the Chi square test and Fisher's exact test; P values <0.05 were considered statistically significant. The odds ratio (OR >1.96), which reflects the likelihood of a subject carrying a specific allele and the 95% confidence interval (95% confidence interval) were calculated.

RESULTS

Serological tests showed patients divided into two inactive chronic, eight active chronic and 40 inactive HBsAg carrier (chronic HBV infection). The results of HLA complex were achieved by using line probe assay (Figures 1 to 4) for 50 patients with chronic HBV infection

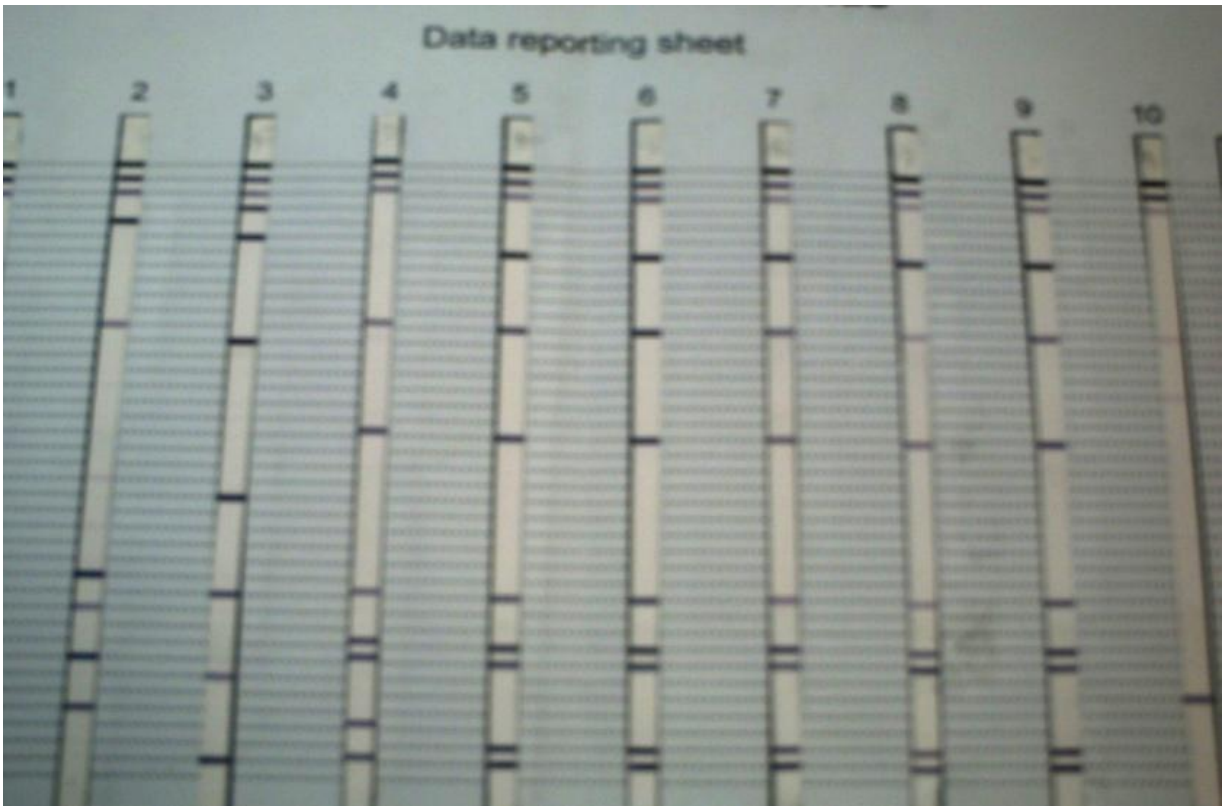

Figure 3. The reactivity pattern of the HLA-DRB1 probes. Positive reactivity probes on the INNO-LiPA HLA-DRB1 PLUS strips were identified by using the plastic reading card, then the DRB1 types were detected by using a version of the LiPA interpretation software. 2 = 111202, 3 = 010203, 4 = 1320, 5 to 9 = 030101, 10 = 130301.

and 50 healthy control group have a clearer integrative idea on impact of HLA (class II, HLA- DRB1 PLUS) on the susceptibility and resistance to HBV infection. The present study indicate that alleles DRB1*3 (P=0.001), sub-allele DRB1*030101 (P=0.007) and DRB1*11 (P=0.014) were more frequent in patients and had higher significant than in the control group, and these alleles have highly elevated OR (, and), respectively related to positive cases. Therefore, these antigens will be considered as susceptible to the HBV infection; whereas alleles DRB1*01 (P=0.006), DRB1*7 (P=0.015), sub-allele DRB1*070101 (P=0.015), DRB1*01 (P=0.006) and DRB1*13 (P=0.013) were more frequent in control group and had higher significant than in the patients, therefore, these antigens will be considered as protective to this disease (Table 1). The other types of HLA antigens (DRB1*0308, 110101, 111202 and 160201) had not significantly elevated OR. Furthermore, alleles DRB1*1, 11, and 13 had highly statistical significant at allele levels but non at sub- allele levels (Table 1).

