Human leukocyte antigen typing in Iraqi multiple sclerosis patients

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ABSTRACT

Objectives: To assess the relative frequency of human leukocyte antigen (HLA) class I and class II in Iraqi multiple sclerosis (MS) patients, and to ascertain whether they offer any etiologic or protective role.

Methods: We conducted this study in the Baghdad MS Clinic and Teaching Laboratory Institute, Medical City, Baghdad Teaching Hospital, Baghdad, Iraq from March to July 2004. We enrolled 44 randomly selected MS patients and 69 healthy unrelated age- and sex-matched controls. We carried out HLA class I and class II typing on both groups using the microlymphocytotoxicity test.

Results: The HLA class I typing revealed no consistent association between MS and HLA-A and -Cw, while HLA-B5 and -B44 were found to possibly be risk factors for MS with odds ratio (OR) of 10.2 for -B5 and 4.4 for -B44. The HLA-B35 may form a protective factor with OR of 0.1. The HLA class II typing revealed an etiologic risk for HLA-DR4 (OR=10.3) and a protective effect for HLA-DR2 (OR=0.3) and -DR7 (OR=0.2), and etiologic effect for -DQ1 (OR=3.3) and -DQ3 (OR=3).

Conclusions: The HLA DR4 carries the strongest association with MS in Iraqi patients. This study adds to the well-known diversity of HLA-allelic association of MS in different populations, and emphasizes the complexity of genetic susceptibility to MS.

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Multiple sclerosis (MS) is the most common inflammatory demyelinating disease of the CNS. Although the exact etiology is unknown, current thinking favors the notion that environmental, immunologic, and genetic factors initiate an autoimmune process leading to patchy demyelination in the CNS. Of the important genetic factors that are associated with the susceptibility to the disease, are human leukocyte antigen (HLA) genes.[1] This study assesses the relative frequency of various HLA class I and II antigens in Iraqi MS patients compared with healthy controls to define the alleles that may form risk factors or protective factors in these patients.

Methods. Forty-four randomly selected Iraqi MS patients attending Baghdad MS Clinic, Medical City, Baghdad Teaching Hospital, Baghdad, Iraq were enrolled in the study, along with 69 healthy unrelated age- and sex-matched subjects selected as a control group from a pool of subjects in whom their HLA-typing was tested for paternity determination or organ donation purposes. The study was conducted from March to July 2004, and ethical approval was obtained from the Iraqi Board for Medical Specializations. All the patients had clinically definite relapsing-remitting MS according to Poser's criteria.[2] In addition, all the patients had abnormal brain MRI consistent with demyelination. The HLA class I and II typing was carried out in the Immunology Department in the Teaching Laboratory Institute of Medical City, Baghdad Teaching Hospital using the microlymphocytotoxicity test. This test is based on the serological reaction of HLA-antisera with the correspondent membrane–bound antigen (Ag) on the lymphocyte in the presence of rabbit's complement. If the Ag under test is on the lymphocytes, the complement will be activated by Ag-Ab (antibody) reaction, resulting in a structural alteration of the cell membrane, thereby allowing penetration of stains into the cell. This will show as a positive reaction (dead stained lymphocytes) or negative reaction (live unstained lymphocytes). This reaction was fixed by adding formaldehyde, which

allows reading to be carried out even after several days. In this test, peripheral blood T-lymphocytes were used as the target cells for HLA-I typing (HLA-A, B, C) while B-lymphocytes were used for HLA-II (DR and DQ). The isolation and separation of B-lymphocytes from T-lymphocytes were carried out by the use of nylon wool, which utilizes cell membrane properties of B-cells, whereby B-cells adhere to the nylon wool fibers, whereas T-cells can be easily washed-off. This separation method allows B-cell enrichment to 70-80% in the samples prepared. The new HLA nomenclature reflects grafting of newer DNA sequence information on the older system that is based on serology. For example, class I alleles are given a single designation that indicates locus, serologic specificity, and sequence-based subtype, for example, HLA-A*0201 indicates subtype 1 of the serologically defined allele HLA-A2. Our study utilizes the older system, which is based on serology.

