Original Research Article

Association of HLA-DRB1*1501-DQA1*0102-DQB1*0602 haplotype in patients with multiple sclerosis in Khuzestan province

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ABSTRACT

The aim of the present study was to reveal a part of genetic profile of multiple sclerosis (MS) in Khuzestan province. The contribution of HLA-DR2 haplotype (HLA-DRB1*1501-DQA1*0102-DQB1*0602) to MS risk is confirmed. This study included 187 MS patients and 192 healthy individuals. HLA alleles were genotyped, using the polymerase chain reaction amplification with sequence specific primers (PCR-SSP) method. The statistical analyses were performed by SPSS software. The results of this study demonstrated that distribution of HLA-DQA1*0102$^-$-DQB1*0602$^+$ haplotype was statistically different between patients and controls. HLA-DRB1*1501$^+$-DQA1*0102$^-$ was more frequent among women although DQA1*0102$^+$-DQB1*0602$^+$ showed negative association of female patients compare with female controls. Significant correlation was observed among DRB1*1501$^+$-DQA1*0102$^-$ with Fars group. HLA-DRB1*1501$^+$-DQA1*0102$^+$ haplotype was positively associated with EDSS steps 5 to 10. These results suggest that HLA-DRB1*1501$^+$-DQA1*0102$^-$ haplotype may involve in MS susceptibility among women.

***Keywords*:** Multiple sclerosis; HLA-DR2; PCR-SSP; HLA-DRB1*1501; HLA-DQA1*0102; HLA-DQB1*0602

INTRODUCTION

Multiple sclerosis (MS) is a presumed heterogeneous neurodegenerative disease of the central nervous system (CNS), commonly affecting young people. It is one of the most common neurological disorders. The pathogenesis and etiology of this disease is still unknown though susceptibility to the disease depends on a complex interaction between environment and genetic factors [1]. The major histocompatibility complex (MHC) class II family encodes cell surface glycoproteins that present peptides to cytotoxic T cells in which activation of $CD4^+$ autoreactive T cell and their activation into Th1-phenotype are a crucial event in the initial steps and also in long-term evolution of disease. In human, this gene family is located in the short arm of chromosome 6p21.3. Even though there are many genes that appear to be associated with MS and have been confirmed in different studies, whereas the impact of other genes is still under discussion [2]. The importance of human leukocyte antigen (HLA) complex class II genes to develop MS has widely been investigated but the findings are controversial and might be influenced by the diversity in the ethnicity and geographic location of the studied populations. The association between HLA complex genes and the course of MS remain unclear [3]. The studies carried out in the regions with a high prevalence of the HLA-DRB1*15 allele showed that this allele was related to the earlier of the disease, female sex, and worse health outcomes [4-7]. Genetic susceptibility to MS is associated with HLA-DR2 haplotype (HLA-DRB1*1501-DQB1*0102-DQB1*0602) in many population studies [8].

Considering that the frequency of HLA alleles varies significantly among different ethnic groups; the HLA-DR2 haplotype studies could be helpful in the selection of patients for different therapeutic options. It is also important to state that the HLA allelic phenotype among different MS populations is a mandatory requirement for more effective therapy [8, 9].

This study was done to assess haplotype diversity of HLA-DR2 genes in two healthy and MS patient populations, that were included Arabs and non-Arabs. The Expanded disability status scale (EDSS) is a method of quantifying disability in multiple sclerosis and monitoring changes in the level of disability over time. The scale was developed by a neurologist called John Kurtzke in 1983 [10]. Using blood samples from both groups of individuals from Khuzestan province, genetic variation was

examined in three loci including HLA-DRB1*1501, DQB1*0102 and DQB1*0602.

Considering that the heterogeneity carriage of HLA-DR2 in MS patients can affect individual responses to drugs; so identification of genetic susceptibility for MS using HLA ethnicity association can be useful.

