The Implications of HLA Phenotypes in Inflammatory Bowel Disease.

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الخلاصة

من العوامل المهيأة للإصابة بمرض الأمعاء الالتهابي(IBD) هي العوامل الوراثية , ومنها مورثات مستضدات الكريات البيض البشرية التي يحتمل أنها تلعب دورا" في زيادة الأستعدادية للإصابة بهذا المرض بسبب دور الجزيئات المنتجة من هذه المورثات في الاستجابة المناعية . أشارت العديد من الدراسات إلى وجود ارتباطات بين الطرز المظهرية لهذه الجزيئات مع إحدى الحالتين الرئيسيتين لهذا المرض وهما التهاب القولون التقرحي (UC) ومرض كرون , (CD) لكنها لا زالت مثيرة للجدل . ولغرض تقييم مثل هذه الارتباطات , ولإثبات دور هذه الجزيئات كعامل مسبب (EF) أو عامل وقائي , (PF) أجريت الدراسة الحالية على واحد وسبعون مريضا" عراقيا" عربيا" (55 مصابا"بمرض UC و 16 مصابا" بمرض (CD بالمقارنة مع سبعون من الأصحاء كمجموعة ضابطة . خضع الجميع إلى فحص سمية الخلايا اللمفاوية المصلي باستخدام مجموعة من الأضداد وحيدة النسيلة لهذه المستضدات الصنف الأول والثاني . أظهرت النتائج وجود ارتباطا" موجبا" مهما" من الناحية الإحصائية مع مستضدات الصنف الأول التالية : A23 A(16+66), B27, بنسب تكرار 18.9, 9.4 , 4.8 على التوالي , مزيدة بذلك احتمالية لعبها لدور مهيأ لهذا المرض , في حين كان المستضد DQ2 من مستضدات الصنف الثاني الوحيد الذي يلعب نفس الدور بنسبة تكرار 2.6 . من جهة ثانية , أظهرت المستضدات DR8 : A2, Bw4, Cw5, DR3,ارتباطا" سلبيا" قد يلعب دورا" وقائيا" ضد المرض المذكور حيث تكررت بنسب 0.3, 0.1, 0.2, 0.1, 0.4 على التوالي . يستنتج من هذه الدراسة دورا" لهذه المستضدات من كلا الصنفين في رفع أو خفض استعدادية الأشخاص للإصابة بمرض الأمعاء الالتهابي . ويوصى بإجراء دراسة جينية لتحديد دور هذه المورثات بصورة أدق .

Abstract

Susceptibility to Inflammatory Bowel Disease (IBD) is, in part, genetically determined, and the HLA genes are candidate for a role in the genetic susceptibility to this disease, because their products play a central role in the immune responses. Multiple studies have reported associations between HLA phenotypes and either Ulcerative Colitis (UC) or Crohn's Disease (CD), the major two disorders of IBD, but much of these data are still controversial. To estimate overall associations between HLA phenotypes and this disease, and to establish

the probable etiologic or protective functions conferred by these molecules, a total of 71 Iraqis, Arabs patients (55 with UC and 16 with CD) compared with ethnically matched, 70 healthy control group were assayed for a panel of monoclonal antibodies for HLA class I and II, using microlymphocytotoxicity test. Among class I molecules; A23, A(16+66), and B27, were positively associated with the disease (Odds ratio: 18.9, 9.4, and 4.8, respectively), conferred etiologic roles. The DQ2 was the only one of class II molecules that played same role (OR 2.6). On the other hand, A2, Bw4, Cw5, DR3, and DR8, were found to be negatively associated with IBD suggesting a protective role at respective ORs; 0.4, 0.1, 0.2, 0.1, and 0.3. Thus, IBD is associated with specific class I and II molecules that may play roles in the etiology or in prevention of this disease. Further studies required to determine allelic variants of HLA-genes in this study.