DISCUSSION

The present study results indicate that the frequency of DRB1*13 allele was significantly associated with healthy control, therefore, this antigen will be considered as protective to HBV infection. Recently, the low frequency of the HLA class II allele DRB1*13 in patients with chronic HBV were confirmed in Gambia (Thursz et al., 1995), Germany (Diepolder et al., 1998), Taiwan (Wu et al., 2004), Korea (Ahn et al., 2000) and India (Amarapurpar and Kankonkar 2003). Patients with HLA-DR13 can mount a more vigorous CD4+ T cell response to HBV core antigen during acute HBV infection and that progression to chronic HBV is rare in that group. It is tempting to speculate that the HLA-DR13 molecule could be more proficient for the presentation of HBV core epitopes than other HLA-DR molecules (Diepolder et al., 1998).

The results of present study indicate that the frequency of DRB1*07 allele was higher in control group than patients with chronic HBV infection. In contrast, HLA DRB1*07 allele is associated with HBV clearance in Chinese (Han et al., 2005), HBV chronicity among Turkish populations (Karan et al., 2002), non-response to HBV vaccination (Wu et al., 2004) and with the susceptibility of the infants to intrauterine HBV infection (Yuan et al., 2008). Host HLA polymorphism is an important factor in determining the outcome of HBV infection (Yasuda, 1988). Failure of HBV vaccination has been tied to dysfunction of both Th1 cells and specific B

