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Polymorphisms of HLA Class I and II Alleles in Iraqi Patients with Hepatic Hydatid Infection

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ABSTRACT

Background: *Hepatic hydatid disease (HHD) is a parasitic zoonosis caused by larval stage of Echinococcus tape worm. In addition to environmental factors, genetic constitution of hosts seems to play a crucial role in acquiring the infection and developing disease.* ***Aims:*** *This study was carried out to investigate the association of HLA-class I and class II (A, B, DR and DQ) alleles with HHD by genotyping in Iraqi patients, as well as to provide information about genotypes that confer susceptibility or resistance to develop the disease.* ***Materials and Methods:*** *Twenty patients with HHD, their age range (20-50) years and 20 healthy controls their ages were matched with the patients were enrolled in this study. Blood was collected from patients and controls, DNA was extracted from blood samples, and then HLA-class I and class II genotyping was performed by polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSO).* ***Results:*** *The present findings showed that frequencies of HLA-A*32 (65%; P=0.011), DRB1*11 (60%; P=0.004) and DQB1*03 (70%; P=0.007) alleles are significantly higher in patients than controls, while the frequency of DRB1*04 was significantly decreased in patients when compared to controls (25% vs. 75%; P=0.002). Furthermore, the current study could not observe significant differences in the frequencies of HLA-B alleles between patients and controls.* ***Conclusions:*** *We concluded that HLA-A*32, DRB1*11 and DQB1*03 alleles might contribute to the increased susceptibility to HHD and DRB1*04 could be a protective marker against the disease.*

Keywords: Hydatid disease, Hepatic hydatid disease, HLA-class I and II, PCR-SSO

INTRODUCTION

Infectious diseases, particularly parasitic diseases are becoming increasingly prevalent in the modern world. Hydatid disease (HD) or cystic echinococcosis is an ancient parasitic disease defined death if it bursts. Hydatidosis is endemic in many parts of the world with serious impacts on organ function and host survival. It is a near-cosmopolitan zoonosis caused by tapeworms (cestodes) belonging to the family Taeniidae and the genus *Echinococcus* [1-3]. It is a unique parasitic disease that primarily affects the liver, the right lobe being the most common site of involvement. There are many potential intra-hepatic and extra-hepatic complications that affect the liver and other organs [4].

In addition to environmental factors facilitating infection with the parasite, genetic constitution of hosts seems to play a crucial role in acquiring the infection and developing disease signs and symptoms. An appropriate example would be the exposure of many individuals to the parasite, with only some of them manifesting illness post exposure [5]. The most important determinants of genetic susceptibility to HD are located in the major histocompatibility complex (MHC) or the human leukocyte antigen (HLA) gene area on the short arm of chromosome 6, it is a kind of genetic marker of human beings [6]. The HLA system, initiator of immune responses, has been reported to have associations with many diseases worldwide. Numerous studies in Iraq reported associations between HLA and diseases [7-14]. The HLA component of the immune system, encoded by highly polymorphic genes that vary across racial/ethnic groups, has been suggested to be a biologically based risk factor for HD and thus may explain some of its variation by race/

ethnicity [5]. A number of HLA alleles have been reported to be associated with the occurrence of HD [15,16], while others were reported to be associated with protection against the parasite [15,17]. So, this prompted us to investigate the frequency of HLA (-A, -B, -DR, -DQ) with HHD by genotyping in Iraqi patients, as well as to provide information about genotypes that confer susceptibility or resistance to develop the disease.

