Positive Anti-neutrophil Cytoplasmic Antibodies (ANCA) as a function of Human Leukocyte Antigens (HLA) in Inflammatory Bowel Disease

***Hummady AL-Hilaly** **College of Med.\ Dept. of Microbiol.\ Univ. of AL-Qadisiya*
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ألخلاصة

لقد أثبتت الدراسات الحديثة التي تتعلق بوجود ارتباطات مستقلة بين كل من مرضي التهاب القولون الالتهابي (UC) ومرض كرون (CD) مع مستضدات الخلايا البيض البشرية بصنفيها الأول والثاني، أثبتت وجود اختلاف وراثي للأستعدادية للإصابة بهذين المرضين اللذان يمثلان الحالتان الرئيستان لمرض المعي الالتهابي، ماعزز هذا الاختلاف وجود أضداد هيولي العدلات (ANCA) في هؤلاء المرضى وعوائلهم دون غيرهم. لاختبار علاقة ظهور هذه الأضداد بالعوامل الوراثية المتمثلة بمستضدات الخلايا البيض البشرية، تم اختبار 42 عراقيا" مصابا" بمرض المعي الالتهابي (IBD) (30 مصابا" بالتهاب القولون التقرحي و 12 مصابا" بمرض كرون) ، قورنوا مع 32 من الأصحاء العراقيين كمجموعة ظابطة. أستخدم فحص سمية الخلايا اللمفية الدقيق لتنميط مستضدات الخلايا البيض بصنفيها الأول والثاني، فيما أجري فحص الروز المناعي المرتبط بالأنظيم ELISA للتحري عن أضداد ANCA . لم يظهر فحص p.ANCA (حول الهيولي) ايجابية" في أي من أفراد المجموعة الظابطة، في حين أعطى 25 (83.3%) و 4 (33.3%) من مرضى UCو CD، على التتالي، نتيجة" موجبة" للفحص. أما الضد c.ANCA (الهيولي) فقد أعطى 7(8.75 %) من المجموعة الظابطة نتيجة موجبة لهذا الفحص في الوقت الذي كان 7(23.3%) و 2(16.6%) من مرضى UC و CD ، على التتالي، يحملون هذا الصنف من الأضداد. أرتبط وجود الضد p.ANCA ارتباطا ايجابيا" مع نسب تكرار المستضدات A11، DR2، DQ1 ، DQ2 عند مستويات EF (عامل مسبب) : 0.151 ، 0.185 ، 0.241 ، 0.215 ، على التتالي. من جانب آخر، كان هناك ارتباطا" سلبيا" للضد أعلاه مع تكرار المستضدات : B12 ، CW4 ، DR1 مسببة" حينما أظهرت نسب تكرارية 16.2 ، 7.3 ، 10.6 ، على التتالي. كما أظهرت المستضدات DR4 ، DQ3 أدوارا" مشابهة" . أما بالنسبة للضد c.ANCA ، فقد ارتبطت أيجابية الفحص بتكرار المستضدات A11 ، A(28+34) ، CW6 ارتباطا" ايجابيا" في مصول المرضى عند مستويات EF المتتالية: 0.342، 0.229، 0.326، في حين لم يرتبط هذا الضد سلبا" مع أي من المستضدات . نستنتج من هذه الدراسة أن وجود وعدم وجود أضداد ANCA بين مرضى المعي الالتهابي تستند الى اختلافات وراثية.

