"Possible Association of HLA Class-I molecules with Rheumatoid Arthritis in Iraqi Patients"

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Summary:

Background: *Rheumatoid Arthritis (RA) is a multisystem inflammatory disease, which primarily affects synovial joints.*
Aim: *to shed light on the possible association of HLA class-I antigens in RA patients in Iraq.*
Subject & methods: *Microlymphocytotoxicity assay has been used to assess HLA-typing of 249 blood samples of 80 RA patients, 62 patient controls (SLE) & 107 healthy normal controls.*
Results: *This study revealed that HLA-A10, B22, B40, Cwl & Cw7 might play a role in RA susceptibility with significant differences between RA and controls (P<0.0001, 0.009, 0.007, 0.005 & 0.002). On the other hand negative association was observed significantly with HLA-A2 (PO.0001) and B14 (PO.028).*
Conclusion: *high RF positivity participated in increasing the inflammatory effects which probably reflects the acute phase response by high level of CRP as this study pointed to.*

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Introduction:

Rheumatoid Arthritis (RA) is a multisystem inflammatory disease, which primarily affects synovial joints. The population prevalence is 0.5-1% (Gabriel, 2001). Many twins studies denote that the heritability of RA is 40-60% (Silman, *et.al.* 1993). Recurrent risk modeling suggested that the genetic susceptibility be encoded by a limited number of genes with significant genetic epistasis (Genin, *et. al.* 1998). These genes are localized at MHC region. The contribution from the MHC was estimated to be ~ 30% of the total genetic effect (Deighton, *et. al.* 1989). Only few studies denoted an association between RA and Class-I antigens in most populations have investigated so far. However, a positive association with the Antigen (A9) was reported in Iranian patients (Nikbin, et. al. 1977), while an increased frequency of B22 and B54 were reported in Japanese RA patients (Maeda, et. al. 1981). Moreover'positive association was observed between A10, A31, B8, B27, B60 & Cw3 (Ad'haiah, 1990). This study was established to shed light on the possible association of HLA class-I antigens and RA patients in Iraq.

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Patients & Methods:

The present study included 80 Arab, Iraqi RA patients compared with 62 (SLE) as patient controls and 107 healthy controls both of the same ethic .All patients were diagnosed according to the revised criteria of the American Rheumatism Association (Arnett,, et. al. 1988 and revised one by Klippel et.al. 2001). Microlymphocytotoxicity assay has been applied for HLA-typing (Terasaki and McClelland, 1964) and modified by Dick, et.al. 1979 and Bender, 1984. Health assessment questionnaire included beside, ESR, hemoglobin level, CRP and RP quantitative estimation. CRP estimated by agglutination technique while RF was estimated by ELISA quantitative method for all sera samples.

Statistical Analysis:

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

Results:

Age distribution and Gender effect showed a high frequency of disease among 40-49 years of age groups as has been listed in table 1.

Table 1. Distribution of RA patients by age and gender in comparison with control groups

Age Groups (Years)	Studying groups					
	RA patients		Patients Controls		Healthy Controls	
	No.	%	No.	%	No.	%
≤20	4	5	4	6.5	7	6.5
20-30	7	8.8	20	32.3	29	27.1
30-39	19	23.8	23	37.1	29	27.1
40-49	28	35	13	21	23	21.5
50-59	15	18.8	1	1.6	13	12.1
60-69	7	8.8	1	1.6	6	5.8
Gender						
Female	70	87.5	55	88.7	53	49.5
Male	10	12.5	7	11.3	54	50.5
Total	80	100	62	100	107	100

Considering gender effect, the current study

revealed that 87.5% of RA patients were female in 3.3: 1 female: male ratio.

Lymphocytotoxicity assay results were listed in the table below:

Table 2.A Frequencies of Class-I HLA-A molecules among RA patients in comparison with healthy controls.

