A Study of Human Leukocyte Antigens-G (HLA-G), cANCA and pANCA in Inflammatory Bowel Diseases Patients

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الخلاصة

مرض المعي الالتهابي هو اعتلال التهابي متناوب للأمعاء يشمل مرض كرون والتهاب القولون التقرحي. إن حدوث الاصابة بهذا المرض لم يزدد فقط في البلدان الغربية بل شمل آسيا أيضا. إن مرض كرون والتهاب القولون التقرحي يصنفان كأمراض أمعاء التهابية مزمنة مجهولة السبب إن المسبب والامراضية لهذه الأسباب غير واضحة لحد الان. إن توضيح أسباب المرض جاءت عن طريق دراسات وراثية ووبائية حيث أوضحت أهمية العوامل الوراثية في مسببات هذه الأمراض. لقد كانت مورثات مستضدات الخلايا البيض في الإنسان هي المرشحة بصورة أولية لمثل هذه الدراسات. جمعت أثنين وأربعون عينة معظمهم مرضى يراجعون قسم الجهاز الهضمي والكبد في مستشفى الديوانية التعليمي الكثير منهم يعانون من نزف دموي شرجي ،آلام في منطقة البطن، نقص في الوزن وعند إجراء الفحص بواسطة الناظور تبين إن ثماني وثلاثين منهم يعانون من تقرح القولون وأربعة من مرض كرون كما تمت مطابقة النتائج مع واحد وعشرين من الأصحاء (مجموعة سيطرة) .أجريت الدراسة للفترة من تشرين الثاني 2010 ولغاية أيلول 2011 كان معدل الأعمار في هذه الدراسة (41.1) سنة كما وإن أكثر عمر يحدث فيه المرض هو أربعين سنة فما فوق (55.3% بالنسبة لمرضى تقرح القولون) و(25% بالنسبة لمرضى كرون للأعمار بين كما بينت الدراسة وجود فرق معنوي بين الرجال والنساء بالنسبة لمرضى تقرح القولون (63.2%) و36.8%) للرجال والنساء على التوالي ولمرضى كرون(75% و25%) للرجال والنساء على التوالي أظهرت النتائج إن هنالك فرق معنوي بين مرضى المعي الألتهابي والأصحاء أثناء فحص الـ p.ANCA حيث إن مرضى تقرح القولون (81.5%) أما المرضى المصابين بمرض كرون (25%) أما الأصحاء فكانت النتائج سالبة بالنسبة لهذا الفحص وكذلك الحال بالنسبة لفحص الـ c.ANCA ولكن بنسب أقل للمرضى المصابين بتقرح القولون(31.9%). أما فحص الـ HLA-G فقد أظهر فرقا معنويا بين المجاميع الثلاثة حيث إن المرضى اللذين يعانون من تقرح القولون (47,4%) والمرضى اللذين يعانون من مرض كرون(100%) أما الأصحاء فكانت النتيجة سالبة.

Abstract

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disorder of the bowel, consisting mainly of Crohn's disease (CD) and ulcerative colitis (UC). The incidence of IBD has been rising not only in Western countries, but also in Asia, the exact causes of both diseases are still a mystery.

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A total of forty two Iraqi patients from Al-Diwanyia province (15 females and 27 males) who have been diagnosed by specialist physicians in Al-Diwaniya Teaching Hospital for Gastrointestinal Tract and Hepatic diseases unit, compared with twenty one unrelated, healthy controls (10 females and 11 males). All patients was diagnosed by endoscopy and biopsy from the sites of inflammation (hitopathological method). The patients group was composed of two sub-groups; 38(90.5%) patients with Ulcerative Colitis and 4(9.5%) patients with Crohn's disease. The highest age incidence was above 40 years old (55.3% and 25% of UC and CD patients, respectively), followed by age of 30-39 years. The majority of UC (81.5 %) have shown positive results for p.ANCA, compared with (25 %) of CD patients, while all controls have given negative results for this test. Both of UC(47.4%) and CD(100%) patients shown positive results for HLA-G, while all healthy controls were given negative results for this test.

