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Original Article

Genotyping of HLA Class II Alleles Associated with Some Immunological Markers in Patients with Hydatidosis

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ABSTRACT

Hydatidosis is a worldwide parasitic disease which results in serious health problem and financial loss. Different alleles Human leukocyte antigen (HLA) were found to be associated with the susceptibility to different diseases. The current study aimed to determine the impact of different HLA alleles on the susceptibility to cystic echinococcosis (CE) and on serum levels of some immunological markers. The study involved 30 patients with CE and 20 healthy controls. DNA was extracted from a blood sample taken from each subject. HLA class II gene was amplified using Sequence-specific oligonucleotide primed PCR (SSO-PCR). Molecular typing of HLA alleles DR and DQ was performed using a reverse hybridization Automatic Line probe assay (Auto-LiPA). Enzyme linked immunosorbent assay was used to estimate serum levels of IL-10, TNF-α, IgG4 and IgE in HC-patients and control. There was significant increase in the frequency of HLA-DR*0403 and*0701 in healthy control and patients respectively**.** Furthermore, significant increase in serum levels of IL-10, IL-4, IgE and IgG4 in hydatidosis patients compared to healthy control was recorded. Thus it cab concluded that alleles HLA-DR*0701 and HLA-DR*0403 are associated with susceptibility and resistance respectively to hydatidosis in Iraqi population.

Keywords: HLA, cystic hydatidosis, IL-10, TNF-α, IgG4, IgE

INTRODUCTION

Hydatidosis or cystic echinococcosis (CE) is a parasitic disease caused by infection with the larval stage of the tape worm *Echinococcus granulosus*. The disease has worldwide distribution with only a few areas in the world like Island, Ireland and Greenland are thought to be free from this infection [1]. Due to its zonootic nature, the disease is particularly endemic in rural areas where there is a close contact of man with the domestic animals. In Iraq and many Arab and neighboring countries, CE is considered hyperendemic [2]. Host factors are usually the most critical determinant of CE progression. The outcome of the disease is a result of a complex interaction between the parasite and host immunological and genetic factors. Accordingly, population were categorized into susceptible and resistant to CE [3]. In susceptible group, the disease progress smoothly with the eventual formation of hydatid cyst, while relatively high percentage of the cysts die out after their establishment in the resistant group.

Probably the most effective genes in this regard is the major histocompatibility complex (MHC) or HLA genomic region which is well-known for its high density of genes and polymorphic nature [4]. Gene products of this region are responsible for antigen recognition by T-cells and for controlling of a variety of basic immunologic functions [5]. Since anti-oncospheral antibodies play a major role in parasite killing and they are crucial for the protective immune response against *E. granulosus* [6], it is reasonable to assume that allelic variations in MHC class II gene have a great effect on the susceptibility to *E. granulosus*. Stern and Calvo-Calle [7] stated that there are more than 500 different alleles in this gene each being present at a relative high frequency in a certain population. Many previous studies have established associations between some of these alleles with

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particular parasitic diseases [8,9,10]. The current study aimed to determine the association of different HLA alleles with the susceptibility to cystic echinococcosis (CE) and on serum levels of some immunological markers.

SUBJECT AND METHODS

A case/control study was conducting during the period from Septemper 2014 to February 2015 to demonstrate the association of HLA-Dr allele with the incidence of hydatidosis in Iraqi patient. A total 30 patients (11 males and 22 females, age range 28 to 71 years, average 44.21 years) with confirmed CE (by X-ray and ultrasound examination) and age-matched 20 apparently healthy individuals (13 males and 12 females, age range 30 to 69 years, average 48.16) were recruited for this study. Consent form was obtained from each participant.

Blood Samples: Five ml of venous blood were obtained from each subject, from which 2 ml were kept in EDTA tubes for DNA extraction, and the other 3 ml in plane tubes from which serum was obtained and kept at -20 C until be used.

DNA Extraction and Genotyping: DNA was extracted from whole blood using ready kit (KIAGEN/ Germany) according to the manufacturer's instructions. Sequence-specific oligonucleotide primed PCR (PCR-SSO) method was used for the amplification of HLA-DRBI and HLA-DQ using ready kit (Lipa HLA-DRB, Innogenetics. Murex Biotech Limited, Dartford, UK). . Molecular typing of HLA alleles was performed using a reverse hybridization Automatic Line probe assay (Auto-Lipa) supplied by the same company, in which typing tests were based on the reverse dot blot hybridization (11). Positive probes on each strips were recognized by typing table (provided with the kit).

Serum levels of TNF-α, IL-4, IL-10, IgG4 and IgE: Commercial kits were utilized for estimation of serum levels of IL-10, TNF-α, IgG4 (Demeditec Diagnostic/ Germany) and IgE (Human Gesellschaft fur Biochemica und Diagnostic/ Germany) using automated ELISA apparatus ((Diagnostic Automation Inc, USA) and following the manual protocol supplied with each kit.