Geographic region and ethnicity have a certain impact on the MS immunogenetic features. Considering the ethnicity diversion in Khuzestan, and also there are a few data in this regard in Arabic countries, the recent study can be of great importance. The aim of this study was to analyze the association of the HLA haplotypes with MS, MS type and EDSS. Association with MS was also performed within Arab and non-Arab groups and both genders.

MATERIALS AND METHODS

Altogether, peripheral blood was collected from 187 MS patients who were referred to Khuzestan MS Society. They were 9 to 58 year old patients and written informed consent was obtained from each patient before enrolment to the study. The diagnosis of MS was established according to the widely accepted and revised McDonald criteria [11]. A questionnaire was supplied about age, gender, ethnicity, positive family history, clinical features as well as MS subtype. While clinical parameters estimations and EDSS steps were carried out by experts in the field of neurology. EDSS steps 1.0 to 4.5 refer to people with MS who are able to walk without any aid. EDSS steps 5.0 to 9.5 are defined by the impairment to walking. So in this study, patients were divided into two groups including scale of 0 (no disability) to 4.5 (more severe disability) and 5 to 10 (death due to MS).

The normal population studied included 192 randomly selected, unrelated healthy individuals without any autoimmune disease and familial history from Khuzestan. They were 15 to 50 year old healthy controls. A questionnaire was supplied about parameters, such as ethnicity, gender, positive family history of autoimmune disease and age. The control participants were informed about our study and completed a consent form, as well. Control group was selected by group matching to compare with the patient series on characteristic, such as gender and race; also they were originally from the same geographical region.

Peripheral blood samples were collected from controls and patients in EDTA tubes. Extraction of DNA was conducted by standard salting out protocol. Nanodrop and

Electrophoresis methods were applied for assessing the quality and quantity of extracted DNA; therefore several random genome DNAs were selected for this aim.

HLA typing was analyzed by polymerase chain reaction amplification with sequence-specific primers PCR (PCR-SSP) technology and was done again, if discordant results were achieved. The primer sequences used to amplify the DRBI*1501, DQA1*0102, DQB1*0602 genes and were designed using the IMGT/HLA database (http://www.ebi.ac.uk/) and aligned in NCBI/blast. The primers used for PCR-SSP HLA-DQB1*0602, -DRBI*1501, -DQA1*0102 and MOG genes HLA typing were published else-where [12-14].

Since each allele is determined by the presence of specific PCR product; so the size of PCR product may be useful in the interpretation of the findings.

Each reaction of PCR-SSP is deemed to have worked if the internal control amplification is observed. Internal control for primers was Myelin oligodendrocyte glycoprotein (*MOG*) gene which means in ideal PCR condition, the band be appeared. The sequences of *MOG* primers were published [12]. Finally, the findings were validated by sequencing some samples randomly.

The amplified products were determined by means of agarose gel electrophoresis. Laboratory analysis was carried out in the Genetic laboratory of Shahid Chamran university of Ahwaz.

The allele frequency of the HLA-DRB1*1501, DQA1*0102, DQB1*0602 were already published [12-14]. The frequency of DQA1*0102-DQB1*0602, DRB1*1501-DQA1*0102, DRB1*1501-DQB1*0602 and also HLA-DRB1*1501-DQA1*0102-DQB1*0602 haplotypes were determined as percentage. It should be noted that some typing were missing. So, a few genomes were not typed for all three DRB1*1501, DQB1*0602 and DQA1*0102 alleles. Fisher's exact and Chi-square tests were used for comparing frequency of the mentioned haplotypes between cases and controls. SPSS statistical software (version 16, USA) was used for data analyzing. An odds ratio (OR) with 95 % confidence intervals (CI) and a p-value < 0.05 were considered.

RESULTS

The study was investigated on a total of 379 subjects, containing 187 unrelated MS patients and 192 healthy controls from Khuzestan province and frequency of the most frequent haplotypes regards to HLA-

DRB1*1501, DQA1*0102, DQB1*0602, were evaluated among them.

Summarized characteristics of the mentioned MS patients have been published, before [12-14].