Abstract

The newly described distinct associations of HLA class I and II genes with ulcerative colitis (UC) and Crohn's disease (CD) provide strong evidence for genetic heterogeneity of susceptibility between these two major forms of inflammatory bowel disease (IBD), moreover, the familial distribution of anti-neutrophil cytoplasmic antibodies (ANCAs, a subclinical markers) in patient's families has further implicated the existence of heterogeneity within this disease. To test the hypothesis that heterogeneity indicated by ANCAs has a genetic basis that resides within HLA region, we studied 42 IBD Iraqi cases (30 UC and 12 CD) and an ethnically matched healthy control group (n=35). Microlymphocytotoxicity test was used for classes I and II HLA-typing. ANCAs were detected using an ELISA test. It was observed that controls were all p.ANCA-negatives, while 25 (83.3%) and 4 (33.3%) of UC and CD, respectively, were positives for this test. Only 7 (8.57%) of controls were c.ANCA positives, while 7 (23.3%) and 2 (16.6%) of UC and CD patients were positives, respectively. P.ANACA-positive IBD patient's status was reported to be positively associated with the frequencies of A11, DR2, DQ1, and DQ2 revealing etiology at the respective EFs : 0.151, 0.185, 0.241, and 0.215. In contrast, B12, Cw4, and DR1 were conferred preventive roles (p.ANCA negatives) at significantly decreased frequencies of IOR; 16.2, 7.3, and10.6, respectively, in addition to DR4 and DQ3 which appeared to have similar roles. On the other hand, A11, A(28+34), and Cw6 were observed in positive associations with c.ANCA-positive patients at respective EFs: 0.342, 0.229, and 0.326. Whoever, no allele was detected to prevent the positivity of this test. Thus, genetic HLA-based heterogeneity has been concluded within ANCA positive\ANCA-negative IBD patients.

Introduction

Anti-neutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed against proteins of cytoplasmic granules or other cytoplasmic constituents of neutrophils [1,2]. The staining patterns produced by ANCA

have been described as perinuclear (p.ANCA) which is mainly represented by Myeloperoxidase (MPO), or cytoplasmic (c.ANCA) represented by Proteinase-3 (PR-3)[2]. C.ANCA was originally detected in sera from patients with Wegener's granulomatosis (WG), a disease characterized by necrotizing granulomatosis of the upper and lower airways in conjunction with systemic vasculitis and crescentic glomerulonephritis [3]. P.ANCA was firstly detected in several other idiopathic forms of systemic vasculitis and glomerulonephritis [4]. After that, ANCA have been detected in inflammatory bowel disease[5,6], in which, chronic, relapsing and tissue destructive idiopathic inflammatory condition limited to the large bowel (Ulcerative Colitis –UC-) or occurring anywhere along the alimentary tract (Crohn's Disease –CD-)[7]. Since 1990, numerous reports have confirmed this finding in 50-90 % and in 10-20 % of patients with the first and the second disease, respectively [6,7]. One of the genetic factors that hypothesized to predispose for IBD were the Human Leukocyte Antigens (HLA), which were widely studied in different ethnic groups, however, there were no universally observed associations between any iatrogenic risk allele and this disease [8]. Regarding Iraqi patients, our previous study was depicted the associations between class I and II HLA and this disease [9]. This study was planned to reveal a possible role of HLA molecules in the positive rates for ANCA in those patients, as their were ANCA-positive and ANCA-negative ones.

Materials and Methods

Patients: The study group consisted of 42 IBD Iraqi patients (30 with UC and 12 with CD), in whom the diagnosis was based on well-established clinical endoscopical, and histopathological criteria in Gastrointestinal tract and liver diseases teaching hospital / Baghdad. In addition, a group of 35 apparently healthy, ethnically matched was involved as a control group, the study was carried out during the period Feb.-Jun. 2005.

Methods: Microlymphocytotoxicity test was used for a panel of monoclonal antibodies for HLA-typing (Biotest, Germany) as established by Terrasaki and McClell, 1964 [10], and modified by Dick, et al 1979 [11] and Bender, 1984[12]. The antisera used in this study were listed in table 1.

An indirect ELISA test was used to detect ANCA (p.ANCA represented by anti-MPO, and c.ANCA represented by anti-PR-3) in the sera of both study groups. Kits supplied by (Biomaghrib, Tunisia) for both autoantibodies were utilized. HLA-typing and ANCA tests were kindly carried out in the teaching labs , medical city, Baghdad.

Statistical analysis: Data were computerized and analyzed using SPSS v.13 in linkage with excel. The Odds ratio (OR) represent the value of certain antigens frequency among patients to it's frequency among healthy controls, if it more than two, it regarded to have a positive association, while inverse OR (IOR) of more than two

Table -1: Antisera for HLA-typing used in this study.
*** Indicates serologic specificity.**