Introduction

Inflammatory Bowel Disease (IBD) is the condition that results from inappropriate activation of the immune system in the intestinal mucosa. The etiology of this disease is still enigmatic, whether the trigger of such immune response is genetics, environment, infections, are as-yet unidentified [(1).] Genetic factors implications were emerged from twins studies and from familial aggregations for UC and CD, the two major forms of IBD. Putative associations of this disease and polymorphic genes that are located in the Major Histocompatibility Complex (MHC) on the short arm of chromosome 6 have been subject of intensive researches [(2,3)]. The genetic studies have shown that persons who have certain HLA alleles have a higher risk of specific autoimmune diseases than persons without these alleles, moreover, other alleles were seemed to be protective against other certain diseases [(4,5,6)]. Such associations were widely studied and their explanations were diversely hypothesized; deficiencies of the HLA molecules as a system malfunction that may result when responsible genes falter in their expression [(7)], abnormalities of genes linked to the HLA complex [(8)], non-fulfillment of antigens expression by HLA molecules, increased susceptibility for infectious diseases [(9)], finally, the self reactive T cells may be activated by complex of certain HLA molecules with particular self peptides, a condition of autoimmune diseases [(10,11)].

Whether IBD caused by infections, commensals, or an autoimmune reaction as hypothesized by many authors, the HLA molecules may have a role in the etiology, which was the aim of this study.

Patients and Methods

Between Jan. and Jul. 2005, seventy-one Iraqis, Arab patients group with IBD (55 with UC and 16 with CD) compared with 70 apparently healthy, ethnically matched control group were involved in this study. The diseased individuals have been already had verified diagnosis in the GIT and hepatic diseases teaching hospital in Baghdad, based on well-established clinical, endoscopical, and histological criteria, all were regularly attending for programmed therapy and follow-up.

A questionnaire was designed to gather initial data regarding clinical manifestations, the duration of the disease, surgical interventions in addition to the age and sex.

A volume of 10-20 ml blood was obtained from each individual in both groups and added into heparinized tubes and send to HLA unit in the teaching laboratories/medical city /Baghdad, as soon as possible. For HLA-typing, the microlymphocytotoxicity test was used, a test which established by Terrasaki and McClelland, 1964 [(12)], and modified by Dick, et al., 1979 [(13)], and Bender, 1984 [(14)]. Briefly, the lymphocytes pellet was isolated based on density gradient cooled centrifugation on Lymphoprep solution, then the B cells separated from T cells utilizing nylon wool, both cells were examined for viability using Trypan Blue stain, the T cells were assayed for class I HLA (A, B and C), while B cells for class II (DR and DQ) [(15)]. For this purpose, a panel of monoclonal anibodies for both classes was used, each interact with it's respective antigen on the lymphocytes, the addition of rabbit's complement render the positive cells penetratable by colored stain, Eosin solution 5%, the reaction was fixed by Formaldehyde and examined under inverted phase-contrast microscope.

Results

A total of seventy-one IBD patients (55 with UC and 16with CD) were studied, 39 males and 32 females (1.2:1 ratio). Their distribution over age intervals was shown in fig. 1, where the high numbers (45%) of them were occurred in $\geq$ 40 years old, and 35.3% of them were found at 30-39, while only 19.7% have had either diseases at $\leq$ 30 years old.