A-Ag	Healthy Controls		RA Patients		OR	Inverse OR	P-value	Adjusted P-value	EF	PF
	No.	%	No.	%						
1	23	21.5	14	17.5	0.8	1.3	NS	NS	**	0.048
2	39	36.4	6	7.5	0.1	7.1	0.0001	0.0001	**	0.313
3	19	17.8	7	8.8	0.4	2.3	NS	NS	**	0.099
9	18	16.8	25	31.3	2.2	**	0.022	0.220	0.173	**
10	31	23.0	48	60.0	3.7	**	0.000	0.0001	0.437	**
11	11	10.3	10	12.5	1.2	**	NS	NS	0.025	**
19	42	39.3	25	31.3	0.7	1.4	NS	NS	**	0.116
21	3	2.8	0	0.0	0.2	5.4	NS	NS	**	**
28	16	15.0	17	21.3	1.5	**	NS	NS	0.074	**
36	0	0.0	3	3.8	9.7	**	0.044	0.439	0.034	**
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The above table showed the frequencies of each molecule of class-I HLA alleles in which HLA-A10 appeared in the high frequency among HLA-A molecules [OR=3.7] with P value <0.0001, however other molecules showed high frequencies though non-significant [such as A9 and A36]. Negative association was revealed by A2 with highly significant difference with Inverse OR=7.1 (PO.0001), Hence this Ag was considered as a protective factor. Among HLA-B molecules B22 and B47 showed as risk factors with ORs of 3.0, 1.3 respectively. Both of significant frequencies (P<0.009, 0.007 respectively). Only B14 appeared as a protective with inverse OR=4.2 (PO.028).

Table 2.B Frequency of HLA-B molecules in RA patients with comparison with normal healthy controls.

B-Ag	Healthy control		RA patients		OR	Inverse OR	P-value	Adjusted P-value	EF	PF
	No.	%	No.	%						
5	2	1.9	0	0.0	1.2	**	NS	NS	0.071	**
7	12	11.2	4	5.0	0.4	2.4	NS	NS	**	0.065
8	12	11.2	13	16.3	1.5	**	NS	NS	0.057	**
12	19	17.8	14	17.5	1.0	1.0	NS	NS	**	0.003
13	8	7.5	5	6.3	0.8	1.2	NS	NS	**	0.013
14	15	14.0	3	3.8	0.2	4.2	0.028	0.670	**	0.107
15	7	6.5	5	6.3	1.0	1.1	NS	NS	**	0.003
16	15	14.0	12	15.0	1.1	**	NS	NS	0.011	**
17	11	10.2	16	20.8	2.2	**	NS	NS	0.108	**
18	10	9.3	4	5.0	0.5	2.0	NS	NS	**	0.046
21	44	41.1	36	45.0	0.7	1.4	NS	NS	**	0.030
22	10	9.3	19	23.8	3.0	**	0.009	0.226	0.159	**
27	5	4.7	10	12.5	2.9	**	NS	NS	0.082	**
37	3	2.8	2	2.5	0.9	1.1	NS	NS	**	0.003
40	1	0.9	3	3.8	4.1	**	NS	NS	0.028	**
41	2	1.9	5	6.3	3.5	**	NS	NS	0.045	**
42	2	1.9	0	0.0	0.3	3.8	NS	NS	**	**
47	1	0.9	1	1.3	1.3	**	0.007	0.160	0.003	**
53	1	0.9	0	0.0	0.4	2.3	NS	NS	**	0.022
73	1	0.9	2	2.5	2.7	**	NS	NS	0.016	**
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HLA-Cw alleles participated in increasing the risk of the disease particularly Cwl with OR of 3.0 (P<0.005) while Cw7 revealed a higher frequency than Cwl with OR = 2.7 with higher significant difference (PO.002). Cw6 seems to be protective [OR= 2.7] although it's frequency was not highly significant (P<0.047).

Table 2.C frequency of HLA-Cw molecule in RA patients in comparison with normal controls.

Cw-Ag	Healthy controls		RA Patients		OR	Inverse OR	P-value	Adjusted P-value	EF	PF
	No.	%	No.	%						
1	12	11.2	22	27.5	3.0	**	0.005	0.44	0.183	**
2	17	15.9	15	18.3	1.2	**	NS	NS	0.034	**
3	29	27.1	31	38.8	1.7	**	NS	NS	0.160	**
4	29	27.1	22	27.5	1.0	**	NS	NS	0.005	**
5	29	27.1	12	15.0	0.5	2.1	NS	NS	**	0.142
6	19	17.8	6	7.5	0.4	2.7	0.047	0.380	**	0.111
7	28	26.2	39	48.8	2.7	**	0.002	0.013	0.306	**
8	11	10.5	6	7.5	0.7	1.4	NS	NS	**	0.030
Blank	37		7							

The baseline demographic and clinical variables in 80 patients and controls were listed in table 3.