MATERIALS AND METHODS

Twenty patients with HHD (10 males and 10 females), age range 20-50 years with mean age (31.15 ± 2.37) and 20 healthy individuals as control, their ages and genders were matched with the patients (9 males and 11 females), age range 20-50 years with mean age (30.22 ± 2.40) were enrolled in this study. They were among patients admitted to AL-Kadhumyia Teaching Hospital and Baghdad medical city teaching hospital from February 2012 to till September 2012. The diagnosis was made by the consultant medical staff, which was based on clinical and (X-ray and ultrasound examination). Three ml of blood were withdrawn from each subject under aseptic technique, then transferred into EDTA tube, kept at -20°C for the genotyping of HLA class I and II. The DNA was extracted by using the genome DNA extraction kit (Qiagene/Germany). All DNA was stored at -20°C until tested. HLA-genotyping were performed by the PCR-SSO according to the manufacturer's instructions, this method depends on reverse hybridization, using the PCR-SSO kit (Histo Type/ DNA-SSO Kits-Innogenetics Line Probe Assay, INNO-LiPA, Belgium).

Statistical Analysis

The results were presented in terms of percentage frequencies, and alleles showing variations between patients and controls were further presented in terms of odds ratio (OR). The significance of these differences was assessed by Fisher's exact probability (P). P values of $P<0.05$ were considered statistically significant.

RESULTS

Frequency of HLA class I and II alleles belonging to the patients and healthy subjects are seen in Tables 1-4. Some significant findings can be concluded from this study. The distribution of HLA-class I and II alleles among the total of 60 patients and controls showed a significant increase in frequencies of HLA-A*32 (65%; OR-512; P=0.011), DRB1*11 (60%; OR-13.50; P=0.004) and DQB1*03 (70%; OR-14.54; P=0.007), while the frequency of DRB1*04 (OR-11.00; P=0.002) was significantly decreased in HHD patients group as compared to control group (25% vs. 75%). Furthermore, the current study could not observe significant differences in frequencies of HLA-B alleles between patients and control groups, Table 2.

Table 1 HLA-A alleles frequencies in HHD patients and healthy control group

HLA-A allele	Patients (N=20)	%	Control (N=20)	%	OR	P value
*01	2	10%	3	15%	0.632	NS
*02	9	45%	7	35%	1.52	0.021
*03	0	0.00%	3	15%	0.125	NS
*11	5	25%	4	20%	1.333	NS
*24	3	15%	2	10%	1.588	NS
*30	0	0.00%	4	20%	0.089	NS
*31	1	5%	0	0.00%	3.152	NS
*32	11	65%	2	10%	5.12	0.011
*33	3	15%	8	40%	0.261	NS
*34	2	10%	0	0.00%	5.541	NS
*68	1	0.00%	3	15%	1.125	NS
*92	3	15%	4	20%	1.205	NS
Total	40	100	40	100	-	-
OR: odds ratio; NS: not significant						

Table 2 HLA-B alleles frequencies in HHD patients and healthy control group

HLA-B allele	HHD patients (N=20)	%	Control (N=20)	%	OR	P-value
*01	1	5%	2	10%	0.474	NS
*02	3	15%	0	0.00%	8.2	NS
*03	1	5%	2	10%	0.474	NS
*04	1	5%	2	10%	0.474	NS

*07	1	5%	2	10%	0.474	NS
*08	1	5%	1	5%	1	NS
*13	1	5%	0	0.00%	3.154	NS
*15	2	10%	2	10%	1	NS
*26	1	5%	0	0.00%	3.154	NS
*27	2	10%	5	25%	2.111	NS
*32	4	20%	1	5%	4.75	NS
*35	5	25%	4	20%	1.333	NS
*38	0	0.00%	1	5%	0.317	NS
*40	3	15%	0	0.00%	8.2	NS
*41	1	5%	0	0.00%	3.154	NS
*44	1	5%	0	0.00%	2.085	NS
*47	2	10%	3	15%	0.63	NS
*49	2	10%	3	15%	0.63	NS
*50	1	5%	1	5%	1	NS
*51	2	10%	1	5%	2.111	NS
*52	4	20%	4	20%	1	NS
*55	3	15%	6	30%	0.185	NS
Total	40	100	40	100		