Association and frequency of DQA1*0102-DQB1*0602, DRB1*1501-DQA1*0102, DRB1*1501-DQB1*0602 and also DQA1*0102-DRB1*1501-DQB1*0602 haplotypes were investigated in MS patients and compared with healthy controls (Table 1).

Table 1. Association of HLA haplotypes with MS in Khuzestan province

HLA haplotypes	Patients		Controls		*P* value	OR	95% CI
	positive haplo	Total	positive hapl	Total			
DRB1*1501^{+}- DQB1*0602^{+}	50 (27.3)	183	34 (28.6)	119	0.813	0.940	0.562-1.571
DRB1*1501^{+}- DQA1*0102^{+}	43 (23)	187	36 (30.5)	118	0.145	0.680	0.405-1.143
DQA1*0102^{+}- DQB1*0602^{+}	75 (40.3)	186	88 (45.8)	192	0.279	0.799	0.531-1.201
DRB1*1501^{+}- DQB1*0102^{-}	35(19)	186	13 (11)	119	0.065	1.890	0.954-3.743
DQA1*0102^{-}- DQB1*0602^{+}	32 (20.5)	156	23 (12)	188	0.037*	1.851	1.032-3.321
DRB1*1501^{+}- DQB1*0602^{+}- DQA1*0102^{-}	16 (9)	185	7 (6)	116	0.406	1.474	0.587-3.700
DQA1*0102^{+}-DRB1*1501^{+}- DQB1*0602^{+}	33 (17.7)	186	23 (20.2)	114	0.600	0.853	0.472-1.543

* Significant P values; Statistical significance was at p < 0.05. Values are expressed as No. (%)

Table 2. Analysis of association between HLA haplotypes with Fars ethnic of Khuzestan province

HLA haplotypes	Patients		Controls		*P* value	OR	95% CI
	positive haplo	Total	positive hapl	Total			
DRB1*1501^{+}- DQB1*0602^{+}	25 (21.8)	115	12 (27.9)	43	0.415	0.718	0.322-1.597
DRB1*1501^{+}- DQA1*0102^{+}	26 (22.4)	116	14 (30.4)	46	0.286	0.660	0.307-1.41903
DQA1*0102^{+}- DQB1*0602^{+}	49 (42.6)	115	39 (51.3)	76	0.237	0.704	0.394-1.261
DRB1*1501^{+}- DQB1*0102^{-}	22 (19.5)	113	3 (6.5)	46	0.042*	3.465	0.983-12.212
DQA1*0102^{-}- DQB1*0602^{+}	23 (20)	115	9 (12)	74	0.161	1.806	0.785-4.155
DRB1*1501^{+}- DQB1*0602^{+}- DQA1*0102^{-}	11 (10)	107	3 (8)	39	0.638	1.375	0.363-5.214
DQA1*0102^{+} -DRB1*1501^{+}- DQB1*0602^{+}	20 (18)	113	8 (19.5)	41	0.797	0.887	0.357-2.206

* Significant P values; Statistical significance was at p < 0.05. Values are expressed as No. (%)

Table 3. Analysis of association between HLA haplotypes with Arab ethnic of Khuzestan province

HLA haplotypes	Patients		Controls		*P* value	OR	95% CI
	positive haplo	Total	positive hapl	Total			
DRB1*1501^{+}-DQB1*0602^{+}	15 (22.05)	68	18 (33.33)	54	0.164	0.566	0.253-1.267
DRB1*1501^{+}-DQA1*0102^{+}	16 (22.2)	72	16 (32)	50	0.227	0.607	0.269-1.370
DQA1*0102^{+}-DQB1*0602^{+}	27 (38)	71	36 (44.5)	81	0.423	0.767	0.401-1.468
DRB1*1501^{+}-DQB1*0102^{-}	13 (18)	72	8 (16)	50	0.767	1.157	0.440-3.038
DQA1*0102^{-}-DQB1*0602^{+}	12 (17)	71	10 (12)	83	0.391	1.485	0.600-3.676
DRB1*1501^{+}-DQB1*0602^{+}-DQA1*0102^{-}	5 (7)	78	5 (10)	51	0.481	0.630	0.173-2.297
DQA1*0102^{+} -DRB1*1501^{+}-DQB1*0602^{+}	14 (19.5)	72	12 (24)	50	0.546	0.764	0.319-1.830