Statistical analysis was carried out using SPSS version 10.5 computer software in association with Excel, XP version. The strength of association between disease status and HLA antigens is expressed through odds ratio (OR), which vary from less than one (negative association) to more than one (positive association). In the latter case, an etiological fraction (EF) was given, which indicates how much of a disease is "due to" the disease-associated factor. In the former case, a prevention fraction (PF) was given, which indicates how much of a disease is prevented by the disease-associated marker. Both PF and EF can vary between zero (no association) and one (maximum association). To avoid a chance occurrence of an association (due to many comparisons), the P was multiplied by the number of antigens tested at each HLA-locus to obtain the corrected probability (Pc). A *P*-value ≤0.05 was considered significant.

Results. The patients comprised 20 males and 24 females with age range from 19-53 years (37.3±8.6). Distribution of cases according to onset is shown in **Table 1**, which shows that the mean age of onset is 29.5±7.8, and the maximum age of onset was 20-29 years. Duration of disease varies between 1-20 years with an average of 7.8 ± 6.3. Consanguinity among parents of patients was found in 18 patients (40.9%). Presentation of patients at onset of disease (first clinical attack) is shown in **Table 2**, with bilateral leg weakness being the most common mode of presentation followed by left leg weakness. Both HLA-A locus and HLA-Cw locus testing did not reveal significant association with the disease as shown in **Table 3**. Within the HLA-B locus, it was found that the presence of HLA-B5 might form an etiological risk factor for the disease, this is followed by HLA-B44. The HLA-B35 has a protective effect (**Table 3**). **Table 4** shows HLA- class II (DR and DQ) typing in the patient and control groups. It revealed that HLA-DR4 represents an etiological risk factor, while DR2 and DR7 had a protective effect. For HLA-DQ typing, DQ1 and DQ3 showed etiologic risks. Univariate models showed no consistent association between MS disease status and HLA-A and HLA-Cw Ag, and so only HLA-B, DR, and DQ antigens were eligible for studying the observed and expected frequencies. This method compares the observed (actual) HLA-Ag frequencies among MS cases with the expected frequency if the cases were similar to healthy control in HLA-Ag distribution. This is shown in **Table 5**, which revealed that HLA-DR4 Ag shows that the expected frequency is smaller than the observed, and had the smallest *P*-value so this Ag is considered as the one with the strongest association with the risk of having the disease. Analysis of intralocus and extralocus combinations of antigens in MS does not reveal any significant combination, probably because of small sample size.

Table 1 - Distribution of cases by age of onset and sex.

Age of onset (years)	Males n (%)	Females n (%)	Total n (%)
< 20	2 (10)	0 (0)	**2 (4.5)**
20 – 29	12 (60)	12 (50)	**24 (54.6)**
30 – 39	2 (10)	8 (33.3)	**10 (22.7)**
40 – 49	4 (20)	4 (16.7)	**8 (18.2)**
Total	**20 (100)**	**24 (100)**	**44 (100)**

Table 2 - Distribution of patients by clinical presentation at onset of the disease.

Presentation	Males n (%)	Females n (%)	Total n (%)
Bilateral leg weakness	3 (15)	10 (41.6)	**13 (29.6)**
Left sided leg weakness	5 (25)	4 (16.7)	**9 (20.4)**
Blurred vision only	4 (20)	3 (12.5)	**7 (15.9)**
Blurred vision and leg weakness	4 (20)	2 (8.3)	**6 (13.6)**
Numbness	2 (10)	2 (8.3)	**4 (9.1)**
Right sided leg weakness	1 (5)	1 (4.2)	**2 (4.5)**
Blindness	0 (0)	1 (4.2)	**1 (2.3)**
Uncontrolled urination	1 (5)	0 (0)	**1 (2.3)**
Vertigo	0 (0)	1 (4.2)	**1 (2.3)**
Total	**20 (100)**	**24 (100)**	**44 (100)**

Discussion. It is agreed that the susceptibility to MS may be inherited, however, it does not follow any

Table 3 - Depicts the observed numbers, percentage frequencies of human leukocyte antigen (HLA) class I (A, B, C) for multiple sclerosis (MS) patients, as compared with healthy controls.

