Analysis of HLA-A*03 in Multiple Sclerosis Patients in Khuzestan Province, Iran

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Abstract

Background: Multiple sclerosis (MS) is known as chronic inflammatory causes demyelination and results in neurodegenerative disease in central nervous system (CNS), which is known with progressive disability. MS is accepted and is a multifactorial disorder that is a result of genetic and environmental factors interaction. The set of genes coding for MHC (major histocompatibility complex) molecules exert the effect on disease development. HLA-A*03 (HLA-A3) is one of HLA class I alleles, which is associated with MS.
Objectives: Our goal is to survey the frequency of this allele in MS patients from the Khuzestan province.
Methods: HLA-A*03 alleles association was investigated in 200 MS patients in the province of Khuzestan then compared with 195 healthy controls. HLA-typing was performed by polymerase chain reaction (PCR) amplification with SSP-PCR method.
Results: Frequency of HLA-A*03 was significant among MS patients (47% vs. 26%, P < 0.001). There was no significant correlation among this allele and sex, course of disease, initial symptoms, and expanded disability status scale (EDSS).
Conclusions: Our findings suggested that HLA-A*03 allele has a significant association with MS, which is in line with other studies, and also confirmed that the presence of this allele could increase the risk of MS. Furthermore, positive association between the HLA-A*03 allele and the Arab population in the province of Khuzestan was found. CIS patients followed up for three years demonstrated that all patients were converted to RRMS.

Keywords: Multiple Sclerosis, Human Leucocyte Antigen (HLA), HLA-A*03

1. Background

Multiple sclerosis (MS) is neurodegenerative disease, which affects the central nervous system (CNS) (1), Furthermore, MS is an autoimmune disorder, which interferes with the immune system causing myelin sheath degradation. Symptoms of MS involves: vision, sensory and motor systems, balance and coordination, bowel/bladder/sexual, and cognition disorders (2, 3).

EDSS (expanded disability status scale) of patients was assumed from 0 to 10 based on the Kurtzke expanded disability status scale (4).

MS is clinically separated into four diverse categories: RRMS (relapsing-remitting), SPMS (secondary-progressive), PRMS (progressive-relapsing), and PPMS (primary-progressive) (5). A total of 85% of individuals who identified with MS have primary signs or attacks, which is mentioned to clinically isolated syndrome (CIS).

Average onset of MS is between 20 and 40 years old (5). The MS incidence in childhood and after the age of 50 is rare (6). The peak of MS incidence is around the age of 24 (7). Women have two or three-fold more prevalence of multiple sclerosis than men (6).

Both environmental and genetic factors trigger MS (8). The first idea regarding genetic factors was raised in the 1890s (9, 10). In the 1920s, worldwide investigation of MS prevalence demonstrated a racial and ethnic difference, which may indicate a genetic influence (11).

Several genes contribute in MS susceptibility such as major histocompatibility complex genes (1).

Major histocompatibility genes, such as HLA, are integrated in normal immune response. The term HLA refers to the human leucocyte antigen system (12). Several of these genes play leading role in numerous immunological progressions (13).

Certain alleles of HLA have been studied in MS suscep-

tibility. HLA-A3 (HLA-A*03) is one allele of class I HLA, which is firmly related with MS ([1]).

HLA-A*03 frequency in the Khuzestan province has not yet been reported. Ethnicity diversity in Khuzestan and limited published data for local Arabs and nearby located Arab countries is the turning point of our data.

Our goal was to consider the association between HLA-A*03 in MS patients and healthy controls from the Khuzestan province as well as study this allele in different ethnicities (Arab and Persians). Furthermore, we examined the possible relationship of HLA-A*03 allele with course of disease, primary symptoms, and EDSS.