HLA-I				HLA-II	
A	B		C	DR	**DQ**
A1+36+80 *	Bw4	B39	Cw1	DR1	**DQ1**
A2	B5(51+52)	B40(80+61+48)	Cw2	DR2(15+16)	**DQ2**
A3	Bw6	B41	Cw3	DR3	**DQ3**
A9(23+24)	B7(73)	B44	Cw4	DR4	**DQ4**
A10(25+26+34+66)	B8	B45	Cw5	DR5(11+12)+8	
A11	B12(44+45)	B47+40	Cw6	DR6(13+14)+3	
A23	B13	B49(52)	Cw7	DR7	
A24	B14(64+65)	B51		DR8	
A25	B15+57	B55		DR10	
A(26+66)	B16(38+39)	B56		DR11	
A(28+34)	B17(57+58)	B57		DR11+13	
A29	B18	B60+48		DR(13+12+1)	
A30	B21(49+50)	B62+75		DR8+12, 1404	
A(31,30)	B22(54+55+56)	B73		DR14	
A(23,25)	B27	B70(10+21+62)		DR15	
A33	B35+53			DR17(13)	
A34	B37			DR52(8)	
	B38			**DR52**	

regarded to be in a negative association with the condition under test, in our case, the positive ANCA. The OR should attained significant value ($P \leq 0.05$). The antigen considered contributor to positive ANCA if it had Etiologic Fraction (EF) ≥ 0.15, and be

prevent attaining such result if it had Prevention Fraction (PF) of ≥ 0.15 [13].

Results

The positive rates for p.ANCA and c.ANCA in patients and healthy control groups were depicted in table-2, p.ANCA was never given positive in controls, while 83.3% of UC and 33.3% of CD patients were resulted positives for this auto-antigen. Whereas, the c.ANCA positives were seen in lower frequencies among all study groups (8.57%, 23.3%, and 16.6% in controls, UC, and CD, respectively).The positive rates for p.ANCA as a function of both classes HLA molecules were presented in table-3. Only antigens that revealed an OR (positive associations) or an Inverse OR (negative associations) of ≥ 2 were depicted in this table. The antigens B12, Cw4, and DR1 were observed in significant IORs of 16.2, 7.3, and 10.6, respectively. Moreover, although B12 seemed to be of no preventive role for this test, the Cw4 and DR1 were did so at PFs of 0.193 and 0.354, respectively. Many other molecules were contributed to increased or decreased incidence of p.ANCA positives, but those played significant roles were A11, DR2, DQ1, and DQ2 which conferred etiology the respective EFs of; 0.151, 0.185, 0.241, and 0.215. On the other hand, DR4 and DQ3 were contributed to prevent the positives at PFs of 0.158 and 0.197, respectively.

Table-2: The frequencies of positive ANCAs among study groups.

	Healthy controls (n=35)		Ulcerative Colitis (n=30)		Crohn's disease (n=12)	
	N	%	N	%	N	%
Positive p.ANCA	**0**	**0**	**25**	**83.3**	**4**	**33.3**
Positive c.ANCA	**7**	**8.57**	**7**	**23.3**	**2**	**16.6**

In the same context, table-4 was listed the frequencies of class I and II molecules among patients based on positives and negatives

c.ANCA test. As shown in the table, A11 antigen played the same role that in p.ANCA, it significantly increased the positive rates for this test (OR 4.6) contributing to such results at EF of 0.342. Similar functions were detected in the frequencies of A(28+34), which repeated at OR 3.7, and EF 0.229, and Cw6 which repeated at OR 7.7 and EF 0.326, the latter was attained significance even after adjustment of P. value. In addition, many other HLA alleles were observed in increased or decreased frequencies, but didn't revealed statistical significance neither in ORs nor in EF/PF roles.

Discussion

The high prevalence of p.ANCA-positive UC patients compared with the low one that in CD patients in this study was compatible with early studies; in 1990, two different groups reported the presence of ANCA in sera from 48% and 59% of UC patients, respectively[5, 6]. Since then, p.ANCA were also detected in CD, although the prevalence (10-20 %) in patients with this disease was much lower than that in UC patients, and in primary sclerosing cholangitis (50-85 %), a chronic cholestatic liver disease that is strongly associated with IBD [14, 15, 16], a matter which recently carried authors to create serological diagnostic markers consisted of ANCA, Anti-*Saccaromyces cerevesiae* Antibodies (ASCA), and Anti-outermembrane Protein C (OMPc) of *E.coli*, in the diagnosis and distinction between UC and CD [17, 18, 19].