* Significant P values; Statistical significance was at p < 0.05. Values are expressed as No. (%)

Table 4. Association of HLA haplotypes with EDSS in MS patients of Khuzestan province

HLA haplotypes	EDSS (1-4.5)		EDSS (5-10)		*P* value	OR	95% CI
	positive haplo	Total	positive hapl	Total			
DRB1*1501^{+}-DQB1*0602^{+}	45 (27.6)	163	2 (25)	8	0.872	1.144	0.223-5.879
DRB1*1501^{+}-DQA1*0102^{+}	32 (19.4)	165	4 (50)	8	0.037*	0.241	0.057-1.014
DQA1*0102^{+}-DQB1*0602^{+}	66 (41.2)	160	2 (25)	8	0.361	2.106	0.412-10.76
DRB1*1501^{+}-DQB1*0102^{-}	31 (20)	156	2 (25)	8	0.724	0.744	0.143-3.866
DQA1*0102^{-}-DQB1*0602^{+}	31 (19)	165	0 (0)	8	0.176	1.060	1.018-1.103
DRB1*1501^{+}-DQB1*0602^{+}-DQA1*0102^{-}	16 (9)	173	0 (0)	8	0.368	1.051	1.015-1.088
DQA1*0102^{+}-DRB1*1501^{+}-DQB1*0602^{+}	29 (18)	158	2 (25)	8	0.638	0.674	0.129-3.512

* Significant P values; Statistical significance was at p < 0.05. Values are expressed as No. (%)

The results showed that the frequency of the HLA-DQA1*0102^{-}-DQB1*0602^{+} haplotype (carrying DQB1*0602 allele but not HLA-DQA1*0102) increased significantly between the patients compared with the control group (20.5 % vs. 12 %, *P* = 0.037, OR = 1.851 [95 % CI = 1.032-3.321]) and the haplotype may have effect on MS susceptibility although more studies with bigger sample sizes, on other populations,

need to be done to confirm our results. Also, the findings demonstrated that the frequency of other haplotypes was almost similar among MS patients and controls and no correlation was found among them and multiple sclerosis statistically, as it was shown in Table 1.

Furthermore, significant correlation was observed among DRB1*1501^{+}-DQA1*0102^{-} with Fars ethnic (19.5 % vs. 6.5 %, *P* = 0.042, OR = 3.465 [95 % CI =0.98-12.2]) though logistic regression analysis failed to report any association between other mentioned variants and both ethnics (Table 2 and 3). Moreover, HLA-DRB1*1501^{+}-DQA1*0102^{+} haplotype was positively associated with EDSS steps 5 to 10 (50 % vs. 19.4 %, *P* = 0.037, OR =0.241 [95 % CI =0.057-1.01]) although no more association was found between the rest of the haplotypes and level of disability, as it was shown in Table 4. We also analyzed the association of DQA1*0102-DQB1*0602, DRB1*1501-DQA1*0102, DRB1*1501-DQB1*0602 and HLA-DRB1*1501-DQA1*0102-DQB1*0602 haplotypes with multiple sclerosis in females and males, separately.

DISCUSSION

Multiple Sclerosis is a complex neurodegenerative autoimmune, classified as sporadic and familial types. An association between MS and HLA II was first noted in 1973 [15]. This study evaluate the frequency and association of DQA1*0102-DQB1*0602, DRB1*1501-DQA1*0102, DRB1*1501-DQB1*0602 and also HLA-DRB1*1501-DQA1*0102-DQB1*0602 haplotypes with susceptibility to MS in Khuzestan province. Positive association between HLA-DQA1*0102^{-}-DQB1*0602^{+} and MS was found; however, since recombination, genetic drift, latitude, epistasis, founder effect and so on might effect on the results; so to confirm the results, more studies with bigger sample sizes on different ethnic groups need to be conducted.