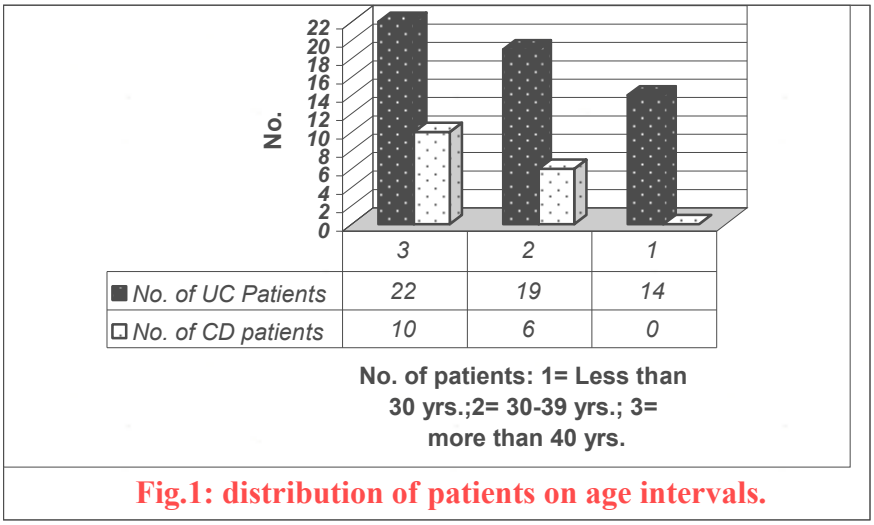

Fig.1: distribution of patients on age intervals.

Figure 2 shows that 45% of the patients were had disease duration of ≤ 5 years, while 36.6% and 18.3% of them were developed either diseases for 5-9 and ≥ 10 years ago, respectively. HLA-typing results obtained for HLA class I are summarized in table 1. Among HLA-A alleles, A23 and A (26+66) were the only antigens that are observed to be significantly increased in their frequencies in IBD patients compared with healthy controls (OR.s; 18.9 and 9.4, P≤: 0.006 and 0.043, respectively), however, other increased antigen frequencies were not attained statistical significance. On the other hand, A2 was reported in low frequency in those patients suggesting preventive factor of PF 0.292.

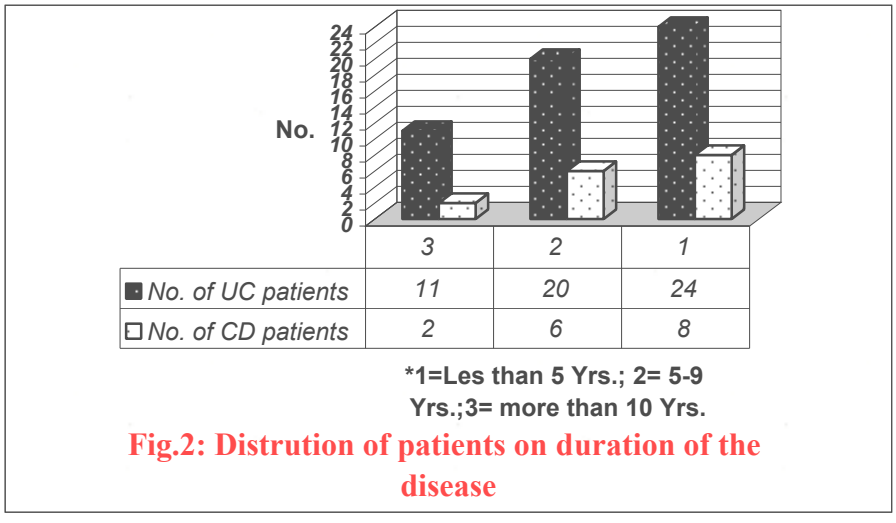

Fig.2: Distrution of patients on duration of the disease

Table 1-a: Frequencies of HLA-A, their Odds ratio, Etiologic and Preventive fractions in IBD patients compared with healthy controls.