HLA A Locus	Cases (MS) n (%)		Healthy controls n (%)		OR	Inverse OR	*P*-value	Adjusted *P*	EF	PF
1	13	(29.5)	11	(15.9)	2.2	-	0.09NS	-	0.162	-
2	19	(43.2)	29	(42.0)	1.0	-	0.9NS	-	0.020	-
3	12	(27.3)	18	(26.1)	1.1	-	0.89NS	-	0.016	-
9	16	(36.4)	18	(26.1)	1.6	-	0.25NS	-	0.139	-
10	13	(29.5)	11	(15.9)	2.2	-	0.09NS	-	0.162	-
11	2	(4.5)	10	(14.5)	0.3	3.6	0.11NS	-	-	0.104
28	0	(0.0)	3	(4.3)	0.2	4.7	0.17NS	-	-	-
29	1	(2.3)	1	(1.4)	1.6	-	0.75NS	-	0.008	-
30	3	(6.8)	10	(14.5)	0.4	2.3	0.22NS	-	-	0.082
32	0	(0.0)	4	(5.8)	0.2	6.1	0.1NS	-	-	-
33	3	(6.8)	7	(10.1)	0.6	1.5	0.55NS	-	-	0.036
34	1	(2.3)	0	(0.0)	4.8	-	0.21NS	-	0.018	-
Blank	5		10							
B Locus	**Cases (MS) n (%)**		**Healthy controls n (%)**		**OR**	**Inverse OR**	***P*-value**	**Adjusted *P***	**EF**	**PF**
5	17	(38.6)	4	(5.8)	10.2	-	0.000	0.004	0.349	-
7	9	(20.5)	7	(10.1)	2.3	-	0.13NS	-	0.115	-
8	11	(25.0)	9	(13.0)	2.2	-	0.11NS	-	0.138	-
12	2	(4.5)	0	(0.0)	8.2	-	0.07NS	-	0.040	-
14	2	(4.5)	4	(5.8)	0.8	1.3	0.77NS	-	-	0.013
16	1	(2.3)	0	(0.0)	4.8	-	0.21NS	-	0.018	-
17	1	(2.3)	3	(4.3)	0.5	2.0	0.57NS	-	-	0.021
18	3	(6.8)	4	(5.8)	1.2	-	0.83NS	-	0.011	-
27	3	(6.8)	1	(1.4)	5.0	-	0.17NS	-	0.054	-
35	1	(2.3)	15	(21.7)	0.1	11.9	0.018	0.68NS	-	0.199
40	0	(0.0)	1	(1.4)	0.5	1.9	0.59NS	-	-	-
41	6	(13.6)	5	(7.2)	2.0	-	0.27NS	-	0.069	-
42	0	(0.0)	1	(1.4)	0.5	1.9	0.59NS	-	-	-
44	16	(36.4)	8	(11.6)	4.4	-	0.003	0.1NS	0.280	-
45	1	(2.3)	2	(2.9)	0.8	1.3	0.84NS	-	-	0.006
49	0	(0.0)	2	(2.9)	0.3	3.3	0.31NS	-	-	-
50	1	(2.3)	3	(4.3)	0.5	2.0	0.57NS	-	-	0.021
55	3	(6.8)	0	(0.0)	11.7	-	0.030	1.11NS	0.062	-
62	0	(0.0)	4	(5.8)	0.2	6.1	0.1NS	-	-	-
Blank	6		37							
Cw Locus	**Cases (MS) n (%)**		**Healthy controls n (%)**		**OR**	**Inverse OR**	***P*-value**	**Adjusted *P***	**EF**	**PF**
1	2	(4.5)	1	1.4	3.2	-	0.34NS	-	0.031	-
2	1	(2.3)	6	8.7	0.2	4.1	0.2NS	-	-	0.066
3	1	(2.3)	2	2.9	0.8	1.3	0.84NS	-	-	0.006
4	10	(22.7)	15	21.7	1.1	-	0.9NS	-	0.013	-
5	2	(4.5)	4	5.8	0.8	1.3	0.77NS	-	-	0.013
6	10	(22.7)	15	21.7	1.1	-	0.9NS	-	0.013	-
7	15	(34.1)	18	26.1	1.5	-	0.36NS	-	0.108	-
Blank	41		61							

