Possible association of HLA class I and II molecules with Ulcerative colitis in Iraqi patients

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Abstract

Background: The etiology and pathogenesis of ulcerative colitis has not yet been elucidated. However, attention has been brought to the belief that a genetic factor plays a crucial role in the development of the disease.

Objectives: This study was established to shed light on the possible association of HLA class I and class II antigen with ulcerative colitis.

Methods: The study included 105 subjects .It comprised of 35 ulcerative colitis patients and 70 apparently healthy controls. Lymphocytotoxicity assay has been used to assess HLA- typing.

***Results*:** Comparison between ulcerative colitis patients and healthy control showed positive significant associations of HLA –B14, B27 and DR52 antigens with patients, however, only B27 maintained a significant P value after adjustment. Meanwhile the frequencies of HLA -B35, B41 and Cw6 were decreased in patients when compared with control group.

Conclusions: These findings demonstrated that HLA –B14, B27 and DR52 might play a role in ulcerative colitis susceptibility, while HLA-B35, B41 and Cw6 may confer protective effects against ulcerative colitis.

Keywords: Ulcerative colitis, Genetic factors, HLA.

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Introduction

Ulcerative Colitis (UC) and Crohn's Disease (CD) are classified as the chronic idiopathic forms of Inflammatory Bowel Disease (IBD). Ulcerative Colitis is an inflammatory disorder that affects the rectum and colon [1, 2]. All races and ethnic groups in the world may have UC; however, there is a clear higher incidence in developed countries compared with the less-developed one. Although the etiology and pathogenesis of this disorder has not yet been elucidated, there has been a significant progress in recent years regarding the potential genetic and environmental factors that contribute to the pathogenesis of UC in animal models and human disease [3].

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The important clue has come from genetic-epidemiological studies, which have suggested a role of genetic factors in the etiology of the disease. Human Leukocyte Antigen (HLA) genes are a good candidate for such role, Stasangi *et al* [4] reported that genes of major histocompatibility complex are implicated as important inherited determinants of susceptibility to UC and may also influence the pattern of disease. Another study done by Trachtenberg *et al* [5] demonstrated an association between UC and HLA class II genes, and mentioned that the interaction between DR and DQ may determine the extent of a disease risk, on the other hand, Shorter and associates [6] observed a limited association between UC and the expression of HLA –B5.

The large majority of reported association studies in UC have denoted a positive association with HLA –DR2 and a negative association with HLA-DR4. The most significant association between this disease and HLA–DR2

have been observed in Japanese population, moreover, it has been observed in pooled Caucasian population studies [7,8]. The present study was established to shed light on the possible association of HLA class I and class II antigen with ulcerative colitis.

Subjects and Methods

Subjects:

The present study included 35 Iraqi UC patients (16 females and 19 males; mean age was 41.6±15.7 years (ranged between 18-83 years). They were attending the Gastroenterology and Hepatology Teaching Hospital". The result of them was compared with the results of 70 healthy (age and gender matched) control group.

HLA-Typing

Microlymphocytotoxicity assay was applied for HLA-typing as described by Terasaki and MeClelland [9] and modified by Dick *et al*, and Bender [10, 11]. The isolation of lymphocytes from the whole blood by a single step centrifuged technique (density gradient centrifugation). T and B-lymphocytes were separated by a nylon wool column before testing for HLA DR and DQ antigens.

Statistical analysis

Univariate analysis was applied for the data depending on logistic regression and the results were reported as odds ratio (ORs). The results were presented as percentage frequencies, and the analysis included estimating the EF and PF values. An estimate was considered statistically significant if its P value was less than an α level of significance of 0.05. Adjustment for the hypothetically proposed increase in the alpha level of significance in case of multiple comparisons of different HLA antigens was done by multiplying the P value by the number of comparisons in each HLA locus (Emery, 1976 and Sorlie, 1995).

Results

A total of 35 patients were typed for HLA class I and II antigens. The frequency distribution of various class I and II antigens for studied groups are presented in table (1). Comparison between UC patients and healthy controls showed several antigens deviations in their frequencies. HLA-B14, B27 and DR52 were observed with increased frequencies in the patients (25.7, 34.3 and 17.1%, respectively) versus healthy controls (5.7, 4.3 and 4.3% respectively), with P-values of 0.007, <0.001 and 0.039 respectively, however, only B27 maintained a significant P value after adjustment. In contrast HLA-B35, B41 and Cw6 were observed with decreased frequencies in patients when compared with controls.

