HLA Class-I Molecules in Iraqi Patients with Rheumatoid Arthritis. A Sporadic & Familial study

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Summary:

Background: *Rheumatoid Arthritis (RA) is a common autoimmune disease of unknown etiology and of poorly understood pathophysiology. In spite of the obvious association of HLA class-I alleles in RA susceptibility both in sporadic and familial cases, there are few documented studies.*

Objective: *the aim of study is an attempt to explore the association of class I HLA molecules with familial and sporadic cases of RA.*

Subject & methods: *Microlymphocytotoxicity assay was used to assess HLA-typing of 80 sporadic RA patients and 53 probands in 25 families to compare them with 94 healthy relative and 62 patients with SLA and 107 normal persons as controls.*

Results: *the results showed that A10, B22, B47, Cwl & Cw7 molecules are associated significantly with the disease among sporadic patients, and a highly significant statically differences between patients and control groups (P< 0.0001, 0.009, 0.007, 0.005 & 0.002 respectively. Only B5 & B21 were found in familial RA (P<0.003 & 0.012 respectively). On the other hand highly significant negative association was noticed with A2, B4, B14 & Cw6 (P<0.0001, 0.006, 0.028 & 0.047 respectively) among sporadic RA compared with only B12 & B16 (P<0.035 & 0.023 respectively) among familial RA. However, A2 & Cw6 were found in significant frequencies but not statistically significant (Inverse OR=2.2 & 5.3 respectively).*

Conclusion: *HLA-typing indicated the prevalence of the disease among those with HLA-A10, B22, B47, Cwl and Cw7, while familial patients possess only B5and B21. However, A10, Cwl and Cw3 were found in significant frequencies, but were not statistically significant.*

Introduction:

Rheumatoid Arthritis (RA) is a common human autoimmune disease of unknown etiology and poorly understood pathophysiology. Population and family studies have established the important contribution of genetic factors to RA susceptibility and disease progression. In spite of the obvious association between HLA class-I alleles in RA susceptibility, a few studies were carried out in this respect (Ad'haiah, 1990). Moreover, it was reported that HLA-A molecules were significantly linked to RA (Marlow, *et. al.* 1997). While, Sels, et. al. (1997) pointed that siblings with maternal A2, B51 haplotype showed a positive association. Further more, HLA class-I alleles A2, B27, and B35 showed significant frequencies in the affected offspring (Moròldo, *et. al.* 1998).

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In this study, we attempted to shed light on the association between class-I HLA molecules and patients with RA in familial and sporadic cases.

Patients & Methods:

Microlymphocytotoxicity assay was used to asses HLA-typing of 80 Iraqi Arabs sporadic patients, 53 probands of 25 families, and 94 of their healthy relatives and comparing them with 62 patients with SLE and 107 healthy subjects as control. All patients were diagnosed according to the revised criteria of the American Rheumatism Association. Mycrolymphocytotoxicity assay has been applied for HLA-typing (Terasaki and McClelland, 1964) and modified by Dick, et. al. 197, and Bender, 1984. Health assessment questionnaire was used and RF, ESR, Hb level and CRP were estimated.

Statistical Analysis:

Univariate analysis has been applied for the data depending on logistic regression and the results were reported as odds ratio (OR), which represented the increased or decreased risk for RA.

Results:

The complete demographic pictures of studied groups are listed in the table below:

Table 1. Clinical and demographic features of studying groups

Clinical & demographic features	*Sporadic RA*	*Family related RA*	*Healthy relatives*	*Patient controls*	*Healthy controls*
Female: Male ratio	7: 1	1.5: 1	1: 1.6	7.9: 1	1: 1.02
Age of onset (Mean)	35(±12.5)	28(±13.3)	(-)ve.	26(±10.1)	(-)ve.
Duration of RA (years)	0.1-30	0.1-35	(-)ve.	0.2-29	(-)ve.
Rf positivity (%)	73 (91.3)	51 (96.2)	55 (58.5)	15 (24.2)	1 (0.94)
CRP positivity (%)	39(73.6)	35(71.4)	4(13.8)	29(47.5)	2(1.9)
Total No. of cases	80	53	94	62	107

Table 1. shows that the majority of patients were females in family RA (87.5% vs. 60.4%), as compared to sporadic RA female: male ratio 7: 1 vs. 1.5: 1 respectively.

Age of onset was younger among the familial patients (28 years) than among sporadic patients(35 years) for both male and female. Using ELISA technique, RF positive was higher among familial RA (96.2%) than that for sporadic patients (91.3%), with a significant difference between affected individuals and controls. CRP estimation showed significant elevation in the sera of patients [sporadic and familial] in comparison with control groups.

Table 2 shows that among HLA-A molecules, A10 and A3 6 were considered as risk factors in sporadic patients since they were observed in a highly significant frequencies. However, none of these antigens seems to be significant factors in familial RA although were found in high frequencies.

Negative association was observed between A2 in sporadic and familial RA, and although non-significant with the latter, but highly significant compared with controls (P<.0001).

This table revealed that, HLA-B molecules may play a role in the disease susceptibility; since B5, B21, B22, B27, B40, B41, B47 and B73 existed in high frequencies among patients than in controls [OR=2.4, 2.2, 4.2, 3.0, 2.9, 4.1, 3.5, 1.3 and 2.7].

