

Association between HLA-DQB1 alleles and HAM/TSP patients in Khorasan Province

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ABSTRACT

Objective(s): HTLV-1 is the first human retrovirus with limited endemic regions in the world. The epidemiological studies have shown that the genetic background and immune response to the virus have a significant role in HTLV-1-associated diseases. Among the genes are involved in HTLV-1 infection, the role of human leukocytes antigen (HLA) have been studied in different population. In the present study we examined the association between HLA-DQB1 alleles and HTLV-1 infection in HAM/TSP patients, HTLV-1 carriers and healthy controls in north east of Iran, Mashhad.

Materials and Methods: The blood samples of 16 patients with HAM/TSP, 20 HTLV-1 carriers, and 30 healthy individuals were taken and DNA was extracted by salting out method. HLA-DQB1 typing was performed using PCR-SSP method and the frequency of HLA-DQB1 alleles were compared by Fischer Exact Test.

Results: There was a significant difference between HAM/TSP patients and healthy controls in the frequency of HLA-DQB1*07 ($P=0.004$, $RR=7$). Furthermore, we found that possession of HLA-DQB1*02 or HLA-DQB1*05 increased the risk of disease 1.5 times.

Conclusion: The data presented here suggest that both HLA-DQB1*07 and HLA-DQB1*06 are associated with disease development.

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Introduction

Host genetic factors are major determinants of susceptibility to infectious disease in humans (1). HTLV-I is a persistent virus, infecting 10–20 million people worldwide. Most infected people remain healthy, but 1–2% develops a progressive paralytic myelopathy (HTLV-1-associated myelopathy; HAM/TSP) and a further 2–3% develops an aggressive T cell leukemia/lymphoma. The reasons for the different outcomes of infection are unknown. HAM/TSP is a chronic debilitating inflammatory disease of the central nervous system, characterized by axonal damage and demyelination, most pronounced in the midthoracic spinal cord. Furthermore some of the self-immune diseases such as uveitis and polymyositis are found to be resulted from this virus (2-5). The endemic regions affected with this virus include the south-west of Japan, Caribbean Islands, and some regions of the USA (6). The other parts with less prevalence of the virus are

Taiwan and some regions of Africa and Israel. (7-9). Mashhad in the north of Iran has been recognized as a new endemic region of the virus (10, 11). HAM/TSP is similar to multiple sclerosis in some aspects. The onset is with no evident symptom. The symptoms include weakness or dryness in one or both feet, backache and no control on urine. The sensory changes are usually weak but peripheral neuropathy may happen. One-third of the patients become paralyzed after 10 years and one half of them are not able to walk (12). On the contrary to ALT in which the number of the male patients is a little more, HAM affects the female disproportionately. High proviral load and being female are two factors of high risk of HAM/TSP and the relative risk of HAM/TSP increases when the proviral load becomes clearly more than one copy in any PBMCS (13, 14). Both the class I MHC proteins (HLA-A, -B, and -C), which present viral peptides for recognition by virus-specific CTL, and class II MHC proteins (HLA-DR and

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-DQ), which present peptides to CD4 T cells, are likely to be important in the immune response to HTLV-I infection (15, 16 and 17). The results of several previous studies have suggested associations between various HLA class I or class II alleles and susceptibility to T cell leukemia/lymphoma or HAM/TSP (18-22). However, small sample size, mixed ethnicity, lack of adequate controls, or lack of staging in these studies precluded a definite conclusion. If the relationship between HTLV-1 infection and Class II is considered as the factor of susceptibility to disease, it may help in understanding pathogenesis of HAM/TSP. The supplied peptides of axon antigens bonded to the products of MHC Class II activate CD4⁺ on the surface of T lymphocytes and the activated CD4⁺ can cross the blood-cerebral obstacle and identify the cell affected with virus. The activated CD4⁺ causes apoptosis through producing cytokines and damage to the cell affected with virus and the peripheral cells may play a role in CNS (23, 24).