Table 3 HLA-DR alleles frequencies in HHD patients and healthy control group

HLA-DR allele	HHD patients N=20	%	Control N=20	%	OR	P-value
*02	1	5%	1	5%	1	NS
*03	5	25%	6	30%	0.92	NS
*04	5	25%	15	75%	11	0.002
*06	5	25%	4	20%	1.33	NS
*07	2	10%	3	15%	0.63	NS
*10	1	5%	0	0.00%	2.08	NS
*11	12	60%	1	5%	13.5	0.004
*13	4	20%	4	20%	1	NS
*15	1	5%	0	0.00%	2.08	NS
*16	2	10%	3	15%	0.63	NS
*63	1	5%	3	15%	1.12	NS
*90	1	5%	0	0.00%	2.08	NS
Total	40	100	40	100	-	-

Table 4 HLA-DQ alleles frequencies in HHD patients and healthy control group

HLA-DQ allele	HHD patients (N=20)	%	Control (N=20)	%	OR	P-value
*02	5	25%	9	45%	2.085	NS
*03	14	70%	2	10%	14.54	0.007
*04	1	5%	0	0.00%	2.08	NS
*05	8	40%	8	40%	1	NS
*06	3	15%	9	45%	0.21	NS
*07	3	15%	2	10%	1.58	NS
*08	4	20%	4	20%	1	NS
*13	0	0.00%	3	15%	0.12	NS
*60	2	10%	3	15%	0.63	NS
Total	40	100	40	100	-	-

DISCUSSION

Several associations between various pathologies and specific HLA antigens have been reported. However, the role of HLA polymorphism in infectious diseases has not yet been fully explored. Various ethnic groups have been studied to determine HLA association for a number of diseases including HD or cystic echinococcosis. The current study revealed that HLA-A*32, DRB1*11 and DQB1*03 alleles are significantly higher in patients group than controls group, while the frequency of DRB1*04 was significantly decreased in patients when compared to control. On the other hand, there is no significant difference in frequencies of HLA-B alleles between patients and control groups.

Bulent, et al. reported increase in the antigen frequencies of HLA-DRB1*15, HLA-DQB1*02, 06, 07 in the hepatic echinococcosis patients compared with those in the control group, and they indicated that susceptibility to HHD in the Turkish population is essentially HLA class II and poorly class I mediated, with HLA-26, and DRB1*015, DQB1*02, 06, 07 with more allele distribution in the patient group [18]. In previous Iraqi study using serological method conducted by Al-Joofy, et al. found significant increased trend of HLA-A28 and A-11, -B18 and B-35 in patients with this disease as compared with healthy control. On the other hand, HLA-B14 and B27 antigens evidently presented a certain resistance to these invasions [19]. In contrast with our result Mahdi, et al. showed that HLA-DQB1*03 allele is positively associated with the resistance to HD, so he concluded that DQB1*03 molecules are associated with the level of immune response to parasite antigens [20]. However, Azab, et al. reported that HLA-DR11 is positively associated with the occurrence of cysts <5 cm in patients with HD and carriers of DR-3 and DR-11 are at high risk for unilocular echinococcosis [16]. In Lebanon, Chakhtoura, et al. reported that HLA-B*14 and HLA-DRB1*01 to have a protection role against echinococcosis [21]. The phenomenon of immunological or constitutional resistance may be dependent upon a potential immunogenetic predisposition with a potential HLA association [22]. The presence of different HLA antigens among different studies of other societies and our study may be due to ethnic differences among world population and/or could be due to small sample of patients taken in this study, or could be due to mirage among ethnic groups of Iraqi societies from very previous generations.

CONCLUSION

This study concluded that HLA-A*32, DRB1*11 and DQB1*03 alleles might contribute to the increased susceptibility to HHD and DRB1*04 could be a protective marker against the disease.

CONFLICT OF INTEREST

We declare that we have no conflict of interests.

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