Discrepancies between different studies regarding prevalence of this marker may emerged from different techniques used by researchers for this purpose, reviewed in [20], whoever, the newly distinctive pure p.ANCA (MPO) and c.ANCA (PR-3), had been, to our knowledge, firstly reported in Iraq.

Table-3: The frequencies of HLA molecules and their roles in positive rates of p.ANCA among IBD patients.

	p.ANCA				OR	Inverse OR	P	Adjusted P	EF	PF
	Positive		Negative							
	N	%	N	%						
HLA-A antigen										
A1+36+80	9	29	2	18.1	2.0	**	0.33 *[NS]*	**	0.140	**
A9 (23+24)	2	6.4	2	18.1	0.3	3.3	0.17 *[NS]*	**	**	0.117
A10 (25+26+43+66)	2	6.4	2	18.1	0.4	2.5	0.27 *[NS]*	**	**	0.098
A11	8	25.8	1	9	2.6	**	0.24 *[NS]*	**	0.151	**
A(26+66)	2	6.4	0	0	3.4	**	0.28 *[NS]*	**	0.053	**
A30	3	9.6	0	0	2.2	**	0.49 *[NS]*	**	0.061	**
HLA-B antigen										
B5 (51+52)	4	12.9	0	0	6.0	**	0.1 *[NS]*	**	0.110	**
B7 (73)	2	6.4	1	9	0.5	2.1	0.44 *[NS]*	**	**	0.058
B12 (44+45)	0	0	1	9	0.1	16.2	0.019	0.51 *[NS]*	**	**
B17 (57+58)	3	9.6	0	0	3.4	**	0.28 *[NS]*	**	0.053	**
B45	2	6.4	0	0	2.6	**	0.41 *[NS]*	**	0.035	**
B49 (52)	3	9.6	0	0	3.4	**	0.28 *[NS]*	**	0.053	**
B55	2	6.4	1	9	0.5	2.1	0.44 *[NS]*	**	**	0.058
B(60+48)	2	6.4	0	0	2.6	**	0.41 *[NS]*	**	0.035	**
HLA-Cw antigen										
Cw4	1	3.2	3	27.2	0.1	7.3	0.030	0.21 *[NS]*	**	0.193
Cw5	3	9.6	0	0	3.4	**	0.28 *[NS]*	**	0.053	**
Cw6	4	12.9	3	27.2	0.4	2.2	0.26 *[NS]*	**	**	0.123
HLA-DR antigen										
DR1	2	6.4	5	45.5	0.1	10.6	0.002	0.035	**	0.354
DR2 (15+16)	10	32.2	2	18.1	2.4	**	0.22 *[NS]*	**	0.185	**
DR4	3	9.6	3	27.2	0.3	3.5	0.1 *[NS]*	**	**	0.158
DR5 (11+12)+8	2	6.4	2	18.1	0.3	3.3	0.17 *[NS]*	**	**	0.117
DR7	4	12.9	1	9	2.2	**	0.49 *[NS]*	**	0.061	**
DR12+8	0	0	1	9	0.1	9.2	0.08 *[NS]*	**	**	**
DR14	2	6.4	0	0	2.6	**	0.41 *[NS]*	**	0.035	**
DR53	4	12.9	1	9	2.2	**	0.49 *[NS]*	**	0.061	**
HLA-DQ antigen										
DQ1	9	29	1	9	6.7	**	0.08 *[NS]*	**	0.241	**
DQ2	10	32.2	2	18.1	3.5	**	0.12 *[NS]*	**	0.215	**
DQ3	6	19.3	4	36.4	0.4	2.4	0.15 *[NS]*	**	**	0.197

Table-4: The frequencies of HLA molecules and their roles in positive rates of c.ANCA among IBD patients.