Both MHC class I and class II molecules are important in determining the recognition of HTLV-I peptides and for the generation of an effective immune response. The predominant cytotoxic T lymphocyte response (CTL) against Tax protein, which is necessary for viral expression, can be restricted by a wide variety of MHC class I molecules in HTLV-I infected subjects. It has been suggested that no single MHC class I allele is associated with HAM/TSP (15, 25). In Japanese HTLV-I-infected subjects has been demonstrated that expression of HLA-A*02 and HLA Cw*08 alleles reduced the proviral load of HTLV-1 and consequently the risk of HAM/TSP, while HLA-B*5401 allele increased the proviral load and the risk of HAM/TSP in HTLV-I carriers. Furthermore, HLA-DRB1*0101 increased the risk of HAM/TSP in carriers in the absence of HLA-A*02 (18, 21).

The aim of this study was to investigate the frequency of HLA-DQB1 alleles in HTLV-I-infected individuals and healthy controls resident in Mashhad.

Materials and Methods

Study population

The present case-control study was conducted in Mashhad-Iran. The samples were selected by convenience sampling method. The studied subjects were 66, including 16 HAM/TS patients, 20 asymptomatic carriers and 30 healthy controls. 23 % of controls were female. The mean age of controls was 57±12.52. Sixteen of 20 HAM/TSP patients were female (60%) and 72% of HTLV-I carriers were female. The mean age of patients was 44.14±14.62 and the mean age of HTLV-I carriers was 51 ±10.22.

All patients fulfilled established criteria for HAM/TSP when they examined by neurologist and HTLV-I carriers had symptoms of HTLV-I-associated diseases. All of the HAM/TSP patients, HTLV-I

carriers and healthy controls had the same ethnic background. The study protocol was approved by the ethics committee of Mashhad University of Medical Sciences. Informed consent was obtained from all participants. 10 cc blood was taken from all the subjects for DNA extraction and serum preparation.

Serological assay

Serum samples were screened for HTLV-I antibody by enzyme linked immunosorbent assay (ELISA) according to the manufactures' instruction (Diapro, Italy) and reactive samples were confirmed by western blotting (Genelab Diagnostic, Singapore).

HLA-DQB1 typing

DNA was extracted by non-enzyme salting out method using Biogen kit (Mashhad-Iran) according to manufacturer's instruction. Polymerase chain reaction with sequence-specific primers (PCR-SSP) method was carried out to determine HLA-DQB1 alleles according to the previously published method (26).

Briefly, seven alleles of HLADQB1 were detected using specific primers in eight PCR reactions. This procedure currently detected DQB1 alleles including 0501-0504, 0601-0609, 0201, 0301-0305, and 0401-0402 which corresponding to the serological specificities DQ4, DQ5 and DQ6 alleles, whereas the DQ2, DQ7, DQ8 and DQ9 specificities were amplified by two primer mixes. We used at least 2 primers (Primm-Italy) for each reaction (Table 1, 2).

Results

The frequency of HLA-DQB1 alleles was calculated in HAM/TSP patients, asymptomatic carriers and healthy control groups. The frequency of HLA-DQB1*07 was significantly higher in patients with HAM/TSP (75%) compared with healthy controls (30%) ($P=0.004$, OR=8.52) (Table 1).

The HLA-DQB1*02 and DQB1*05 was positive in 7 HAM/TSP patients, while in healthy control group 24 subjects were positive. Significant difference was observed between two groups ($P=0.125$ and RR=6.24). Table 2 shows the distribution of these alleles in study population.

The frequency of both HLA-DQB1*07 and HLA-DQB1*06 alleles in HAM/TSP patients was more than the ones if they infected with more than the ones free of it if they are infected with HTLV-1 (P -value=0.0009 and RR=10.95) (Table 3).