2. Methods

A total of 200 MS patients, according to the McDonald criteria, confirmed in the Khuzestan multiple sclerosis society, were studied in Shahid Chamran University, (159 females and 41 males with the mean age as 32.2 years and age range 16 - 57). Clinical symptoms estimation was conduct by expert neurologist. Furthermore, we included 195 unrelated healthy controls excluded for any possible autoimmune-disease and history of MS in the family to this study (165 women and 30 men, mean age was 27.4, and age range was 14 - 63). The control group was selected from the same geographical zone and matched with patients on characteristics such as ethnicity, gender, and age. All contributors were informed regarding the aim of the study and consent form were completed by all.

A total of 5 mL of blood was drawn with the consent of patients and controls and collected in EDTA tube.

DNA extraction and purification was performed by high pure PCR template preparation kit from Roch Company, Germany.

2.1. Primers

HLA-A*03 allele in MS patients and health controls was investigated by sequence specific primer polymerase chain reaction (SSP-PCR). Primers sequences was applied to amplify the HLA-A*03 designed according to IMGT/HLA database (http://www.ebi.ac.uk/) and checked out by NCBI/blast database (www.ncbi.nlm.nih.gov). Forward and reverse primers sequences for HLA-A*03 was: Forward: 5'-AGCGACGCCGCGAGCCA-3' and Reverse: 5'-CACTCCACGCACGTGCCA-3'. The size of PCR product was 630 bp.

Single specific primer-polymerase chain reaction (SSP-PCR) reaction is confirmed with internal control amplification. The MOG (Myelin oligodendrocyte glycoprotein) gene was applied as internal control, which results in 356 bp fragment. Internal control primers were designed

by the web primer design program, batchprimer3, which is accessible at (http://probes.pw.usda.gov/batchprimer3/). Primers were aligned in ncbi/blast. The primer sequences are: Forward: 5'-GGGACCAATTCTGTGTCACC-3' reverse: 5'-TGAACCCAGAAGTCACTCACA-3'.

Touchdown PCR method was used to increase the specificity of primer annealing. PCR conditions are shown in Table 1.

After gel electrophoresis running, random direct sequencing was used for result confirmation.

2.2. Statistical Analysis

To examine the susceptibility of HLA-A*03 allele in MS disease, χ^2 was calculated and Fisher's exact test was accomplished using SPSS for Windows V. 22.0. P value less than 0.05 was accepted as significant.

3. Results

HLA-A*03 allele was compared in 200 patients with 195 healthy controls by SSP-PCR method. Demographic and clinical profiles of MS patients and controls is illustrated in Table 2.

HLA-A*03 allele frequencies were more significant in the patient than control group ($P < 0.001$). Results are shown in Table 3 and agarose gel electrophoresis (PCR image) is shown in Figure 1.

We compared this allele in different ethnicities, Arab and Persian patients, and found the significant differences between them (Arab patients have more HLA-A*03 allele, ($P = 0.001$), since this significance not seen in healthy controls). This result is shown in Table 4.

HLA data compared with clinical categories included: disease course, EDSS, and clinical symptoms such as sensory symptoms, motor symptoms, brainstem-cerebellum, visual disturbance, urinary symptoms, and intestinal
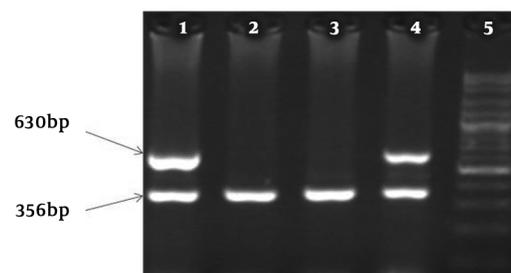
Figure 1. Agarose gel shown one or two bands. Lanes 1 and 4 are positive samples, lanes 2 and 3 are control samples, and lane 5 is 100 bp marker. HLA-A3 PCR product was 630 bp and control gene PCR product was 356 bp.

