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Original Article

Differential Genomics Output and Susceptibility of Iranian Patients with Unilocular Hydatidose

*Mahdi MOSAYEBI¹, Abdolhossein DALIMI ASL², Mohammad MOAZZENI³,
Ghasem MOSAYEBI⁴

1. Dept. of Parasitology, Medical Faculty, Arak University of Medical Sciences, Arak, Iran
2. Dept. of Parasitology, Medical Sciences Faculty, University of Tarbiat Modarres, Tehran, Iran
3. Dept. of Immunology, Medical Sciences Faculty, University of Tarbiat Modarres, Tehran, Iran
4. Dept. of Immunology, Medical Faculty, Arak University of Medical Sciences, Arak, Iran

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*Correspondence Email:
M.Mosayebi@Arakmu.ac.ir

Abstract

Background: The objective of this study was to investigate HLA-DRB1* and DQB1* allelic polymorphisms in Iranian patients with hydatidose. This is the first survey dealing with the correlation between HLA-DRB1* and DQB1* alleles and cystic echinococcosis in Iranian patients.

Methods: The study was carried out on 56 patients with confirmed cystic echinococcosis and 30 apparently healthy individuals living in Arak- Markazi Province by HLA-DRB1 and DQB1 typing through PCR-SSP method. The first step was to identify the patients and blood sampling. DNA was prepared from whole blood and PCR-SSP with 31 primer mixes for per sample was used. PCR reaction mixtures were loaded in agarose gels and bands were observed under UV illumination and gel document after electrophoresis. Analysis of results was carried out with specific softwares and frequency and interpretation tables for calculation of *P*-value in χ^2 test were provided via Fisher's exact test. Significant samples were analyzed by logistic regression and odds-ratios were calculated.

Results: A statistically significant positive association was found between HLA-DQB1*03 and the resistance to cystic echinococcosis ($P < 0.02$) (odds-ratio=2.87).

Conclusion: Immunogenetic susceptibility to unilocular hydatidose varies according to the HLA antigens in Arak, Markazi Province, and DQB1*03 molecules are associated with the level of immune response to parasite antigens.

Introduction

Hydatidose is an important disease resulting from infection with larvae of the dog tape worm, *Echinococcus granulosus*, and a serious global zoonotic disease of human and various herbivores acting as intermediate hosts (1). This disease is prevalent in sheep raising countries in various parts of the world, including the Middle East (2).

In Iran, human hydatidose is one of the most important endemic parasitic diseases particularly in areas along the Alborz and Zagros Mountains and the adjacent provinces (3, 4).

Many studies have shown resistance or susceptibility to infectious diseases (5), particularly in parasitic diseases (6,7). Cystic and alveolar echinococcosis, for example, is restricted by individual host factors and immunologic responses (8-13). The variable susceptibility of humans to cystic echinococcosis has led to the categorization of patients into a susceptible group who develop the disease and a resistant group in whom it is not detected (14). In resistant individuals, a proportion of hydatid cysts die sometime after initial establishment, and 13.6% of cysts disappear or collapse spontaneously (15). This has been attributed to human leukocyte antigens (HLA) in which a group of major histocompatibility antigens are directly involved in acquired immune responses. HLA molecule is responsible for peptide presentation to T lymphocytes and it triggers the activation of different cells involved in immune response (16-18). HLA DRB1 gene is the most polymorphic of the human HLA class II genes, making it a powerful marker for individual identification (19).

The aim of the present study was to determine the frequencies of possible HLA-DRB1*-DQB1* haplotypes associated with susceptibility and protection in Iranian patients with hydatidose.

Materials and Methods

Patients

Blood samples were drawn from 86 individuals who either belonged to an endemic area

in Markazi Province or had history of hydatid surgery (N=56) or apparently healthy individuals living in that area (N=30) in 2007. The blood samples were collected in citrated tubes and stored at -70°C until examination.

DNA extraction

DNA was prepared from 100 ul of whole blood freeze alternatively by denaturation/precipitation using DNA extraction solution (DNG plus- Cinnagen Inc.).

PCR-SSP

The primers were designed for DRB1 and DQB1 'low-resolution' typing by PCR amplification with sequence-specific primers (PCR-SSP) considering all the currently recognized DRB1 and DQB1 alleles (INNO-TRAIN Co., Germany). This resolution was achieved by performing 23 PCR reactions per individual for recognition of DRB1 alleles and eight PCR reactions per individual for DQB1. The primer cocktails consisted of DRB1*, DQB1*, and HGH (human growth hormone) primers as internal control. PCR amplification was carried out in a mastercycler (Eppendorf, Germany).