Statistical Analysis: The Statistical Package for the Social sciences (SPSS, version 14) was used for statistical analysis. The association between the different alleles and the development hydatidosis was calculated through adjusted odd ratio and 95% confidence intervals using Chi-square test. Serum levels of cytokines and immunoglobulins were quantitative variables, but were non-normally distributed as shown by Shapiro-Wilk test. These variables are better to be analyzed by non-parametric test, and median instead of mean was calculated. The Mann-Whitney test was used to further explore the significance of difference in median between each pair of study groups. A *P*-value < 0.05was considered statistically significant.

RESULTS

HLA-DR Genotyping: Only two alleles of HLA-DR loci had significant influence on susceptibility to CE infection. The first one was HLA-DR*0403 which had higher frequency among healthy control (35%) compared to HC-patients (6.67%) (OR= 0.158, *P*=0.02). The other allele was HLA-DR*0701 which had 30% frequency among HC-patients with none among healthy control carrying this allele (OR=18.116, *P*=0.007) (table 1). **HLA-**

DQ Genotyping: Table 2 shows the genotyping of HLA-DQ loci. None of the tested allele showed significant influence on susceptibility or protection against CE.

Serum levels of Cytokines and Antibodies: Patients with CE showed higher median serum levels of IL-10 and IL-4 (10.25 pg/ml, 2.04 g/ml respectively) than healthy control group (0.0 pg/ml for each) with significant difference for both cytokines. Nevertheless, there was no significant difference in TNF-α between patients and controls (17.0 pg/ml and 14.15 pg/ml respectively, *P*=0.078). Median IgG4 and IgE concentrations from patients were 4.96 IU/ml and 29.05 IU/ml respectively which are significantly higher than that of controls (2.96 IU/ml and 0.0 IU/ml respectively) (table 3).

Table 1: HLA-DR typing of the most frequent HLA-DR in Hydatidosis patients and healthy controls

HLA-DR allele	Hydatidosis patients	Control	OR	P value
***0308**	4 (13.3%)	5 (25%)	0.479	NS
***0319**	2 (6.67%)	0 (0%)	3.596	NS
***0403**	2 (6.67%)	7 (35%)	0.158	.020
***0435**	1 (3.33%)	1 (5%)	0.661	NS
***0456**	2 (6.67%)	2 (10%)	0.649	NS
***0459**	1 (3.33%)	4 (20%)	0.186	NS
***0701**	9 (30%)	0 (0%)	18.116	.007
***0717**	4 (13.33%)	3 (15%)	0.849	NS
***1101**	1 (3.33%)	1 (5%)	0.661	NS
***1107**	3 (10%)	0 (0%)	5.218	NS
***1109**	2 (6.67%)	2 (10%)	0.649	NS
***1122**	2 (6.67%)	0 (0%)	3.596	NS
***1137**	2 (6.67%)	0 (0%)	3.596	NS
***1165**	1 (3.33%)	1 (5%)	0.661	NS
***1302**	2 (6.67%)	0 (0%)	3.596	NS
***1374**	0 (0%)	3 (15%)	0.082	NS
***1401**	2 (6.67%)	0 (0%)	3.596	NS

OR: odds ratio, NS: non-significant

Table 2: HLA-DQ typing of the most frequent HLA-DR in Hydatidosis patients and healthy controls

A-DQ allele	Hydatidosis patients	Control	OR	P value
***0202**	12 (40%)	3 (15%)	3.378	NS
***0203**	2 (6.67%)	0 (0%)	3.596	NS
***0204**	6 (20%)	5 (25%)	0.748	NS
***0301**	3 (10%)	1 (5%)	1.655	NS
***0302**	4 (13.33%)	2 (10%)	1.257	NS
***0303**	2 (6.67%)	0 (0%)	3.596	NS
***0305**	1 (3.33%)	2 (10%)	0.376	NS
***0308**	1 (3.33%)	3 (15%)	0.254	NS
***0320**	3 (10%)	0 (0%)	5.218	NS
***0321**	7 (23.33%)	6 (30%)	0.712	NS
***0402**	1 (3.33%)	1 (5%)	0.661	NS
***0501**	4 (13.33%)	0 (0%)	6.962	NS
***0502**	2 (6.67%)	2 (10%)	0.649	NS
***0503**	4 (13.33%)	1 (5%)	2.208	NS
***0602**	1 (3.33%)	1 (5%)	0.661	NS
***0603**	1 (3.33%)	3 (15%)	0.254	NS
***0608**	1 (3.33%)	0 (0%)	2.085	NS

OR: odds ratio, NS: non-significant

Table 3: Serum levels of TNF-α, IL-10, IL-4, IgG4 and IgE in CE patients and healthy controls