Table 1. PCR Conditions

Step	Cycle Repeat	Function	Temperature (°C)	Time
1	1	Initial denaturation	96	1 min
2	5	Denaturation	96	20 s
		Annealing	70	45 s
		Elongation	72	25 s
3	21	Denaturation	96	25 s
		Annealing	65	50 s
		Elongation	72	72 s
4	4	Denaturation	96	30 s
		Annealing	55	1 min
		Elongation	72	90 s
5		Elongation	20	1 min

Table 2. Demographic and Clinical Profiles of Multiple Sclerosis Patients and Controls

Groups	Sex, No. (%)	Ethnicity, No. (%)	Age[a] (y)	Age Range (y)	Age at Onset[a] (y)
MS patients	Female: 159 (79.5); male: 41 (20.5)	Arab: 60 (44.4); Persian: 75 (55.6)	31.6 ± 7.9	16 - 57	27.57 ± 6.80
Controls	Female: 165 (84.6); male: 30 (15.4)	Arab: 98 (55.6); Persian: 78 (44.3)	29.5 ± 10.13	20 - 63	-

[a] Values are expressed as mean $\pm$ SD.

Table 3. Frequency of HLA-A*03 Allele in MS Patients and Controls

HLA-A*03 Allele	Patient, No. (%)	Control, No. (%)	P Value
+	93 (46.5)	51 (25.5)	
-	107 (53.5)	144 (73.5)	< 0.001
Total	200	195	

symptoms, which also showed no significant relation with HLA-A*03. Results are shown in Table 5.

4. Discussion

In 1970s, linkage analysis indicated diverse variations in HLA loci, which affect the susceptibility of MS ([14-16]). The first discovered loci was HLA-A*03 and HLA-B7 genes ([17]). In the recent two decades, susceptibility loci for MS, through the HLA region, were replicated in numerous populations. Supplementary influence of class I HLA loci (HLA-A*03) doubling the risk for MS has been described ([18]). A study performed in Spain in 2005, indicated that frequency of HLA-A*03 allele was 41% in MS patients and 24% in health controls ([18]). The study performed by Harbo et al., in 2004, indicated that the combination of HLA-DR15 and HLA-A3 alleles have a high significant association with MS ([19]). In 2012, Kankonkar et al., reported HLA-A, B, C and DQB1, DRB1 frequency in MS patients in India and the HLA-A*03 allele indicated a significant association with MS ([20]).

In this study, we genotyped MS southwest Iranian patients with evaluation of HLA-A*03 significance in these patients.

We found that HLA-A*03 frequency in these patients is significantly high. Females are more susceptible for MS than males. In comparison to other studies, our study confirmed that the existence of this allele can increase the risk of MS in southwest Iranian population.

The most common symptoms of this disorder involve interruption of vision, motor and sensory systems, coordination and balance (brainstem-cerebellum), bowel/bladder/sexual, and cognition ([2], [3]). In this study, the effect of HLA-A*03 loci on clinical variables such as sex, course of disease, initial symptoms, and EDSS was evaluated. All of them showed no significant relation with HLA-A*03, which is in line with the study of Bayati et al., in 2008, in Iran ([21]).

According to the results of Sharafaddinzadeh et al., incidence and prevalence of MS is low among Arab people in comparison with the Persian (non-Arab) part of the Khuzestan province population ([22]), that is why we studied the HLA-A*03 in different ethnicities (Arab and non-Arab) and found a significant association between this allele and Arab patients.

We studied MS courses in these patients as a clinical

Table 4. Compare HLA-A*03 Allele in MS Patients and Controls in Different Ethnicity

HLA-A*03 Allele	MS Patients			Controls		
	Arab	Persian	Number	Arab	Persian	Number
+	40	28	68	26	17	43
-	20	47	67	72	61	133
Total	60	75	135	98	78	176
P value		0.001			0.292	