Introduction

Inflammatory Bowel Disease (IBD) comprises those conditions which tend to be chronic or relapsing immune activation and inflammation within gastrointestinal tract (GIT). Ulcerative Colitis (UC) and Crohn's disease (CD) are the two major forms of this disease with unidentified etiopathology[1]. The UC and CD are chronic, idiopathic, inflammatory diseases of the GIT that share common symptoms such as diarrhea, abdominal pain, fever, and weight loss. Ulcerative colitis involves all or part of the colon, whereas, Crohn’s disease commonly involves the terminal ileum and proximal colon [2, 3]. The distinct difference being that Crohn’s can affect any part of the gastrointestinal tract, from mouth to anus, with most beginning in the terminal ileum; while Ulcerative Colitis is restricted to the colon. The exact causes of both are still a mystery [4]. It is generally believed that chronic IBD occurs in genetically predisposed individuals who are exposed to unknown environmental and microbial triggers. However, the genetic links provide only a partial explanation for disease development as the majority of patients with IBD have neither a family history nor a

known genetic defect [5]. HLA-G, a gene located within the major histocompatibility complex (MHC) at the short arm of Chromosome 6. HLA-G structures act as regulatory factors of immune responses operating in several inflammatory conditions [6]. Diagnostic laboratories are frequently asked to add this to their repertoire of tests. In previous studies ANCAs were present in 50 to 85% of patients with UC but also in 10 to 20% of patients with CD [7]. The pANCA pattern of perinuclear staining around the nuclei of neutrophils is the second type of pattern noted. The pANCA pattern is the result of positively charged molecules that migrate to the edge of the nuclei of neutrophils[8]. HLA-G is a non-classical, has been referred to as a non-classical gene or class Ib because of its structural relation to classic MHC class Ia (HLA-A, HLA-B, HLA-C)products because they are immunologically functional peptides presenting heterodimeric glycoproteins noncovalent associated with β2 microglobulin which are linked to the Major Histocompatibility Complex (MHC) locus on chromosome 6. Studies have demonstrated a tolerogenic function for HLA-G molecule against innate and adaptive cellular immune responses[9,10].

Material and Method

Patients

A total of forty two patients from AL-Diwanya province 27 males and 15 females) with inflammatory bowel disease (38 UC and 4 CD patients) who have been diagnosed by specialist physicians in AL-Diwanya Teaching Hospital for Gastrointestinal Tract and Hepatic diseases unit, depending clinical features, endoscopy, and biopsy for histopathology. All were regularly attending the consultant clinic for treatment and follow-up during the period November 2010 to September 2011. Members of the two groups were subjected to the following assays;

1- HLA-G
2- pANCA
3- cANCA

Sampling

Under sterile conditions, 5-10 ml of blood were drown from each member of the study groups using vein puncture immediately into disposable tubes without anticoagulant, stand in room temperature for 30 minutes to obtain clotted blood, then centrifuged at 2000 RPM for 10 minutes, then the sera dispensed in aliquots, and kept at -20 ^{0}C till used.
ELIZA technique used for all previous tests.

HLA-G assay:

Principle;

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for HLA-G has been pre-coated on to a microplate. Standards and samples are pipetted into the wells and any HLA-G present is bound by the immobilized antibody. An enzyme-linked monoclonal antibody specific for HLA-G is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of HLA-G bound in the initial step. The color development is stopped and the intensity of the color is measured, the optical density of each well determined within 30 minutes, using a microplate reader set to 450 nm.

Results and Discussion

The patients group was composed of two sub-groups; 38(90.5%) patients with Ulcerative Colitis and 4(9.5%) patients with Crohn's disease. The study groups are represented in table (1). The mean of ages in this study is (41.1) years old, which compatible with the mean of age in (Barahona-Garrido *et al*., 2009), and differ from the mean of age in other studies [12, 13, 14]. The higher frequencies of CD patients(50%) in age intervals occurred in (30-39) years old, while the UC patients shown higher frequencies (55.3%) than CD patients (25%) in age intervals above forty years old, while in age intervals less than thirty years old there are no significant in frequencies of UC and CD.

Table 1: Age intervals of Ulcerative Colitis, Crohn's Disease patients involved in this study.

Age in years groups (mean=41.1)	Ulcerative Colitis		Crohn's disease	
	No.	%	No.	%
<30	9	23.7	1	25
30-39	8	21	2	50
>40	21	55.3	1	25
Total	38	100	4	100

Table (2) show the significant differences between male and female in both UC and CD patients as (63.2% and 36.8%) in male and female of UC, respectively and (75% and 25%) in male and female of CD patients, these results are different from what depicted in the other reviewed studies (12,15,16).

Table 2: Gender of Ulcerative Colitis, Crohn's Disease patients and Healthy control involved in this study.

Gender	Healthy Controls		Ulcerative Colitis		Crohn's disease	
Female	10	47.6	14	36.8	1	25
Male	11	52.4	24	63.2	3	75
Total	21	100	38	100	4	100

A significant difference has also emerged when the two subtypes of UC have distributed over the gender of patients as shown in figure (1). A comparable results reported are compatible with other study (21,17,18,12,19,20) that 70% of patients had proctitis.