Variables	Hydatidosis Patients		Healthy Controls		P-value
	Median	Range	Median	Range	
TNF-α (pg/ml)	17.0	0.0-89.2	14.15	0.0-39.0	0.078
IL-10 (pg/ml)	10.25	0.0-83.5	0.0	0.0-11.8	<0.001
IL-4 (pg/ml)	2.04	0.0-62.0	0.0	0.0-0.0	0.017
IgG4 (IU/ml)	4.96	2.24-7.85	2.69	7.45-1.54	0.001
IgE (IU/ml)	29.05	0.0-71.7	0.0	0-36.9	0.078

Pg: pictogram, IU: international Unit, the statistical analysis was based on Mann-Whitney test

DISCUSSION

This study aimed to investigate the association of different HLA class II alleles with the incidence of hydatidosis among Iraqi patients. Two alleles appeared to have significant effect on the susceptibility to CE. The first one, HLA-DR*0403, was a protective allele (OR= 0.158) which implies that carriers of this allele are 6.329-fold less likely to be infected with CE compared to non-carriers under the same circumstances. The other allele was HLA-DR*0701 which associated with increased susceptibility to CE (OR= 18.116). That means carriers of this allele are 18.116-fold more likely to be infected with CE compared to non-carriers under the same circumstances.

These results are not in accordance with many previous studies. In Egypt, Azab et al. [12] found HLA-DR3 to be associated with the occurrence of isolated pulmonary cysts, multiple cysts, cysts >5 cm in size, non-cured disease and with a common radiological picture of hydatid cyst. Chakhtoura et al. [13], in Lebanon, reported that HLA-B*14 and HLA-DRB1*01 to have a protection role against CE. In Yemen, Al-Ghoury et al. [14] showed that HLA-DR1, DR8, and DR52 were associated with some resistance to CE among Yemeni patients while HLA-DR16 was associated with susceptibility to the disease. Interestingly, Li et al. [8] found that HLA-DR*0701allele (which is confers susceptibility to CE in our study) was significantly higher in control group than in Chinese patients with alveolar echinococcosis, while HLA-DRB1*040 was associated with increased susceptibility to the disease. Based on these disparities, it seems that every ethnic group have its specific profile of HLA alleles regarding the association of these alleles with certain diseases.

To explain the significant association of the two alleles (HLA-DR*0403 and HLA-DR*0701) with the susceptibility to CE, some review to the basic principles of immune system is required. The T-cell receptors (TCRs) are designed to recognize antigens displayed by cell surface HLA molecules. Allelic variation of HLA gene will affect the efficiency by which HLA molecule could interact with TCR and subsequent activation of the T-cell. Particularly, the genetic alteration in loci encoding for side-chain binding pockets has the greatest effect on such interaction. That is because this pocket determines which peptide sequences can accommodating in the biding site [7]. Accordingly, it is expected a specific HLA allele to influence certain disease in a same manner (susceptibility or protection) in different ethics. However, this is not the case, and even certain alleles be protective for a disease in one population and increase the susceptibility to the same disease in other population (as in case of HLA-DR*0701 against CE in Chinese and Iraqi population). The explanation for such discrepancy is probably related to linkage disequilibrium (LD). That is, an allele does not work solely. Rather it associates with many other alleles in the chromosome 6 forming a certain haplotype that determine the status of the host.

Supporting this assumption is the cytokine profile and Ab titers. Regarding cytokines, previous studies pointed out an elevation in Th1 cytokines in patients with chronic hydatidosis [15,16] which is the case of almost all patients in this study. The significance elevation of IL-4 and IL-10 in patients compared to healthy control indicates that there is a usual activity of CD4+ cells and these cells recognized *E. granulosus* antigens which were presented by HLA antigens with eventual activation of Th1 subset. The non-significant difference in TNF-α levels may be explained by the fact that this cytokine does not elevate in chronic hydatidosis.

One of the characteristic immunological feature of hydatidosis is the induction of strong antibody response involving the four most common isotypes of antibodies [16]. Immunoglobulin G4 is usually formed following repeated or long term exposure to antigens and allergens [17]. The highly significant difference in serum levels of this Ig between patients and control in the current study reflects the chronic status of the disease and indicates there is no modulation in the humoral immune response due to different HLA-DR alleles.

Specific anti-echinococcus IgE antibodies are induced by different parasitic antigens which are usually tissue allergens released during death and degradation of parasite or parasitic secretary and excretory metabolic products [18]. In order for a B lymphocyte to switch to IgE production it needs two signals provided by a Th2 cell in the form of the cytokines interleukin (IL-4/IL-13) and ligation of the CD40 (19). The relatively high serum levels of IgE in our hydatidosis patients indicate a normal function of Th2 with adequate production of the required cytokines. Collectively, these data suggest that different HLA-DR alleles have different effect against the same disease in different populations, and this effect is accomplished though linkage disequilibrium with other alleles.

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Competing Interest

The authors did not declare any competing interests.

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