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***J. Nat. Prod. Plant Resour.*, 2017, 7 (1): 15-20**
(http://scholarsresearchlibrary.com/archive.html)

ISSN: 2231-3184
CODEN (USA): JNPPB7

Polymorphisms of HLA Class I (A and B) Alleles in Iraqi Patients with Hydatid Disease

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ABSTRACT

Hydatid disease or cystic echinococcosis is a parasitic infection caused by the larval stage of tape worm is one of the most significant zoonosis all around the world. In addition to environmental factors, genetic constitution of hosts seems to play a crucial role in acquiring the infection and developing disease. This study was carried out to investigate the association of HLA-class class I (A, B) with hydatid disease by genotyping in Iraqi patients, as well as to provide information about genotypes that confer susceptibility or resistance to develop the disease. Thirty patients with hydatid disease their age range (16-57) years and twenty 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 genotyping was performed by polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSO).

*The present findings showed that HLA-A *0273 allele is significantly higher in control group (20%) than patients group (0%), (P=021). Furthermore the current study could not observe significant differences in frequencies of HLA-B alleles between patients and control groups. We concluded that HLA-A *0273 allele may might indicate resistance to disease among patients, and the lack of association between patients group and HLA alleles could reflect racial genetic variation in HLA allelic frequencies.*

Keywords: Hydatid disease, HLA-Class I, Genotyping.

INTRODUCTION

Infectious diseases particularly parasitic diseases are becoming more and more prevalent in the modern world. Hydatid disease (HD) or cystic echinococcosis is an ancient parasitic disease defined death if it bursts. Hydatidosis is prominent disease of the Mediterranean basin 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. This parasitic disease is very common but largely neglected [1,2]. The two major species of medical and public health importance are Echinococcus granulosus and E. multilocularis, which cause cystic echinococcosis and alveolar echinococcosis [3].

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 [4]. 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 [5]. The HLA system, initiator of immune responses, has been reported to have associations with many diseases worldwide. Numerous studies in Iraq reported associations of HLA and diseases [6-13]. 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 [4]. A number of HLA alleles have been reported to be associated with the occurrence of HD [14, 15], while others were reported to be associated with protection against the parasite [14, 16]. So this

prompted us to investigate the association of HLA-class class I (A, B) with HD by genotyping in Iraqi patients, as well as to provide information about genotypes that confer susceptibility or resistance to develop the disease.

RESEARCH METHODS

Thirty patients with HD (10 males and 20 females), age range 16-57 years and 20 healthy individuals as control their ages were matched with the patients 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).

Two ml of venous blood were withdrawn from each subject under aseptic technique, then transferred into two EDTA tube (1.5 mg/ ml), kept at -20ºC for the genotyping of HLA class I (A and B). The DNA was extracted by using the genome DNA extraction kit (Qiagene/ Germany). All DNA was stored at -20°C until tested. HLA-A and -B 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 AND DISCUSSION

This study was performed on 30 patients with HD and 20 healthy controls. The demographic characteristics of patients group and controls group included in this study are presented in Table 1. There are no statistical significant differences in age and gender was existed between two study groups. The mean age of patients was of 34.15±2.37 years and for healthy controls was 35.64±2.40 year, (Table 1). The females constitute the majority of the patients 66.7 % while the males patients were 33.3 %. Furthermore; 3(10 %) of the patients had a positive family history of the disease, while 21(90 %) of the patients had negative family history, as shown in Table (1).

The comparison between patients group and controls group showed that HLA-A *0273 is higher in control group than patients group 20% and 0% respectively ,which considered significant statistically (P=021), Table 2. Furthermore the current study could not observe significant differences in frequencies of HLA-B alleles between patients and control groups, Table 3.

Table 1: Demographic characteristics of the studied groups.

		Study groups		**P-value**
		HD Patients n=30	**Healthy control** n=20	
Age				
Age (years)	Range	(16-57)	(20-55)	
	Mean	34.15	35.64	0.890NS
	SE	2.37	2.4	
Gender	Male	10 (33.3%)	8(40%)	
	Female	20(66.7 %)	12(60%)	0.754 NS
Family history	Positive	3 (10%)	0 (0.0%)	
	Negative	21 (90%)	20 (100%)	

SE= Standard error; NS=Non significant (p>0.05).

Table 2: HLA-A Genotyping in HD patients in comparison with healthy control group.