Table 5. Clinical Categories in Compare with HLA-A*03 Allele in MS Patients

Clinical Information	Number	A3 +	A3 -	P Value
Disease course				0.235
RRMS	175	88	87	
PPMS	2	3	5	
SPMS	1	4	5	
RPMS	0	1	1	
CIS	10	4	6	
EDSS				0.314
0	27	14	13	
1 - 3	123	67	56	
3.5 - 5	18	10	8	
+5	8	4	4	
Symptoms				
Sensory symptoms	80	43	37	0.281
Motor symptoms	86	39	47	0.238
Brainstem-cerebellum	48	26	22	0.396
Visual disturbance				
Pain	87	46	21	0.25
Visual field defects	46	26	20	0.235
Urinary symptoms				
Frequency	38	19	19	0.504
Incontinence	18	8	10	0.356
Intestinal symptoms				
Constipation	43	25	18	0.184

symptom. This criterion is determined by the treating physician based on factors such as the number of attacks and the distances between them, during the course of the disease, the severity, and speed of progression. In this population, more than 80% of patients showed RRMS, while RPMS have less frequency, which is in line with other studies (23).

CIS is the result of only one incident of demyelination in one area, or several areas of the CNS, which lasts for at least 24 hours. Individuals who experience CIS may or may not go on to develop MS (24). From the patient population that we studied, 10 were diagnosed as CIS. We've followed these patients for three years and observed that all of them have been converted to RRMS.

4.1. Conclusions

Since the HLA-A*03 allele frequency is significantly high in southwest Iranian MS patients, there is probably a positive connection between this allele frequency and MS susceptibility in this population.

We observed that patients with Arab ethnicity are less than Persians, however, HLA-A*03 in Arabs are significantly more than Persians patients. Therefore, we can probably evaluate the HLA-A*03 allele as one of the MS susceptibility factors in Arabs.

In general, our purpose is to determine the simple part of the genetic profile of MS in the province of Khuzestan. We think that HLA-A*03 can be suggested as a potent genetically effective factor in the MS population.

Furthermore, more researches on additional HLA alleles and larger population are needed to recognize the role of MHC alleles in MS patients as well as find other genetic risk factors to help us identify the MS susceptibility.