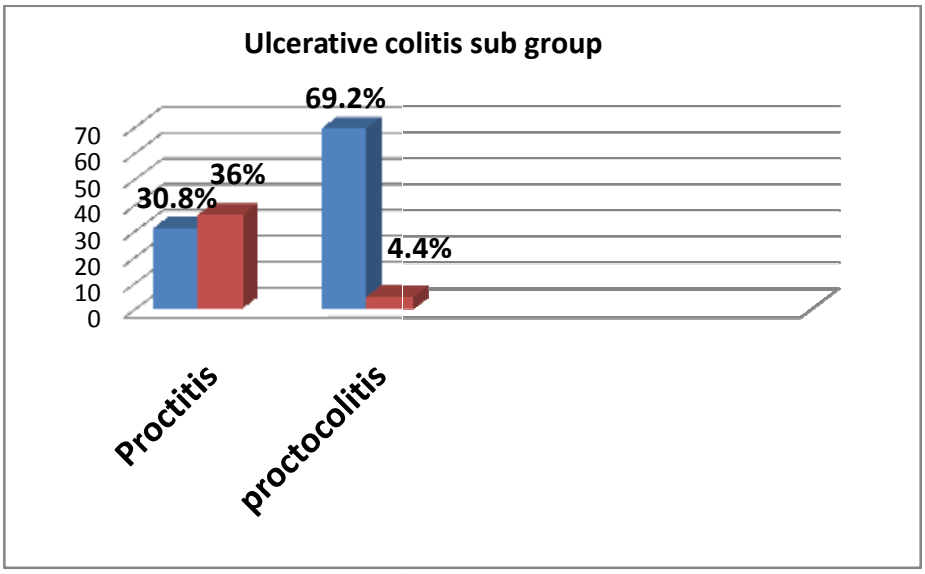

Figure (1): Frequency distribution of Ulcerative colitis subtypes according to the gender in this study.

The positive rates for p.ANCA were assayed in all study groups and the results were depicted in table (3). More than two thirds (81.5 %) of UC patients showed positive results for p.ANCA, compared with 25 % of CD patients, while all controls were given negative results for this test, a condition which given rise a significant differential criteria between; each of UC, CD and healthy controls at p. value of ≤ 0.05.

Table 3: The significance of positive p.ANCA in differentiation between the three study groups (UC, CD, and Controls).

Positive cytoplasmic-Antineutrophil cytoplasmic antibodies(p.ANCA)	Healthy Control (n=21)		Ulcerative Colitis (n=38)		Cronh's disease (n=4)	
	No.	%	No.	%	No.	%
	0	0	31	81.5	1	25

On the other hand, the lowered positive rates of c.ANCA among the three study groups have eliminated such differential significance as that of p.ANCA, with an exception observed between UC and CD at p ≤ 0.05 as show in table 4, these records

are in discrepancies with that some studies (11,14,22,23) and compatible with others(24,25,26) Terjung *et al*. (2004); Yamamoto-Furusho *et al*. (2006).

Table 4: The significance of positive c.ANCA in differentiation between the three study groups (UC, CD, and Controls).

Positive Perinuclear-Antineutrophil cytoplasmic antibodies(c.ANCA)	Healthy Controls (n=21)		Ulcerative Colitis (n=38)		Cronh's disease (n=4)	
	No.	%	No.	%	No.	%
	0	0	15	31.9	1	25

The positive rates of HLA-G in three study groups are shown in table (5). Both of UC(47.4%) and CD(100%) patients shown positive results for HLA-G, while all healthy controls were given negative results for this test, a condition which given rise a significant differential criteria between the UC and CD compared with healthy controls ($p \leq 0.05$). The results confirmed a different HLA-G expression between the diseases, with a spontaneous secretion of HLA-G in CD patients but not in UC and healthy subjects. Our study has documented a clear difference in the production of HLA-G molecules in IBD patients, confirming the presence of a different etiology and immune response mechanisms in UC and CD, similar results were reported by Rizzo *et al*. (2008)[28]. The association of UC with HLA genes is stronger than CD [27].

Table (5): The frequencies positive HLA-G over different study groups (UC, CD, and healthy control).

Positive HLA-G	Healthy control (n=21)		Ulcerative colitis (n=38)		Crohn's disease (n=4)	
	No.	%	No.	%	No.	%
	0	0	18	47.4	4	100

A significantly different HLA-G expression in intestinal biopsies from CD and UC patients. In detail, the results have shown the modulation of HLA-G molecules in UC but not in CD

biopsies. Overall, the data suggested a functional role for HLA-G molecules in IBD diseases and proposed the different HLA-G expression as a potential diagnostic marker to distinguish CD and UC().

Conclusions

In the context of the present study results, the findings has been concluded:

1-There were no differences in IBD incidence between males and females, however, the age of onset appeared later in Iraqi patients than that recorded in Western countries?

2- A clear difference has been appeared in the production of HLA-G molecules in IBD patients that is the association of CD with HLA-G genes is stronger than with UC.

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