HLA-A allele	Hydatidosis patients	%	Control	%	OR	IOR	EF	PF	P value
*0101	2	6.67%	4	20.00%	0.322	3.109	-4.22	0.81	NS
*0106	4	13.33%	0	0.00%	6.962	0.144	3.43	1.41	NS
*0109	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*0123	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*0130	5	16.67%	2	10.00%	1.596	0.627	1.87	2.15	NS
*0201	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*0205	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*0236	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*0247	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*0273	0	0.00%	4	20.00%	0.06	16.636	0	0	0.021
*0276	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*0290	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*0301	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*0309	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*0315	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*0341	1	3.33%	1	5.00%	0.661	1.513	-0.51	0.34	NS
*1106	1	3.33%	1	5.00%	0.661	1.513	-0.51	0.34	NS
*1122	2	6.67%	0	0.00%	0.661	1.513	-0.51	0.34	NS
*1127	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*2402	3	10.00%	3	15.00%	0.636	1.571	-1.71	0.63	NS
*2405	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*2408	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*2418	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*2429	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*2607	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*2625	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*2909	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*3001	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*3002	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*3016	1	3.33%	2	10.00%	0.376	2.658	-1.66	0.62	NS
*3017	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3020	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*3027	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*3105	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3106	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*3121	1	3.33%	1	5.00%	0.661	1.513	-0.51	0.34	NS
*3122	2	6.67%	2	10.00%	0.649	1.541	-1.08	0.52	NS
*3201	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3205	3	10.00%	0	0.00%	5.218	0.192	2.43	1.7	NS
*3207	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3210	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS

*3317	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3324	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3406	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*6606	0	0.00%	3	15.00%	0.082	12.2	0	0	NS
*6802	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*6825	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*6827	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*6844	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*6845	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*9201	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*9204	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*9279	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS

Table 3: HLA -B Genotyping in HD patients in comparison to healthy control group.

HLA-B allele	HD patients	%	Control	%	OR	IOR	EF	PF	P value
*0103	1	3.33%	1	5.00%	0.661	1.513	-0.51	0.34	NS
*0193	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*0205	2	6.67%	1	5.00%	1.14	0.877	0.25	-0.33	NS
*0206	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*0309	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*0412	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*0708	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*0801	5	16.67%	1	5.00%	2.804	0.357	3.22	1.45	NS
*0810	5	16.67%	3	15.00%	1.078	0.927	0.36	-0.57	NS
*0816	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*1302	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*1517	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*1529	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*1561	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*1806	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*1825	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*1832	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*2609	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*2702	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*2718	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*2730	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*3232	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3504	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*3524	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*3536	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*3544	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*3551	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3581	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3591	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS

*3801	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3813	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*3814	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*3905	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*3948	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*4006	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*4053	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*4101	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*4105	4	13.33%	0	0.00%	6.962	0.144	3.43	1.41	NS
*4212	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*4414	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*4702	2	6.67%	0	0.00%	3.596	0.278	1.44	3.25	NS
*4901	2	6.67%	1	5.00%	1.14	0.877	0.25	-0.33	NS
*5001	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*5108	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*5118	1	3.33%	4	20.00%	0.186	5.364	-4.36	0.81	NS
*5123	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*5129	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*5141	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*5152	0	0.00%	2	10.00%	0.121	8.243	0	0	NS
*5153	1	3.33%	1	5.00%	0.661	1.513	-0.51	0.34	NS
*5158	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*5201	3	10.00%	0	0.00%	5.218	0.192	2.43	1.7	NS
*5211	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*5313	0	0.00%	1	5.00%	0.213	4.692	0	0	NS
*5501	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*5524	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*5531	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*5956	1	3.33%	0	0.00%	2.085	0.48	0.52	-1.08	NS
*9531	0	0.00%	1	5.00%	0.213	4.692	0	0	NS

DISCUSSION AND CONCLUSION

Various ethnic groups have been studied to determine HLA association for a number of diseases. The current study revealed that HLA-A *0273 is significantly higher in control group than patients group and could be protective factor against disease, while there is no significant differences in frequencies of HLA-B alleles between patients and control groups. In contrast previous study conducted by Al-Joofy [17] reported 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 [16].

The phenomenon of immunological or constitutional resistance may be dependent upon a potential immunogenic predisposition with a potential HLA association [18]. 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 society from very previous generations. This study concluded that HLA-A *0273 allele may might indicate resistance to disease among patients, and the lack of association between patients group and HLA alleles could reflect racial genetic variation in HLA allelic frequencies.

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