OR - odds ratio, EF - etiologic fraction, PF - protective fraction, NS - non significant

Mendelian inheritance. While familial aggregation of MS is well documented,[3] this study showed only one patient with a positive family history, a female aged 21 years, one of identical twins, with her father dying a few years ago, and her sister dying during childhood due to MS. Interestingly, consanguinity among parents of our patients is common (~41%). However, we cannot make clear conclusions about this finding because it was not actually looked for in the control group in the initial design of the study, and because it is a common phenomenon in our society. For these reasons, a study on a larger sample of MS patients compared with a population-based control group is needed to address this issue. Several studies reported that identical twins of MS have 1/3 chance of developing the disease, while non identical twins carry low risk, similar to non twin siblings.[4] In this study, we tried to ascertain the role of a well-known genetic marker that is often associated with this disease, namely, HLA genotypes. Our study showed no significant association between HLA-A and -Cw loci and the disease. In contrast, studies on British and Norwegian patients showed increased prevalence of A3 with the highest risk being conferred by its combination with DR2,[5] also in Iran an increased incidence of HLA-A24 was shown.[6] Odinak et al[7] reported increased prevalence of A10 in the western region of Russia, while there is negative association with possible protective effect of A2 (RR=0.47), A11 (RR=0.31), and A30 (RR=0.01).[7] The current study showed that HLA-B5 and HLA-B44 may form an etiologic risk. This apparent etiologic risk of HLA-B5 is also shared by Spanish patients (OR 2.85) who also showed increased frequency of B41 (RR 7.65).[8] Other studies in Britain showed significant association with B7,[9] and a Russian study found that patients who have B7, B12, and A3 ran an aggravating course of the disease.[10] Conversely, our study showed a possible protective effect for B35, while Odinak et al[7] found an etiologic effect for B35 (RR=2.6), B7 (RR=2.05), and B13 (RR=3.59), while B18 conferred protective effect (RR 0.35). These studies, along with our study suggest a modulating effect for HLA-class I alleles on the susceptibility to MS with various markers in different population groups, and this effect is not limited to the well-known HLA-II alleles, a phenomena that has previously been observed in animal models only.[11] It is well known that the class II-DR region is the most consistently identified factor in MS, yet DP and DQ may also play a role.[12,13]

We studied HLA-DR and DQ, HLA-DP was not studied because of technical difficulties. We found

Table 4 - Depicts the observed numbers, percentage frequencies of human leukocyte antigen (HLA) class II (DR, DQ) for multiple sclerosis (MS) patients, as compared with healthy controls.