	c.ANCA									
	Positive		Negative							
	N	%	N	%	OR	Inverse OR	P	Adjusted P	EF	PF
HLA-A antigen										
A9 (23+24)	0	0	3	8.8	0.2	4.3	0.18*[NS]*	**	**	**
A11	4	44.4	4	11.7	4.6	**	0.016	0.23*[NS]*	0.342	**
A23	0	0	4	11.7	0.2	5.9	0.1*[NS]*	**	**	**
A24	2	22.2	7	20.5	0.5	2.2	0.35*[NS]*	**	**	0.127
A(26+66)	0	0	2	5.9	0.3	2.9	0.35*[NS]*	**	**	**
A(28+34)	3	8.8	3	8.8	3.7	**	0.06*[NS]*	**	0.229	**
A29	1	11.1	0	0	10.7	**	0.06*[NS]*	**	0.057	**
HLA-B antigen										
B7 (73)	0	0	3	8.8	0.3	3.6	0.25*[NS]*	**	**	**
B17 (57+58)	2	22.5	1	2.9	3.8	**	0.2*[NS]*	**	0.092	**
B18	1	11.1	1	2.9	3.6	**	0.38*[NS]*	**	0.046	**
B22 (54+55+56)	1	11.1	1	2.9	3.6	**	0.38*[NS]*	**	0.046	**
B38	0	0	4	11.7	0.2	5.9	0.1*[NS]*	**	**	**
B55	0	0	3	8.8	0.3	3.6	0.25*[NS]*	**	**	**
B56	2	22.5	1	2.9	7.7	**	0.11*[NS]*	**	0.109	**
B(60+48)	2	22.5	1	2.9	7.7	**	0.11*[NS]*	**	0.109	**
HLA-Cw antigen										
Cw2	1	11.1	4	11.7	0.5	2.2	0.48*[NS]*	**	**	0.070
Cw3	0	0	3	8.8	0.2	4.3	0.18*[NS]*	**	**	**
Cw5	0	0	2	5.9	0.3	2.9	0.35*[NS]*	**	**	**
Cw6	3	8.8	2	5.9	7.7	**	0.005	0.038	0.326	**
HLA-DR antigen										
DR1	1	11.1	5	14.7	0.3	2.9	0.33*[NS]*	**	**	0.109
DR4	3	8.8	3	8.8	2.3	**	0.29*[NS]*	**	0.107	**
DR5 (11+12)+8	0	0	3	8.8	0.2	4.3	0.18*[NS]*	**	**	**
DR7	0	0	4	11.7	0.2	5.1	0.14*[NS]*	**	**	**
DR8	2	22.5	1	2.9	3.8	**	0.2*[NS]*	**	0.092	**
DR8+12, 1404	0	0	0	0	3.4	**	0.4*[NS]*	**	**	**
DR10	1	11.1	4	11.7	0.4	2.6	0.39*[NS]*	**	**	0.089
DR11	1	11.1	0	0	10.7	**	0.06*[NS]*	**	0.057	**
DR15	0	0	2	5.9	0.5	2.2	0.49*[NS]*	**	**	**

In addition, our study have investigated the hypothesis that the presence or absence of ANCA within IBD patients has a genetic basis. It has been early noted that the frequency of positive ANCA in UC patients was varied from population to another, it was 50% in France [21], and England [22], and 70% in most US Caucasian patients[23], such ethnic differences may be, in part, well-determined

by the genetic differences, presumably those linked to HLA class I and II antigens in those patients.

We hadn't noticed class I associations studies in all articles we reviewed, so, interesting results those depicted in our study that concerning A11, which may increased the positive rates of auto-antibodies for both antigens, a role which contrasted by B12 function, this result may be newly reported. In addition, there were other antigens play a predisposing roles for c.ANCA, *i.e.*, A(28+34) and Cw4. The etiologic role played by DR2 and the preventive one played by DR1 and DR4 observed in this study, were compatible with the unique study results that of Yang, et al, 1993 [(24)], who reported that ANCA-positive UC patients had a significantly increased frequency of DR2 compared with ANCA-negative controls and with ANCA-negative patients, furthermore, they have observed that the latter group had an increased frequency in DR4 compared with ANCA-positive patients. In our study, DQ1 and DQ2 antigens were shown to have increasing function contrasting by decreasing one that of DQ3, results that hadn't observed elsewhere. In our previous study [(9)], we demonstrated associations between IBD incidences and HLA molecules, of interest, such molecules didn't participated in roles in this study, suggesting that the presence/absence of ANCA in the sera of those patients have HLA-based entities.

In conclusion, contribution of subclinical marker (ANCA) and HLA-markers, a genetic heterogeneity may be detected within IBD patients, especially patients with UC, i.e., ANCA-positive / ANCA-negative patients, each have their distinct HLA-features. To confirm this observation, familial-large scale, population-based study was recommended.

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