Table1:Antigens frequency of the HLA class I (A,B and C)and class II(DR and DQ)of the U C patients and healthy controls

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	Ulacerative colitis (n=35)		Healthy controls (n=70)		OR	Inverse OR	Chi	P	Adjusted P	EF	PF
	N	%	N	%							
HLA-A locus											
1	10	28.6	16	22.9	1.4	**	0.41	0.52*[NS]*	**	0.074	**
2	9	25.7	25	35.7	0.6	1.6	1.06	0.3*[NS]*	**	**	0.135
3	4	11.4	14	20.0	0.5	1.9	1.18	0.28*[NS]*	**	**	0.097

9	2	5.7	3	4.3	1.4	**	0.10	0.75[NS]	**	0.015	**
10	4	11.4	1	1.4	8.9	**	3.69	0.05[NS]	**	0.101	**
11	7	20.0	8	11.4	1.9	**	1.37	0.24[NS]	**	0.097	**
23	2	5.7	3	4.3	1.4	**	0.10	0.75[NS]	**	0.015	**
24	12	34.3	13	18.6	2.3	**	3.09	0.08[NS]	**	0.193	**
25	2	5.7	1	1.4	4.2	**	1.33	0.25[NS]	**	0.043	**
26	2	5.7	7	10.0	0.5	1.8	0.53	0.47[NS]	**	**	0.045
28	5	14.3	9	12.9	1.1	**	0.04	0.84[NS]	**	0.016	**
29	0	0.0	3	4.3	0.3	3.7	1.31	0.25[NS]	**	**	**
30	3	8.6	12	17.1	0.5	2.2	1.35	0.25[NS]	**	**	0.094
31	0	0.0	2	2.9	0.4	2.6	0.66	0.42[NS]	**	**	**
32	0	0.0	2	2.9	0.4	2.6	0.66	0.42[NS]	**	**	**
33	1	2.9	4	5.7	0.5	2.1	0.40	0.53[NS]	**	**	0.029
34	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
36	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
Blank	7		15								
HLA-B locus											
5	1	2.9	3	4.3	0.7	1.5	0.13	0.72[NS]	**	**	0.015
7	3	8.6	7	10.0	0.8	1.2	0.06	0.81[NS]	**	**	0.016
8	2	5.7	7	10.0	0.5	1.8	0.53	0.47[NS]	**	**	0.045
13	0	0.0	5	7.1	0.2	6.0	2.64	0.1[NS]	**	**	**
14	9	25.7	4	5.7	5.7	**	7.32	0.007	0.22[NS]	0.212	**
15	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
17	2	5.7	4	5.7	1.0	**	0.00	1[NS]	**	**	**
18	0	0.0	5	7.1	0.2	6.0	2.64	0.1[NS]	**	**	**
21	1	2.9	1	1.4	2.0	**	0.25	0.62[NS]	**	0.014	**
22	1	2.9	0	0.0	6.1	**	2.13	0.14[NS]	**	0.024	**
27	12	34.3	3	4.3	11.7	**	12.69	0.000	0.012	0.313	**
35	0	0.0	12	17.1	0.1	15.2	6.59	0.010	0.33[NS]	**	**
37	0	0.0	2	2.9	0.4	2.6	0.66	0.42[NS]	**	**	**
38	3	8.6	5	7.1	1.2	**	0.07	0.8[NS]	**	0.015	**
39	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
40	0	0.0	2	2.9	0.4	2.6	0.66	0.42[NS]	**	**	**
41	0	0.0	9	12.9	0.1	11.0	5.01	0.025	0.8[NS]	**	**
44	5	14.3	6	8.6	1.8	**	0.80	0.37[NS]	**	0.063	**
45	1	2.9	1	1.4	2.0	**	0.25	0.62[NS]	**	0.014	**
47	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
49	0	0.0	5	7.1	0.2	6.0	2.64	0.1[NS]	**	**	**
50	2	5.7	8	11.4	0.5	2.1	0.85	0.36[NS]	**	**	0.061
51	3	8.6	14	20.0	0.4	2.7	2.12	0.15[NS]	**	**	0.125
52	0	0.0	3	4.3	0.3	3.7	1.31	0.25[NS]	**	**	**
53	0	0.0	3	4.3	0.3	3.7	1.31	0.25[NS]	**	**	**
56	1	2.9	1	1.4	2.0	**	0.25	0.62[NS]	**	0.014	**
57	1	2.9	1	1.4	2.0	**	0.25	0.62[NS]	**	0.014	**
62	1	2.9	1	1.4	2.0	**	0.25	0.62[NS]	**	0.014	**
63	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**