PCR conditions

The samples were cycled for 2 minutes at 96 °C, 15 seconds at 96 °C, 10 times for 1 minute at 65 °C, 20 times for 15 seconds at 96 °C, 50 seconds at 61 °C, 30 seconds at 72 °C, and at 4 °C for an unlimited time. PCR reaction mixtures were loaded in 2% agarose gels with a size marker (GenRuler-Fermentas). Gels were examined under UV illumination and documented by photography (Fig. 1, A&B).

Statistics

Allele frequencies were calculated by χ^2 tests and Fisher's exact test (20-24) using SPSS and INNO TRAIN softwares contributed by INNO-TRAIN Co. for obtaining allele combination.

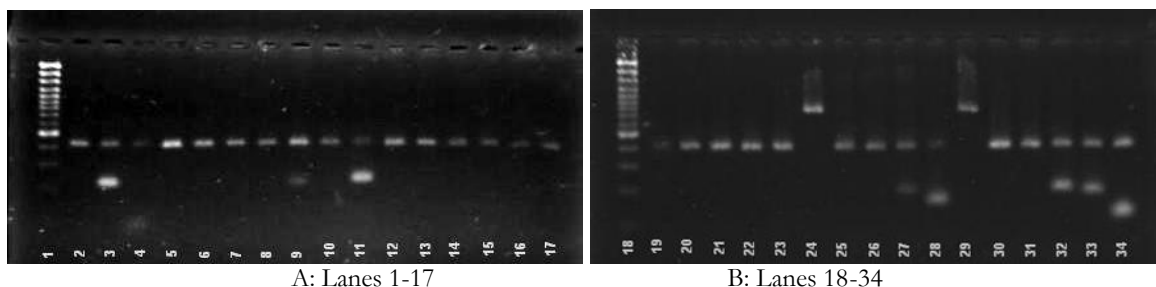


Fig. 1(A & B): PCR-SSP analysis of HLA-DRB1 and HLA-DQB1 alleles/The DNA was amplified using 23 sequence specific primers for DRB1 (lanes 2-13 and 19-30), DRB3 (lane 11), DRB4 (lane 12), DRB5 (lane 13), and 8 sequence specific primers for HLA-DQB1 (lanes 14-17 and 31-34). The allele specific products are visible as intensive bands below the internal positive control (PCR product of the human growth hormone gene) in lanes 3, 9, 11, 27, 28, 32, 33, and 34 (DRB1* 03, 11, 1403, DRB3, DRB1*08, 11, 1202, 13, 1415, DRB1*03, 0820, 13, 1402, 03, 05, 06, and DQB1* 05, 06, 02). Marker 50-1000bp (lanes 1 and 18)

Results

The potential immunogenetic predisposition for susceptibility and resistance to unilocular echinococcosis was investigated by PCR amplification with Sequence Specific Primers, low-resolution. The samples collected were obtained from 56 patients with history of hydatid surgery and 30 healthy individuals and low resolution was achieved by performing 23 PCR reactions per individual for recognition of DRB1* alleles and eight PCR reactions per individual for DQB1*. Overall, 17 different DRB1 alleles were identified, of which DRB1*11 was the most frequent (39.3 vs. 26.7%) and *0308 was less frequent in hydatid patients while DRB1*04 was the most frequent alleles in healthy individuals.

The frequencies of the HLA-DRB1 alleles in patients with unilocular hydatidose were not significantly different from those observed in controls. Similarly, the frequencies of HLA DRB*5, DRB*4, and DRB*3 alleles in hydatid patients did not differ significantly from those observed in healthy individuals.

Among the 6 identified DQB1 alleles, two were predominant: DQB1*02 in patients and *03 in healthy individuals.

Table 1 shows DQB1 alleles gene frequency in Iranian patients with unilocular hydatidose and healthy controls. Some antigen frequencies are clearly different between the two groups,

which highlights their differences. In the patients' samples, antigens, such as DQB1*02, *0203, *0304, *04, and *05 had increased frequencies. On the other hand, DQB1*03 and *06 have lower frequencies.

A significant higher frequency of DQB1*03, was observed in healthy controls compared with patients ($P < 0.02$, OR=2.87).