HLA DR Locus	Cases (MS) n (%)		Healthy controls n (%)		OR	Inverse OR	*P*-value	Adjusted *P*	EF	PF
1	12	(27.3)	17	(24.6)	1.1	-	0.75NS	-	0.035	-
2	6	(13.6)	24	(34.8)	0.3	3.4	0.016	0.29NS	-	0.245
3	10	(22.7)	18	(26.1)	0.8	1.2	0.69NS	-	-	0.043
4	28	(63.6)	10	(14.5)	10.3	-	0.000	0.000	0.575	-
5	0	(0.0)	3	(4.3)	0.2	4.7	0.17NS	-	-	-
6	2	(4.5)	4	(5.8)	0.8	1.3	0.77NS	-	-	0.013
7	2	(4.5)	16	(23.2)	0.2	6.3	0.018	0.32NS	-	0.195
8	6	(13.6)	6	(8.7)	1.7	-	0.41NS	-	0.054	-
10	2	(4.5)	2	(2.9)	1.6	-	0.65NS	-	0.017	-
11	2	(4.5)	6	(8.7)	0.5	2.0	0.41NS	-	-	0.043
17	5	(11.4)	0	(0.0)	19.4	-	0.007	0.13NS	0.108	-
52	2	(4.5)	0	(0.0)	8.2	-	0.07NS	-	0.040	-
53	3	(6.8)	0	(0.0)	11.7	-	0.030	0.54NS	0.062	-
Blank	8		30							
DQ Locus	**Cases (MS) n (%)**		**Healthy controls n (%)**		**OR**	**Inverse OR**	***P*-value**	**Adjusted *P***	**EF**	**PF**
1	21	(47.7)	15	(21.7)	3.3	-	0.005	0.028	0.332	-
2	17	(38.6)	19	(27.5)	1.7	-	0.22NS	-	0.153	-
3	21	(47.7)	16	(23.2)	3.0	-	0.008	0.046	0.319	-
Blank	29		74							

OR - odds ratio, EF - etiologic fraction, PF - protective fraction, NS - non significant

Table 5 - The observed and expected frequencies of HLA-B, DR, DQ loci in multiple sclerosis patients.

HLA B locus	Observed frequency	Expected frequency	*P*-value
5	17	3.2	7.0E-15*
7	9	5.7	NS
8	11	7.3	NS
12	2	0.0	NS
14	2	3.2	NS
16	1	0.0	NS
17	1	2.4	NS
18	3	3.2	NS
27	3	0.8	1.4E-02**
35	1	12.2	5.3E-04
40	0	0.8	NS
41	6	4.1	NS
42	0	0.8	NS
44	16	6.5	1.0E-04
45	1	1.6	NS
49	0	1.6	NS
50	1	2.4	NS
55	3	0.0	4.6E-02
62	0	3.2	NS
HLA DR locus	**Observed frequency**	**Expected frequency**	***P*-value**
1	12	12.6	NS
2	6	17.8	1.6E-03
3	10	13.3	NS
4	28	7.4	2.0E-15
5	0	2.2	NS
6	2	3.0	NS
7	2	11.9	1.9E-03
8	6	4.4	NS
10	2	1.5	NS
11	2	4.4	NS
17	5	0.0	5.7E-05
52	2	0.0	NS
53	3	0.0	4.3E-02
HLA DQ locus	**Observed frequency**	**Expected frequency**	***P*-value**
1	21	13.8	2.7E-02
2	17	17.5	NS
3	21	14.8	NS

NS – non significant, *7.0E-15 = 7.0 x 10^{-15}, **1.4E-02 = 0.014

that HLA-D4 formed an etiologic risk factor, and this allele showed the strongest association with the risk of having the disease. This allele is also shown to be a risk factor in several studies on Arabian,[14] Turkish,[15] and Sardinian[16] patients. The Japanese patients, with negative oligoclonal band, also had increased frequency of this allele, while those with positive oligoclonal band had increased HLA-DR2.[17] Interestingly, this study showed a protective effect for DR2 and DR7, which contradicts several studies that showed increased prevalence of DR2 in British,[9] Scandinavian,[18] French,[19] Spanish,[8] Iranian,[6] Swedish,[11] and Russian patients.[7] Our study demonstrated significant increased frequency of DQ1 and DQ3, a similar increased prevalence of DQ3 along with DR4 was shown in Turkish patients.[15] The HLA-DQ1 was observed in Scottish[20] and Spanish groups of patients.[8] However, a Swedish group of patients carried DQ6 alleles.[11]

This diversity of HLA-allelic association with MS emphasizes the complexity of genetic susceptibility to MS in different populations, and the Iraqi population is no exception. Further studies utilizing the new HLA typing technology on a larger cohort of patients are needed to assess the significance of our findings.

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