	Inflammatory Bowel Disease		Healthy Controls		OR	Inverse OR	P	Adjusted P	EF	PF
	N	%	N	%						
HLAAantigen										
A1+36+80	18	25.4	27	38.6	0.5	1.8	0.09[NS]	**	**	0.177
A2	18	25.4	33	47.1	0.4	2.6	0.008	0.11[NS]	**	0.292
A3	8	11.3	14	20	0.5	2.0	0.16[NS]	**	**	0.099
A9 (23+24)	6	8.5	1	1.4	6.4	**	0.09[NS]	**	0.072	**
A10(25+26+43+66)	7	9.9	7	10	1.0	1.0	0.98[NS]	**	**	0.002
A11	15	21.1	17	24.3	0.8	1.2	0.65[NS]	**	**	0.040
A23	8	11.3	0	0	18.9	**	0.006	0.08[NS]	0.107	**
A24	15	21.1	11	15.7	1.4	**	0.41[NS]	**	0.064	**
A25	5	7	4	5.7	1.3	**	0.75[NS]	**	0.014	**
A(26+66)	4	5.6	0	0	9.4	**	0.043	0.61[NS]	0.050	**
A(28+34)	11	15.5	7	10	1.7	**	0.33[NS]	**	0.061	**
A29	1	1.4	0	0	3.0	**	0.37[NS]	**	0.009	**
A30	7	9.9	10	14.3	0.7	1.5	0.42[NS]	**	**	0.049
A(31, 30)	0	0	0	0						
A(32, 25)	0	0	0	0						
A33	6	8.5	4	5.7	1.5	**	0.53[NS]	**	0.029	**
A34	0	0	0	0						
A-blank (at one haplotyp)	13	18.3	5	7.1						
A-blank(at both haplotypes)	0	0	0	0						
Total	71	100	70	100						

	Inflammatory Bowel Disease	Healthy Controls	OR	Inverse OR	P	Adjusted P	EF	PF

	Inflammatory Bowel Disease		Healthy Controls		OR	In vers	P	Adjusted P	EF	PF
	N	%	N	%						
HLA-B antigen										
Bw4	1	1.4	12	17.1	0.1	14.5	0.011	0.34[NS]	**	0.159
B5 (51+52)	7	9.9	2	2.9	3.7	**	0.11[NS]	**	0.072	**
Bw6	0	0	0	0						
B7 (73)	5	7	8	11.4	0.6	1.7	0.37[NS]	**	**	0.047
B8	6	8.5	9	12.9	0.6	1.6	0.4[NS]	**	**	0.048
B12 (44+45)	2	2.8	0	0	5.1	**	0.16[NS]	**	0.022	**
B13	0	0	3	4.3	0.1	7.4	0.08[NS]	**	**	**
B14 (64+45)	4	5.6	2	2.9	2.0	**	0.42[NS]	**	0.028	**
B(15+57)	1	1.4	2	2.9	0.5	2.1	0.56[NS]	**	**	0.015
B16 (38+39)	9	12.7	9	12.9	1.0	1.0	0.97[NS]	**	**	0.002
B17 (57+58)	4	5.6	8	11.4	0.5	2.2	0.23[NS]	**	**	0.061
B18	2	2.8	6	8.6	0.3	3.2	0.16[NS]	**	**	0.059
B21 (49+50)	4	5.6	3	4.3	1.3	**	0.71[NS]	**	0.014	**
B22 (54+55+56)	2	2.8	5	7.1	0.4	2.7	0.25[NS]	**	**	0.044
B27	19	26.8	5	7.1	4.8	**	0.004	0.11[NS]	0.212	**
B(35+53)	5	7	7	10	0.7	1.5	0.53[NS]	**	**	0.032
B37	1	1.4	2	2.9	0.5	2.1	0.56[NS]	**	**	0.015
B38	8	11.3	3	4.3	2.8	**	0.14[NS]	**	0.073	**
B39	0	0	2	2.9	0.2	5.2	0.16[NS]	**	**	**
B40 (80+61+48)	0	0	2	2.9	0.2	5.2	0.16[NS]	**	**	**
B41	1	1.4	5	7.1	0.2	5.4	0.13[NS]	**	**	0.058
B44	12	16.9	9	12.9	1.4	**	0.5[NS]	**	0.046	**
B45	3	4.2	2	2.9	1.5	**	0.66[NS]	**	0.014	**
B(47+40)	2	2.8	1	1.4	2.0	**	0.58[NS]	**	0.014	**
B49 (52)	4	5.6	3	4.3	1.3	**	0.71[NS]	**	0.014	**
B51	7	9.9	11	15.7	0.6	1.7	0.3[NS]	**	**	0.065
B55	5	7	1	1.4	5.2	**	0.14[NS]	**	0.057	**
B56	3	4.2	1	1.4	3.0	**	0.34[NS]	**	0.028	**
B57	0	0	0	0						
B(60+48)	3	4.2	0	0	7.2	**	0.08[NS]	**	0.036	**
B(62+75)	2	2.8	2	2.9	1.0	1.0	0.99[NS]	**	**	0.000
B70 (10+21+62)	0	0	2	2.9	0.2	5.2	0.16[NS]	**	**	**
B73	0	0	0	0						
B-blank (at one haplotype)	20	28.2	13	18.6						
B-blank (at both haplotypes)	0	0	0	0						
Total	71	100	70	100						