Discussion

This study identified a possible association between HLA class II alleles in human in an area where hydatidose is endemic (25). In particular, this study identified a significant correlation between HLA type and hydatid cysts for class II alleles HLA- DRB1 and DQB1. HLA class II alleles in the DQB1 and DRB1 genes were investigated in Iranian patients with unilocular hydatidose. The hypothesis of an immunogenetic investigation of the infection risk of hydatid cysts was proposed in many previous reports. In this study, the determination of the possible correlation between HLA-antigens specificity and *Echinococcus granulosus* cysts in Iranian patients showed that DQB1*03 carriers are significantly resistant compared with individuals not having DQB1*03. Despite similar life patterns, the number of cystic echinococcosis cases occurring in DQB1*03 carriers was different (Table 1).

Table 1: Frequency of DRB1 and DQB1 alleles in Iranian patients with unilocular hydatidose and healthy controls

Alleles	Patients%	Healthy%	Odds-ratio	P- value
DRB1* 01	10.7	13.3	-	N.S
DRB1* 03	17.9	13.3	-	N.S
DRB1* 0308	1.8	0	-	N.S
DRB1* 04	21.4	33.3	-	N.S
DRB1* 0415	3.6	10	-	N.S
DRB1* 07	25	13.3	-	N.S
DRB1* 08	0	3.3	-	N.S
DRB1* 09	8.1	0	-	N.S
DRB1* 10	1.8	6.7	-	N.S
DRB1* 11	39.3	26.7	-	N.S
DRB1* 12	3.6	3.3	-	N.S
DRB1* 13	23.2	30	-	N.S
DRB1* 1317	1.8	0	-	N.S
DRB1* 14	10.7	10	-	N.S
DRB1* 1410	1.8	0	-	N.S
DRB1* 15	25	20	-	N.S
DRB1* 16	3.6	6.7	-	N.S
DQB1* 02	39.3	26.7	-	N.S
DQB1* 0203	7.1	0	-	N.S
DQB1* 03	41.1	66.7	2.87	P< 0. 02
DQB1* 0304	14.3	3.3	-	N.S
DQB1* 04	3.6	3.3	-	N.S
DQB1* 05	35.7	33.3	-	N.S
DQB1* 06	30.4	36.7	-	N.S
DRB3	4.71	3.83	-	N.S
DRB4	4.46	7.46	-	N.S
DRB5	1.32	3.33	-	N.S

Azab et al. reported that HLA-DR11 is positively associated with the occurrence of cysts <5 cm in patients with unilocular echinococcosis and carriers of DR-3 and DR-11 are at high risk for unilocular echinococcosis (26).

Studies on the correlation between cystic echinococcosis and HLA are mostly on class-I HLA. Class-1 HLA, B5, and B18 antigen carriers are the high-risk group for cystic echinococcosis while HLA –B14 and B27 antigens evidently lead to a certain resistance (9).

Several studies have shown an increased frequency of HLA alleles among alveolar echinococcosis patients as compared with healthy individuals in different geographical areas. It is known that HLA-DRB1*11 is associated with a reduced risk for alveolar echinococcosis de-

velopment (27). By contrast, the same group reported that HLA-DQB1*02 was more frequent in patients with progressive diseases when compared with patients with regressive diseases. Susceptibility to alveolar echinococcosis is strongly associated with the HLA-DRB1*040x allele but HLA-DRB1*0701 is associated with resistant to alveolar echinococcosis (28,29). Gottstein et al. found a slight tendency for susceptibility markers to alveolar echinococcosis, respective to the HLA-DRB1*0901 and HLA-DRB1*1601, 02 genes in Alaska (Yopiks/Inupiat population) (30). Particular DR and DQ antigens are associated with susceptibility to several alveolar echinococcosis (31).

The present study demonstrates that individuals not having DQB1*03 are at high risk for unilocular hydatidose. It also confirms the high incidence of DRB1*11 in patients compared to controls. Since DRB1*11 and other alleles that were more frequent in patients are not in linkage disequilibrium, both of these alleles are possibly synergistically involved in disease development in unilocular hydatidose. This finding shows that immunogenetic susceptibility to unilocular hydatidose varies according to the HLA antigens in Arak, Markazi Province, and DQB1*03 molecules are associated with the level of immune response to parasite antigens.

Conclusion

Susceptibility and resistance to unilocular hydatidose, appear to reflect a complex interaction of parasite and host immunological and genetic factors. This suggestive requires further investigation, such as genome-wide association studies.

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