Discussion

In the present study we examined the frequency of HLA-DQB1 allele in HAM/TSP patients, asymptomatic carriers and healthy controls. Our results indicated that the individuals with DQB1*07 allele are susceptible to be affected with this disease 7 times more than the ones lacking this allele,

Table 1. The frequency of DQB1*07 allele in healthy individuals and patients with HAM/TSP

	Negative for HLA DQB1*07		Positive HLA DQB1*07		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Healthy	21*	70%	9	30%	30	100%
Patient	4*	25%	12	75%	16	100%
Total	25	54.3%	21	45.7%	46	100%

*Significant difference was observed between patients and control ($P=0.004$)

Table 2. The frequency of DQB1*02 and DQB1*05 alleles in healthy controls and patients with HAM/TSP

	Negative for DQB1*02 and DQB1*05		Positive for DQB1*02 and DQB1*05		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Healthy	6*	20%	24	80%	30	100%
Patient	9*	56.3%	7	43.8%	16	100%
Total	15	32.6%	31	67.4%	46	100%

Significant difference was observed between patients and controls ($P=0.112$, OR=6.24)

Table 3. The Frequency distribution of the DQB1*06 and DQB1*07 alleles in healthy individuals and patients with HAM/TSP

	Negative		Positive		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Healthy	43*	97.7%	1	2.3%	44	100%
Patient	11*	68.8%	5	31.2%	16	100%
Total	54	90%	6	10%	60	100%

Significant difference was observed between patients and controls ($P=0.0009$, OR=10.95)

suggesting HLA genes play a significant role in the susceptibility to HAM/TSP disease. Within African population, two antigens determined DR15 and DQ1, occurred at significantly increased frequency among HTLV-I carriers compared with seronegative control subjects (42% versus 22% for DR15 [odds ratio {OR} = 2.7; 95% confidence interval {CI} = 1.0- 7.2] and 78% versus 53% for DQ1 [OR = 3.1; 95% CI = 1.2- 8.5]). Asymptomatic carriers were shown to have an HLA class II allele distribution similar to that of patients with ATL, and the frequencies of the alleles DRB1*1501, DRB1*1101, and DQB1*0602 were significantly greater in patients with ATL and asymptomatic carriers than in patients with HAM/TSP. In addition, haplotypes DRB1*1101-DQB1*0301 and DRB1*1501-DQB1*0602 were significantly increased among patients with ATL compared with patients with HAM/TSP (28).

We also showed that the individuals with HLA-DQB1-2 and HLA-DQB-5 are 1.5 times more susceptible to develop HAM/TSP compared with the subjects who lacked these alleles, therefore, these two alleles may be considered as the protective factors.

Rafatpanah *et al* (19, 20) demonstrated that the frequency of HLA-DRB1*01 was significantly increased in HAM/TSP patients compared with carriers (p 0.028; OR=9.4). The frequency of HLA-Cw*08 was also significantly increased in HAM/TSP patients compared with controls ($P=0.03$; OR=13.5).

It has been showed that there is an association between HLA class I, HLA-A30 allele and HTLV-I infection (15, 20). Kitze *et al* showed that the HAM/TSP patients with particular HLA haplotypes (A24Cw7B7DR1DQ5, A2Cw7B7DR1DQ5, A24Cw-B52DR15DQ6, A11Cw1B54DR4DQ4, and

A24Cw1B54DR4DQ4) express more frequently intrathecal synthesis of antibodies against HTLV-1 synthetic peptides, especially against HTLV-1 env gp21 synthetic peptides (21). An association of HLA-DRB1*0101 with disease susceptibility also was identified, which doubled the odds of HAM/TSP in the absence of the protective effect of HLA-A*02 (22). In the present study, the frequency of HLA-DQB1*07 in HAM/TSP patients was high. It seems that there is a correlation between HLA-DQB1*07 and disease susceptibility. The genotype of HLA-DQB1*06 and 07 was more frequent in patients with HAM/TSP compared with control group (26). This results is different from the results obtained Kitze *et al* (25) who reported that there is a higher risk to develop HAM/TSP among the individuals with HLA-DQ-0105 haplotype. This discrepancy may be associated with the difference in the genetic background of studied population (27).

Conclusion

Although we find an association between HLA-DQB1 alleles and HAM/TSP, however the study should be repeated with more sample size. Furthermore, the study of other HLA class I and class II alleles should be taken into account to understand a more comprehensive role of HLA alleles in disease susceptibility.

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