Table 2.C frequency of HLA-Cw molecule in RA patients in comparison with normal controls.

Cw-Ag	Healthy controls		RA Patients		OR	Inverse OR	P-value	Adjusted P-value	EF	PF
	No.	%	No.	%						
1	12	11.2	22	27.5	3.0	**	0.005	0.44	0.183	**
2	17	15.9	15	18.3	1.2	**	NS	NS	0.034	**
3	29	27.1	31	38.8	1.7	**	NS	NS	0.160	**
4	29	27.1	22	27.5	1.0	**	NS	NS	0.005	**
5	29	27.1	12	15.0	0.5	2.1	NS	NS	**	0.142
6	19	17.8	6	7.5	0.4	2.7	0.047	0.380	**	0.111
7	28	26.2	39	48.8	2.7	**	0.002	0.013	0.306	**
8	11	10.5	6	7.5	0.7	1.4	NS	NS	**	0.030
Blank	37		7							

The demographic picture of Iraqi patients revealed that the mean of age was 45 years in comparison with 32, 34 years for control groups: patients and healthy controls respectively.

RF positivity among patients was significantly higher than that for control groups (90.3%, 24.2% and 0.0% for RA patients, patient controls and healthy controls respectively).

Severity of RA disease that resulted in surgical treatment was highly significant in comparison with controls (6.3% in comparison with 0% for both control groups).

It was appeared that disease onset was 34.7 years for RA while it was earlier among patient controls (mean of onset was 25.8 years).

Most RA patients showed CRP test positivity (79.2%) in comparison with (47.5%) for patient controls. Non of healthy controls showed positive reaction.

Discussion:

In spite of so many studies, which have been carried out on HLA class-II disease association, few indicated the role of class-I allelcs. The current study denoted the role of A10, B22, B47, Cwl and Cw7 as highly significant risk factors. Ad'haiah, 1990 revealed that A10 (26), A31, B8, B27, B60, B62 and Cw3, have the highest frequencies among patients, which shared results of present study in correlation of A10, with the disease. However B8 was observed in high frequency besides B27 though both were non-significant. On the other hand Agrawal, et. al., (1996) declared that RA patients showed an increased frequency of HLA-A2 and B40 Ags compared to healthy controls (P <0,001) at Varanasi, India. The current study indicated the presence of negative association of RA with A2 molecule, while B40 was observed in high frequency in the affected individuals, even though it is non significant (OR=4.I). As have been mentioned previously, Nikbin, et. al. 1977 reported that A9 associated with RA in Iranian patients, while Toyoda, et. al. 1977 observed an increased frequency of B22 in Japanese patients with RA, so Macda, et. al. 1981 did. The last work was comparable to this present work. The demographic picture showed that the mean age of patients was 45.0 ±12.5 years. This result was to some extent similar to that of Rahccm, 2003 (42.1) and lower than that of Anaya, et. al. 2002 (47±12.7); and Pascual, et. al. 2001 (49±2.5) for Iraqi, Colombian and Spanish patients respectively. The lower level, probably due to the fact that the half-life span of Iraqi is lower than that for European. The reflection of this fact appeared clearly in the age of disease onset (34.7) which, seemed to be too young than that of abroad studies such as 41, 42.5, 40.8, 40.4, and 40.5, as had pointed to by: kwoh, et. al. 1996; Timofeev, et. al. 2000; Gran and Nordvag, 2000; Pascual, et. al. 2001 and Anaya, et. al. 2002 respectively. The positivity of RF was observed in 90.2% of patient's sera. While other studies showed 88% in Nebraska, 87% in Germany and 85% in Colombia as has been mentioned by O'dell, et. al. 2002; Fransen, et. al. 2001 and Anaya, et. al. 2002, respectively. The high RF positivity in the current study may be related to high sensitivity of ELISA technique which, has been used rather than latex agglutination test that used by the others. High RF positivity participated in increasing the inflammatory effects which probably reflects the acute phase response by high level of CRP as this study pointed to.

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