Table 1-b: Frequencies of HLA-B, their Odds ratio, Etiologic and Preventive fractions in IBDpatients compared with healthy controls.

Table 1-b was listed the HLA-B27 is the unique that significantly increased in these patients ($p \leq 0.004$) revealing an etiologic fraction of 0.212, while Bw4 was observed as preventive against this diseases at 0.159 PF ($p \leq 0.011$). The HLA-Cw5 was decreased in it's expression (OR 0.2) that may conferred prevention at PF 0.212 ($p \leq 0.003$), which has shown to be significant even after adjustment of P value for ≤ 0.018 (Table 1-c).

	N	%	N	%						
HLA-Cw antigen										
Cw1	6	8.5	2	2.9	3.1	**	0.17[NS]	**	0.058	**
Cw2	8	11.3	5	7.1	1.7	**	0.4[NS]	**	0.045	**
Cw3	6	8.5	11	15.7	0.5	2.0	0.19[NS]	**	**	0.080
Cw4	6	8.5	14	20	0.4	2.7	0.06[NS]	**	**	0.127
Cw5	4	5.6	18	25.7	0.2	5.8	0.003	0.018	**	0.212
Cw6	10	14.1	12	17.1	0.8	1.3	0.62[NS]	**	**	0.036
Cw7	12	16.9	8	11.4	1.6	**	0.35[NS]	**	0.062	**
Cw-blank (at one haplotype)	44	62	66	94.3						
Cw-blank (at both haplotype)	23	32.4	2	2.9						
Total	71	100	70	100						

Table 1-c: Frequencies of HLA-C, their Odds ratio, Etiologic and Preventive fractions in IBD patients compared with healthy controls.

Of HLA-II antigens, the HLA-DQ2 was the only one that gather significance in it's increased frequency among patients (OR 2.6, EF 0.157, P≤ 0.037), whereas, DR3 and DR8 were observed to be negatively associated with this disease suggesting prevention at PF; 0.176 and 0.121, respectively. A number of class II HLA were shown in increased or decreased frequencies, but the differences were not statistically significant (Table 2).

Discussion

The associations between autoimmune-diseases and certain HLA molecules were extensively studied and established by many authors, such associations were varied in strength in different diseases studied [(10)]. For instance, one of the strongest associations is that between HLA-B27.

Table 2: Frequencies of HLA-DR and -DQ, their Odds ratio, Etiologic and Preventive fractions in IBD patients compared with healthy controls.

	Inflammatory Bowel Disease		Healthy Controls		OR	Inverse OR	P	Adjusted P	EF	PF
	N	%	N	%						
HLA-DR antigen										