However, in sporadic patient only B22 and B47 were highly significant. B5, B21 were risk factors in familial patients. B14 was observed in high frequency among healthy controls compared to sporadic patients. There is negative association between familial RA patients and B12 and B16. On the other hand, Cw alleles (Cwl, Cw7) represent an increasing risk for RA since its molecules existed in high frequencies in patients and not in controls: OR=3.0 and 2.7 respectively, which is statically significant (P<0.005 and 0.002 respectively). Cw6 seems to be protective factor [OR=2.7] with significant difference between affected individuals and controls (P< 0.047). However, non of these antigens appeared as a risk factor among familial patients although, Cwl and Cw3 showed the highest frequency [OR= 1.1,1.6 respectively], but it was non-significant statistically. Cw6 is still considered as a protective factor since its frequency was the highest among controls [OR=5.3] although it was statistically non-significant.

Discussion:

It is currently accepted that the incidence autoimmune diseases among females are quite higher than in males. This phenomenon could be due to the hormonal differences between them through their action on the T_hl responses, which are pro-inflammatory may enhance the development of autoimmune disease (Goldsby, *et. al*2000).

The female: male ratio was higher among sporadic patients than in familial cases. This result was comparable to that of Kwoh, *et. al.* 1996. Similar comparable results were observed concerning the age of onset, which was younger among familial affected individuals than in sporadic cases (Kwoh, *et. al.* 1996; Pritchard, 1994). The role of familiality [multiplex families vs. Simplex families] increased the risk of getting RA earlier.

The positive RF was observed in 90.2% of Iraqi patient's sera. This result is slightly higher than other studies, which showed 88% in Nebraska, 87% in Germany, 86% Madrid, and 85% in Colombia (O'dell, *et. al.* 2002; Fransen, *et. al.* 2001, Balsa, *et. al.* 2000 and Anaya, *et. al.* 2002, respectively). The high rate of positive RF in the current study may be related to the high sensitivity of ELISA technique used while other studies used latex agglutination tests. High rate of positive RF clearly reflects the genetic effect in RA as it is higher among familial patients (96.2%) than in sporadic patients (90.3%) (Barrera, *et. al.* 1999) Probands with familial RA were more often RF positive. High rate of positive RF increases the inflammatory effects, which probably reflects the acute phase response shown by high level of CRP in this study.

HLA-typing indicated the prevalence of the disease among those with HLA-A10, B22, B47, Cwl and Cw7 while familial patients possess only B5and B21. However, A10, Cwl and Cw3 observed in high frequencies, although they were statistically non-significant. These results were similar to that of Ad'ahia, 1990, particularly concerning A10 and B5(51).

Negative association was observed with those designated as A2, B14 and Cw6 among sporadic patients while only B12 and B16 were considered as protective factors. These results were comparable to that of Ad'ahia, 1990, particularly for A2 and B16 and varied from other studies, which

reported that B35, A2, B16 (39) were considered as a risk factor (Sels, et: al. 1997; Moroldo, et. al. 1998; Maeda, et. al. 2000).

HLA-B Ag	Sporadic Patients						Familial Patients					
	OR	Inverse OR	P-value	Adjusted P-value	EF	PF	OR	Inverse OR	P-value	Adjusted P-value	EF	PF
5	1.2	**	NS	NS	0.071	**	2.4	**	0.003	0.087	0.331	**
7	0.4	2.4	NS	NS	**	0.065	1.0	**	NS	NS	0.004	**
8	1.5	**	NS	NS	0.057	**	1.4	**	NS	NS	0.069	**
12	1.0	1.0	NS	NS	**	0.003	0.5	2.2	0.035	0.592	**	0.173
13	0.8	1.2	NS	NS	**	0.013	0.9	1.1	NS	NS	**	0.003
14	0.2	4.2	0.028	0.670	**	0.107	3.7	**	NS	NS	0.028	**
15	1.0	1.1	NS	NS	**	0.003	4.5	**	NS	NS	0.069	**
16	1.1	**	NS	NS	0.011	**	0.3	3.1	0.023	0.397	**	0.137
17	2.2	**	NS	NS	0.108	**	0.3	3.6	NS	NS	**	0.061
18	0.5	2.0	NS	NS	**	0.046	1.2	**	NS	NS	0.008	**
21	0.7	1.4	NS	NS	**	0.030	2.2	**	0.012	0.198	0.369	**
22	3.0	**	0.009	0.226	0.159	**	0.8	1.3	NS	NS	**	0.55
27	2.9	**	NS	NS	0.082	**	1.1	**	NS	NS	0.016	**
37	0.9	1.1	NS	NS	**	0.003	0.8	1.3	NS	NS	**	0.07
40	4.1	**	NS	NS	0.028	**	0.4	2.6	NS	NS	**	**
41	3.5	**	NS	NS	0.045	**	3.6	**	NS	NS	0.009	**
42	0.3	3.8	NS	NS	**	**	0.0	**	NS	NS	**	**
47	1.3	**	0.007	0.160	0.003	**	0.0	**	NS	NS	**	**
53	0.4	2.3	NS	NS	**	0.022	0.0	**	NS	NS	**	**
73	2.7	**	NS	NS	0.016	**	2.4	**	NS	NS	0.015	**
Cw-Ag												
1	3.0	**	0.005	0.044	0.183	**	1.1	**	NS	NS	0.033	**
2	1.2	**	NS	NS	0.034	**	1.2	**	NS	NS	0.030	**
3	1.7	**	NS	NS	0.160	**	1.6	**	NS	NS	0.163	**
4	1.0	**	NS	NS	0.005	**	0.8	1.2	NS	NS	**	0.058
5	0.5	2.1	NS	NS	**	0.142	1.1	**	NS	NS	0.024	**
6	0.4	2.7	0.047	0.380	**	0.111	0.2	5.3	NS	NS	**	0.052
7	2.7	**	0.002	0.013	0.306	**	0.9	1.1	NS	NS	**	0.062
8	0.7	1.4	NS	NS	**	0.030	0.4	2.6	NS	NS	**	0.040

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