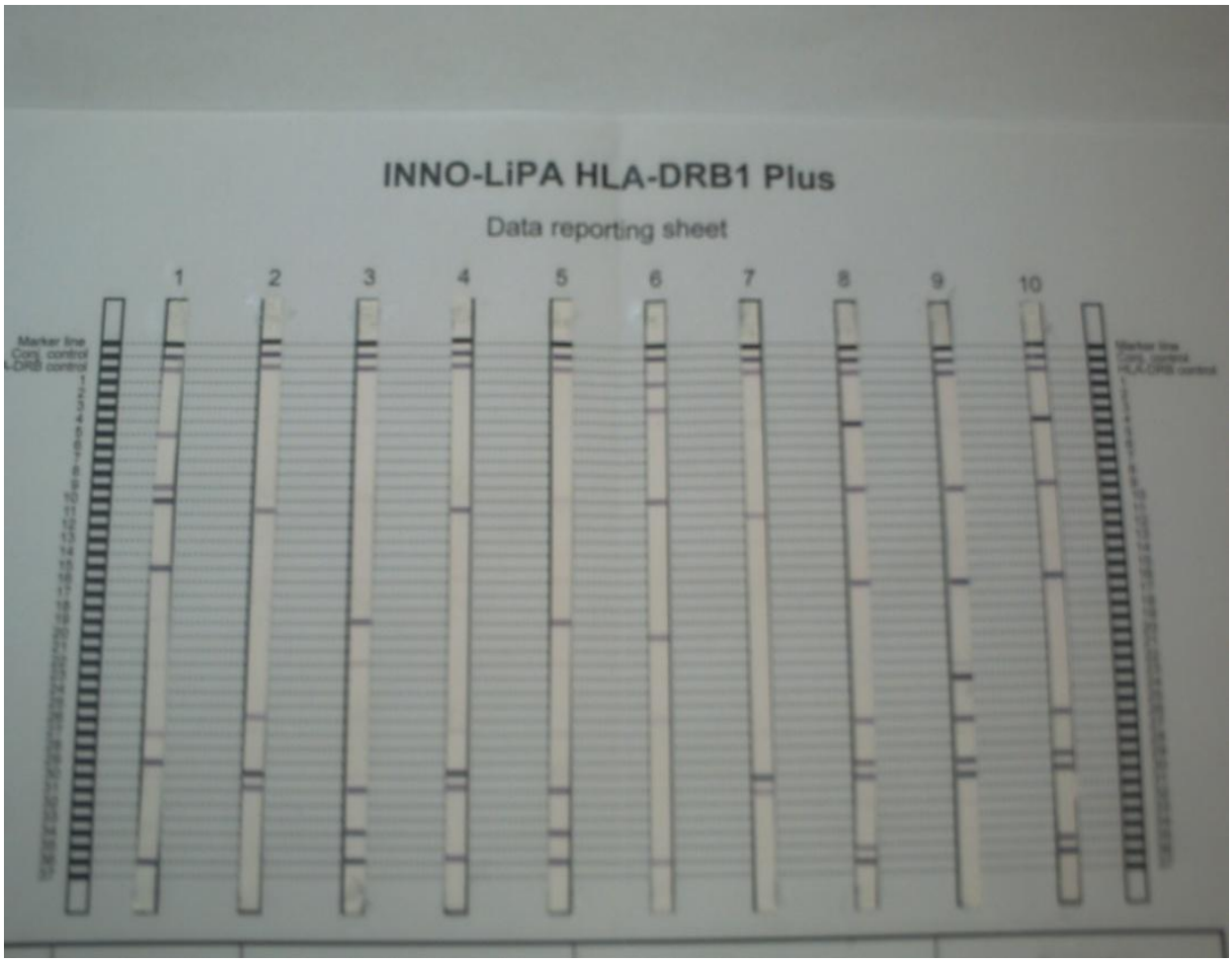

Figure 4. The reactivity pattern of the HLA-DRB1 probes. Positive reactivity probes on the INNO-LiPA HLA-DRB1 PLUS strips were identified by using the plastic reading card, then the DRB1 types were detected by using a version of the LiPA interpretation software. 1 = 140101, 2 = 150104, 3 = 160201, 4 = 1505, 5 = 160201, 6 = 010201, 7 = 150101, 8 = 030101, 9 = 130101, 10 = 030101.

cells (Wu et al., 2004). Furthermore, high positivity's of DRB1*07 allele and HBV genotype C are closely associated with the lower response to interferon-α therapy for chronic HBV (Chu et al., 2005). Also, HLA-DRB1*15 was significantly higher in the HBV genotype B group than in the group C. These findings suggest that there were associations not only between HLA polymorphisms and outcomes of HBV infection but also between HLA polymorphisms and the infected HBV genotypes (Shu-Yun et al., 2006). These findings imply that various HLA molecules could present different HBV epitopes, which may partly come from different HBV genotypes to induce effective immune responses or to cause immune-tolerance responses.

In agreement with Jiang et al. (2003) and Thio et al. (2003), the present study found a significantly higher frequency of the DRB1*03 alleles in patients with chronic HBV; or associated with a poor humoral response to HBsAg vaccination of hemodialyzed caucasian patients in France (Caillat-Zucman et al., 1998) and also in UK (Godkin et al., 2005). Whereas the DRB1*01 was associated with a good response of hem dialyzed caucasian patients in France. Some alleles such as DRB1*03 preferentially induce a Th1 (cellular) response, which would inhibit the Th2 (humoral) response and limit the production of anti-HBsAg antibodies. Conversely, the response would be skewed towards Th2 responsiveness in subject with DRB1*01 (Caillat-Zucman et al., 1998). Furthermore, allele DRB1*11 was associated with susceptibility to HBV infection in African American (Thio et al., 2003) whereas it was associated with protection from persistent HBV infection in China (Jiang et al., 2003). These are due to either host HLA polymorphism or to infected HBV genotypes.

In conclusion, the results of this study confirm that alleles of DRB1*3 and DRB1*11 are associated with HBV as chronic disease. These findings support the theory that HLA class II- restricted helper T cells play an

Table 1. Frequency of HLA-DRB1 PLUS in Iraqi HBV patients.

	Patient	%	Control	%	P value	Odds ratio	95% confidence interval
1	0	0	7	14		-	0.76 to 0.96
010101	0	0	1	2		-	0.94 to 1.02
010201	0	0	4	8		-	0.84 to 0.99
010203	0	0	2	4		-	0.9 to 1.01
3	32	64	15	30			1.79 to 9.57
030101	26	52	13	26			1.33 to 7.14
0308	6	12	2	4			0.62 to 17.07
4	0	0	4	8		-	0.84 to 0.99
040301	0	0	2	4		-	0.90 to 1.01
0404	0	0	1	2		-	0.94 to 1.02
040501	0	0	1	2		-	0.94 to 1.02
7	1	2	8	16		-	0.01 to 0.89
070101	1	2	8	16		-	0.01 to 0.89
8	0	0	1	2	2	-	0.94 to 1.02
080201	0	0	1	2	2	-	0.94 to 0.02
11	10	20	2	4			1.24 to 2. 98
110101	3	6	1	2			0.31 to 31.14
1107	1	2	0	0		-	0.98 to 1.06
111202	6	12	1	2			0.77 to 57.69
13	0	0	6	12		-	0.79 to 0.97
130101	0	0	3	6		-	0.87 to 1.00
130301	0	0	1	2		-	0.94 to 1.02
1320	0	0	1	2		-	0.94 to 1.02
1327	0	0	1	2		-	0.94 to 1.02
14	0	0	2	4		-	0.90 to 1.01
140101	0	0	2	4		-	0.90 to 1.01
15	4	8	2	4			0.36 to 11.94
150101	1	2	0	0		-	0.98 to 1.06
150104	2	4	2	4			0.13 to 7.39
1505	1	2	0	0		-	0.98 to 1.06
16	3	6	3	6			0.19 to 5.21
160101	0	0	1	2		-	0.94 to 1.02
160201	3	6	1	2			0.31 to 31.14
1609	0	0	1	2		-	0.94 to 1.02

HBV, Hepatitis B virus.

important role in HBV as chronic disease.

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