Thereafter, numerous studies have been conducted on the association of HLA antigens and MS; in a number of studies the role of HLA class II, especially DQA1*0102, DQB1*0602, DRB1*1501 have been hypothesized to be the primary HLA genetic susceptibility factor for MS [7]. In a study on a large number of Swedish MS patients showed that HLA-DR2 haplotype was overrepresented among patients [17].

In some immunogenetic studies which focused on high-risk populations, such as Northern European descendants, revealed

that the HLADRB1*1501^{+}-DQA1*0102^{+}-DQB1*0602^{+} haplotype is associated with MS. In most surveys that have been done in many populations the frequency of DQA1*0102^{+}-DQB1*0602^{+}-DRB1*1501^{+} haplotype in patients have been more than that of the controls [18, 19]. A study on Jewish populations showed that HLA-DR2-related haplotype was found to be associated with Ashkenazi Jewish MS patients [20]. A positive association of HLA-DR2 haplotype with MS was shown in Caucasian Turkish population [21]. Contrary to this, we found no correlation of HLA-DR2 with MS.

Findings of a study on the role of HLA class II (DRB1, DQA1 and DQB1) alleles and haplotypes in 43 unrelated Iranian chronic progressive MS showed a positive association with the DRB1*1503^{+}-DQA1*0102^{+}-DQB1*0602^{+} haplotype [22].

It should be noted, we observed that MS susceptibility in patients is associated with DQA1*0102^{-}-DQB1*0602^{+} allelic phenotype. Contrary to this, we found no association with Arab and Persian MS patients which definitely did not depend on an ethnic association. Otherwise, significant association between DRB1*1501^{+}-DQA1*0102^{-} and Fars ethnic was reported. Moreover, no similar study regards to association of the haplotypes with ethnic or sex, was found.

In another study that was carried out at different latitude in Iran, no positive correlation was observed between DRB1*1501^{+}-DQA1*0102^{+}-DQB1*0602^{+} haplotype with sex, initial symptoms, type of disease, EDSS, as well as age at onset, and positive family history of MS [23]. Our findings with regard to sex, type of disease and EDSS are similar to the mentioned study; so, diversity in the ethnicity and geographic location might not make any difference in the results. Furthermore, a study have shown that DQB1*0602^{+}-DRB1*1501^{+} may not influence on MS disease severity. However, this is controversial in different studies. Some studies suggested that this haplotype is associated with severity of MS [24]. In the recent study no association was found between DQB1*0602^{+}-DRB1*1501^{+} and severity of disease.

Study on this population has already shown positive association of HLA-DRB1*1501 with MS [12], negative association of DQA1*0102 with the disease [13], and also in this study, it was revealed that distribution of DRB1*1501^{+}-DQB1*0102^{-}, DRB1*1501^{+}-DQB1*0602^{+}-DQA1*0102^{-}

were not statistically different among cases and controls.

CONCLUSION

The aim of the present study was to reveal a part of genetic profile of MS in Khuzestan province. MS is a multifactorial disorder and several genes are involved in the pathogenesis of the disease. Therefore, it is possible that some other haplotypes in other genes associated with susceptibility to MS in this population. The differences observed between our results and other populations could be due to discrepancy in the genetic profile of the populations, latitude, sample size of studies and environmental factors effect like lifestyles. Small sample size may have an effect on the statistical power. Therefore, our data need confirmation in larger sample size assays. On the other hand, population of Khuzestan province is heterogeneous and consists of various ethnics. Additional polymorphisms exist in the *HLA* gene that might contribute to the susceptibility or activity of MS in this population. Thus, it is suggested that need for a larg number of patients and control subjects. In conclusion, this study gives further support to the importance of replication studies as susceptible loci that might be differ in various ethnic groups. For achieving more documented data, it is suggested to type these haplotypes in MS population in other provinces, specially, in regions considered as high risk for MS.

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