	Inflammatory Bowel Disease		Healthy Controls							
	N	%	N	%	OR	Inverse OR	P	Adjusted P	EF	PF
DR1	10	14.1	14	20	0.7	1.5	0.35[NS]	**	**	0.069
DR2 (15+16)	20	28.2	14	20	1.6	**	0.26[NS]	**	0.102	**
DR3	2	2.8	14	20	0.1	8.6	0.006	0.11[NS]	**	0.176
DR4	8	11.3	11	15.7	0.7	1.5	0.44[NS]	**	**	0.050
DR5 (11+12)+8	6	8.5	3	4.3	2.1	**	0.32[NS]	**	0.044	**
DR6 (13+14)+3	1	1.4	6	8.6	0.2	6.6	0.09[NS]	**	**	0.072
DR7	7	9.9	14	20	0.4	2.3	0.1[NS]	**	**	0.113
DR8	4	5.6	12	17.1	0.3	3.5	0.040	0.76[NS]	**	0.121
DR8+12, 1404	0	0	1	1.4	0.3	3.1	0.36[NS]	**	**	**
DR10	9	12.7	5	7.1	1.9	**	0.28[NS]	**	0.060	**
DR11	1	1.4	3	4.3	0.3	3.1	0.33[NS]	**	**	0.029
DR12	8	11.3	9	12.9	0.9	1.2	0.77[NS]	**	**	0.018
DR12+8	1	1.4	3	4.3	0.3	3.1	0.33[NS]	**	**	0.029
DR(13+12+11+3)	1	1.4	2	2.9	0.5	2.1	0.56[NS]	**	**	0.015
DR14	3	4.2	4	5.7	0.7	1.4	0.69[NS]	**	**	0.015
DR15	3	4.2	3	4.3	1.0	1.0	0.99[NS]	**	**	0.001
DR17 (13)	0	0	1	1.4	0.3	3.1	0.36[NS]	**	**	**
DR52 (8)	13	18.3	5	7.1	2.9	**	0.05[NS]	**	0.120	**
DR53	7	9.9	2	2.9	3.7	**	0.11[NS]	**	0.072	**
DR(11+13)	0	0	0	0						
DR-blank (at one haplotype)	36	50.7	14	20						
DR-blank (at both haplotypes)	1	1.4	0	0						
Total	71	100	70	100						
HLA-DQ antigen										
DQ1	16	22.5	14	20	1.2	**	0.71[NS]	**	0.032	**
DQ2	18	25.4	8	11.4	2.6	**	0.037	0.11[NS]	0.157	**
DQ3	15	21.1	16	22.9	0.9	1.1	0.8[NS]	**	**	0.022
DQ-blank (at one haplotype)	49	69	34	48.6						
DQ-blank (at both haplotypes)	22	31	34	48.6						
Total	71	100	70	100						

and ankylosing spondylitis, where 90% of whites with this disease carry this allele compared with 9% of controls [(11)]. To our knowledge, little studies have been reported about associations between class I HLA and IBD. An early investigation on a random European

population found a significantly increased frequency of HLA-A7 and decreased frequency for HLA-A9 [16]. These results are in discrepancies with ours, However, the results reviewed in [17], which shown increased frequencies for HLA-A9 and B27, a compatibility with our results may be extracted.

A number of studies have been carried out on class II HLA, as they candidate for associations with this disease [18], the most comparable study was the meta-analysis of Stokkers, et al, 2000 [19], in which, 29 study that reported on HLA-DR/DQ phenotypes or allele frequencies in IBD patients compared with healthy controls, these studies were conducted on different ethnic backgrounds worldwide. This analysis revealed increased frequencies for DR4 and DR5 in ORs of 1.18 for each, and R10 in OR of 1.65, while decreased frequencies were calculated for DR1, RD2 and DR3 in ORs; 0.9, 0.83, and 0.71, respectively, were extracted by the same study in addition to increased frequencies for DQ2, DQ3, and DQ4 in respective ORs of ; 1.14, 1.65, and 1.88, and decreased one for DQ1 (OR 0.63). Regarding HLA-II, the results obtained in our study were compatible for certain degree with the meta-analysis above; however, discrepancies may be emerged from genetics, environment, or even the number of cases studied. Recent studies were relied on DNA genotyping for HLA-II rather than the routinely used serological one [20, 21], a matter that may have led to incomparable results, as this technique has not been used in our country yet. Our results emphasize probable linkage between these molecules and IBD. Much larger number of cases, differential UC and CD study is recommended.

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