70	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
Blank	22		23								
HLA-Cw locus											
1	4	11.4	2	2.9	4.4	**	2.74	0.1[NS]	**	0.088	**
2	3	8.6	5	7.1	1.2	**	0.07	0.8[NS]	**	0.015	**
3	1	2.9	4	5.7	0.5	2.1	0.40	0.53[NS]	**	**	0.029
4	3	8.6	16	22.9	0.3	3.2	2.97	0.08[NS]	**	**	0.156
5	0	0.0	2	2.9	0.4	2.6	0.66	0.42[NS]	**	**	**
6	0	0.0	9	12.9	0.1	11.0	5.01	0.025	0.2[NS]	**	**
7	4	11.4	13	18.6	0.6	1.8	0.86	0.35[NS]	**	**	0.081
8	0	0.0	1	1.4	0.7	1.5	0.12	0.73[NS]	**	**	**
Blank	55		88								
HLA-DR locus											
1	1	2.9	8	11.4	0.2	4.4	1.87	0.17[NS]	**	**	0.088
2	12	34.3	17	24.3	1.6	**	1.16	0.28[NS]	**	0.132	**
3	8	22.9	16	22.9	1.0	**	0.00	1[NS]	**	**	**
4	10	28.6	16	22.9	1.4	**	0.41	0.52[NS]	**	0.074	**
5	2	5.7	3	4.3	1.4	**	0.10	0.75[NS]	**	0.015	**
6	0	0.0	4	5.7	0.2	4.8	1.98	0.16[NS]	**	**	**
7	10	28.6	15	21.4	1.5	**	0.65	0.42[NS]	**	0.091	**
8	1	2.9	8	11.4	0.2	4.4	1.87	0.17[NS]	**	**	0.088
9	0	0.0	6	8.6	0.1	7.2	3.26	0.07[NS]	**	**	**
10	5	14.3	8	11.4	1.3	**	0.17	0.68[NS]	**	0.032	**
11	0	0.0	6	8.6	0.1	7.2	3.26	0.07[NS]	**	**	**
12	0	0.0	3	4.3	0.3	3.7	1.31	0.25[NS]	**	**	**
13	1	2.9	4	5.7	0.5	2.1	0.40	0.53[NS]	**	**	0.029
14	2	5.7	3	4.3	1.4	**	0.10	0.75[NS]	**	0.015	**
15	2	5.7	11	15.7	0.3	3.1	1.98	0.16[NS]	**	**	0.106
52	6	17.1	3	4.3	4.6	**	4.26	0.039	0.66[NS]	0.134	**
53	4	11.4	3	4.3	2.9	**	1.78	0.18[NS]	**	0.075	**
Blank	6		6								
HLA-Dq locus											
1	10	28.6	26	37.1	0.7	1.5	0.76	0.38[NS]	**	**	0.120
2	10	28.6	18	25.7	1.2	**	0.10	0.76[NS]	**	0.038	**
3	7	20.0	25	35.7	0.5	2.2	2.65	0.1[NS]	**	**	0.196
4	8	22.9	16	22.9	1.0	**	0.00	1[NS]	**	**	**
Blank	35	100.0	55	78.6							

Discussion

Susceptibility to IBD is partially genetically determined and the HLA genes are candidates for a role in genetic susceptibility to IBD, because their products play a central role in immune response (12).

In the present study, there was a positive significant association of

HLA- B 14, B 2 7 and DR52 in patients with UC as compared with healthy group, however; only B27 maintained a significant P value after adjustment. This result is in agreement with other studies [5, 13, 14], while at variance with the result of Perri and colleagues [15], who demonstrated that patients with UC had a significantly increased frequency of DQ6 and D Q 7 and a decreased frequency of DQ5 and DQ8 when compared with ethnically matched healthy controls, as well as no significant differences in HLA class 1 and HLA-DR antigens was observed. Also present study showed decreased frequencies of HLA-B35, B41 and Cw6 in patients when compared with controls, so, might confer protective effects against ulcerative colitis.

The discrepancies emerged in this study compared with other studies could be, in part, probably due to the highly modified techniques they used for the same purposes, e.g, PCR, however, if the environmental and genetic factors excluded, which yet to be elucidated, the small number of our study groups may be a considerable contributor to these discrepancies.

HLA-B27 showed a significant high frequency in patient group as compared with healthy control. The appearance of HLA-B27 antigen among UC patient who suffering from extraintestinal manifestation (arthralgia), can support involvement of autoimmunologic mechanism in joint inflammation, that B27 antigen was found to be correlated with arthralgia in the UC patients [13]. This result which mimicked what reported by many authors; Tiwana and colleagues [16] referred to a significant HLA associations identified with specific extraintestinal manifestations of IBD, with the best characterized one was the association of Ankylosing Spondylitis (AS) with HLA-B27. Ankylosing spondylitis has been observed to be associated with UC, an association, which may contribute, to differences in endogenous peptide presentation by HLA-B27 subtypes and be relevant in the disease pathogenesis. HLA associations here possibly involve arthritic peptides and molecular mimicry; molecular analysis has shown that Klebsiella microbes possess antigens, which cross-react with self-antigens, such as HLA-B27 and spinal collagens [17]. It is thought that perhaps AS starts when the defenses of the intestine start breaking down and bacteria from the intestine pass into the blood stream directly into the region where the sacroiliac joints are located.

In conclusion, these findings demonstrated that HLA-B14, B27 and DR52 might play a role in UC susceptibility, suggesting HLA-based different etiopathogenesis, in addition some of these molecules might play a role in development of some extraintestinal manifestations observed in UC patients. On the other hand HLA-B35, B41 and Cw6 might confer protective effects against UC.

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