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References

1. Nischwitz S, Muller-Myhsok B, Weber F. Risk conferring genes in multiple sclerosis. *FEBS Lett.* 2011;**585**(23):3789–97. doi: 10.1016/j.febslet.2011.03.037. [PubMed: 21439284].
2. Compston A, Coles A. Multiple sclerosis. *Lancet.* 2002;**359**(9313):1221–31. doi: 10.1016/S0140-6736(02)08220-X. [PubMed: 11955556].
3. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. *N Engl J Med.* 2000;**343**(13):938–52. doi: 10.1056/NEJM200009283431307. [PubMed: 11006371].
4. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). *Neurology.* 1983;**33**(11):1444–52. [PubMed: 6685237].
5. Lublin FD, Reingold SC. Defining the clinical course of multiple sclerosis: Results of an international survey. National Multiple Sclerosis Society (USA) Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis. *Neurology.* 1996;**46**(4):907–11. [PubMed: 8780061].
6. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: The role of infection. *Ann Neurol.* 2007;**61**(4):288–99. doi: 10.1002/ana.21117. [PubMed: 17444504].
7. Ebers GC. Environmental factors and multiple sclerosis. *Lancet Neurol.* 2008;**7**(3):268–77. doi: 10.1016/S1474-4422(08)70042-5. [PubMed: 18275928].
8. Sanfilippo F, Amos DB. An interpretation of the major histocompatibility complex. In: Rose N, Friedman H, Fahey J, editors. *Manual of clinical laboratory immunology.* 3rd ed. Washington DC: Am Soc Microbiol; 1986.
9. Mackay RP. The familial occurrence of multiple sclerosis and its implications. *Ann Intern Med.* 1950;**33**(2):298–320. [PubMed: 15433121].
10. Eichhorst H. [About infantile and hereditary multiple sclerosis]. *Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin.* 1896;**146**(2):173–92. German. doi: 10.1007/bf01882681.
11. Lin R, Charlesworth J, van der Mei I, Taylor BV. The genetics of multiple sclerosis. *Pract Neurol.* 2012;**12**(5):279–88. doi: 10.1136/practneurol-2012-000276. [PubMed: 22976058].
12. Shankarkumar U. The human leukocyte antigen (HLA) system. *Int J Hum Genet.* 2004;**4**(2):91–103. doi: 10.1080/09723757.2004.11885875.
13. Dyment DA, Ebers GC, Sadovnick AD. Genetics of multiple sclerosis. *Lancet Neurol.* 2004;**3**(2):104–10. [PubMed: 14747002].
14. Paty DW, Mervart H, Campling B, Rand CG, Stiller CR. HL-A frequencies in patients with multiple sclerosis. *Can J Neurol Sci.* 1974;**1**(4):211–3. [PubMed: 4441986].
15. Bertrams HJ, Kuwert EK. Association of histocompatibility haplotype HLA-A3-B7 with multiple sclerosis. *J Immunol.* 1976;**117**(5 Pt.2):1906–12. [PubMed: 993587].
16. de Moerloose P, Jeannet M, Martins-da-Silva B, Werner-Favre C, Rohr J, Gauthier G. Increased frequency of HLA–DRw2 and DRw3 in multiple sclerosis. *Tissue Antigens.* 1979;**13**(5):357–60. [PubMed: 91212].
17. Burfoot RK, Jensen CJ, Field J, Stankovich J, Varney MD, Johnson LJ, et al. SNP mapping and candidate gene sequencing in the class I region of the HLA complex: Searching for multiple sclerosis susceptibility genes in Tasmanians. *Tissue Antigens.* 2008;**71**(1):42–50. doi: 10.1111/j.1399-0039.2007.00962.x. [PubMed: 17971048].
18. Leon VJ, Cacho JL, Rodriguez-Perez R, Bowakin D. Study of HLA aleles in multiple sclerosis in Castilla y Leon (Spain). *Hum Immunol.* 2005;**66**(8):40. doi: 10.1016/j.humimm.2005.08.075.
19. Harbo HF, Lie BA, Sawcer S, Celius EG, Dai KZ, Oturai A, et al. Genes in the HLA class I region may contribute to the HLA class II-associated genetic susceptibility to multiple sclerosis. *Tissue Antigens.* 2004;**63**(3):237–47. doi: 10.1111/j.0001-2815.2004.00173.x. [PubMed: 14989713].
20. Kankonkar S, Singhal BS, Shankarkumar U. HLA A, B, Cw. DRB1 and DQB1 alleles in multiple sclerosis patients in India. *Int J Hum Genet.* 2012;**12**(1):37–40. doi: 10.1080/09723757.2012.11886160.
21. Bayati A, Ghabaei M, Amir Sarv Kolahi S. A case control study on genetic susceptibility in multiple sclerosis. *Iran J Neurol.* 2008;**7**(21):143–52.
22. Sharafaddinzadeh N, Moghtaderi A, Majdinasab N, Dahmardeh M, Kashipazha D, Shalbafan B. The influence of ethnicity on the characterists of multiple sclerosis: A local population study between Persians and Arabs. *Clin Neurol Neurosurg.* 2013;**115**(8):1271–5. doi: 10.1016/j.clineuro.2012.11.027. [PubMed: 23273383].
23. Marrie RA. Environmental risk factors in multiple sclerosis aetiology. *Lancet Neurol.* 2004;**3**(12):709–18. doi: 10.1016/S1474-4422(04)00933-0. [PubMed: 15556803].
24. Miller D, Barkhof F, Montalban X, Thompson A, Filippi M. Clinically isolated syndromes suggestive of multiple sclerosis, part I: Natural history, pathogenesis, diagnosis, and prognosis. *Lancet Neurol.* 2005;**4**(5):281–8. doi: 10.1016/S1474-4422(05)70071-5. [PubMed: 15847841].