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Human Leukocyte Antigen-G and Certain Auto-antibodies Profile in Inflammatory Bowel Disease Patients

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Abstract

Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder of the bowel represented by two major forms; Ulcerative colitis (UC) and Crohn's disease (CD). The idiopathic nature of this disease manifests due to irregular intestinal mucosal immune-tolerance. The present study evaluates a possible genetic predisposing factor of the soluble Human Leukocyte Antigen-G (HLA-G) and estimates the auto-antibodies in the sera of a group of IBD Iraqi patients (38 with UC and 16 with CD) during Nov. 2010-Sep. 2011. The sera of the entire experimental group have been examined using commercial ELISA kits. A group of 21 apparently healthy individuals was also included as a control group in this study. The s.HLA-G and Anti-Elastase auto-antibody have found to be produced in all (100%) of CD patients compared with 18 (47.4%) and 24 (63.2%) of UC patients, for the two tests, respectively. Both assays haven't shown positive result in healthy control sera. In addition to these two tests, the Anti-Cathepsin-G and Anti-Lysozyme auto-antibodies were found to be significantly differing in their prevalence among studied groups conferring diagnostic and differential tools. The positive/negative predictive values, sensitivities, and specificities of all tests have also been analyzed. The highest results were occurred in s.HLA-G and Anti-Elastase assays. These two factors were found to have an association with arthralgia development as an extra-intestinal manifestation among UC but not CD patients. The different expression levels of the studied parameters may aid in differential diagnosis and in following-up this group of patients.

Keywords: IBD; HLA-G; Anti-Cathepsin G; Anti-Elastase

Introduction

Inflammatory Bowel Disease (IBD) comprises those conditions which characterized by chronic or relapsing immune activation and inflammation within gastrointestinal tract (GIT). Ulcerative Colitis (UC) and Crohn's disease are the two major forms of this disease with unidentified etiopathology [1]. Both these form are chronic, idiopathic, inflammatory diseases of the GIT that share common symptoms such as diarrhea, abdominal pain, fever, and weight loss. However, pathologically, Ulcerative colitis involves all or part of the colon, whereas, Crohn's disease commonly involves the terminal ileum and proximal colon [2,3]. In the majority of cases, the characteristic manifestations permit to distinguish between these two entities, but in 10% of cases it remains difficult, and there is a need for further diagnostic tools. The extent of the disease may vary depending on factors like advancement of age, inheritance, extra intestinal manifestations, response to treatment, and the natural course of the disease [4].

There is no universal standard for the diagnosis of IBD. A combination of diagnostic criteria would be used for the assessment of the disease, which includes, clinical presentation, endoscopic, radiographic, and pathologic findings. Serological assessments, utilizing antineutrophil cytoplasmic antibody (ANCA) and anti-Saccharomyces cerevisiae antibody (ASCA) have been introduced as a adjunctive role in differentiating ulcerative colitis and Crohn's disease [5]. The Perinuclear-ANCAs auto-antibodies, among many other serological markers, are recently drawing special attention in many auto-immune diseases, especially the atypical p.ANCA; those include auto-antibodies against Cathepsin G, Elastase, lysozyme, Lactoferrin, Catalase, etc. [6].

As chronic inflammation, IBD is generally associated with the immune regulatory factors; one of the anti-inflammatory factors suggested here would be HLA-G. A major histocompatibilty complex class I encoded by a gene on chromosome 6p21, characterized by a limited polymorphism in the coding region with 47 alleles (IMGT HLA database, April 2012) [7]. Among the seven existing isoforms of HLA-G, two are found on the cell surfaces and in the biological fluids. Many authors have recently been focusing on the suggested role of this anti-inflammatory antigen with many auto-immune disorders. The present study has been conducted to measure the serum levels of HLA-G and some atypical pANCAs among a group of Iraqi IBD patients to establish supportive diagnostic, differentiative tools for this mysterious disorder.

Materials and Methods

Subjects

Fifty four patients from Al-Diwaniya province in Iraq (36 males and 18 females) with inflammatory bowel disease; 38 patients with Ulcerative Colitis and 16 with Crohn's disease have participated in this study. A specialist physician at Gastrointestinal Tract and Hepatic diseases unit of Al-Diwaniya Hospital has confirmed the Diagnosis after a thorough examination of clinical features, histopathology, and endoscopy.

According to the data, the patients have regularly attended the consultant clinic for treatment and follow-up during the period from November 2010 to September 2011. Twenty one individuals, who were apparently healthy, without IBD or other abnormalities, acute or chronic diseases, were selected as a control group. Controls were

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Patient's;	Ulcerative Colitis No.=38		Crohn's Disease No.=16	
Gender*	M (%)	F (%)	M (%)	F (%)
	24(63.1)	14 (36.8)	12 (75)	4 (25)
Age group/years				
≤ 30	5 (20.8)	4 (28.5)	1 (8.33)	0 (0%)
30-39	3 (12.5)	5 (35.7)	8 (66.66)	2 (50)
≥ 40	16 (66.6)	5 (35.7)	3 (25)	2 (50)
Mean age at onset/years	(35.31 ± 4.66)		(36.22 ± 2.78)	

* M: Male; F: Female.

Table 1: General characters of IBD patients revealed from the questionnaire.

Extra-intestinal manifestations	Healthy controls (n=21)		cases with UC (n=38)		Cases with CD (n=16)		P (Fisher's exact) comparing:		
	N	%	N	%	N	%	UC to Healthy	CD to Healthy	UC to CD
Arthralgia	0	0.0	14	36.8	4	25.0	<0.001	0.028	0.53 [NS]
Dermatol. problems	0	0.0	6	15.8	0	0.0	0.08 [NS]*	**	0.16 [NS]
Positive test results									
s.HLA-G	0	0.0	18	47.4	16	100.0	<0.001	<0.001	<0.001
Anti-Elastase	0	0.0	24	63.2	16	100.0	<0.001	<0.001	0.005
Anti-Cathepsin-G	0	0.0	21	55.3	4	25.0	<0.001	0.028	0.07 [NS]
Anti-lysozyme	2	9.5	2	5.3	7	43.8	0.61 [NS]	0.024	0.002

[NS]: Not Significant; **Incomparable.

Table 2: Distribution of extra-intestinal manifestations and prevalence of serological markers among IBD patients and healthy control members.

matched with the patients group according to sex, and age. A structured questionnaire was administered in order to determine the patient's ages, sex, duration of the disease, extra-intestinal manifestations, and whether they complained of other diseases.

A volume of 5-10 ml of blood samples was collected to conduct serological tests. The sera were frozen at -80°C. Commercial ELISA kits were used to estimate the serum level of Auto-antibodies for Cathepsin G, Elastase, and Lysozyme according to the manufacturer instructions (Immuchem., Belgium). The serum level of s.HLA-G was estimated using ELISA kit from Cusabio, China. The cut-off values were calculated accordingly for all assays manufactured to be qualitative.

The SPSS (version 17) was used for the statistical analysis; the Fisher's exact was used to test the statistical significances.

Results and Discussion

Iraq has been encountering several challenges in detecting the IBD cases effectively due to poor infrastructure. Physicians often encounter challenges in precisely defining the minute differences between ulcerative colitis and Crohn's disease clinically. As a result they often fail to diagnose it in time.

This study is part of ongoing investigations to establish a complete serological picture of patients with IBD, with a goal of improving the precision and speed of the diagnosis. Ethnicity of the patients is also likely to be playing a role in the cases where this disease is genetically predisposed.

Out of 38 patients affected with UC; 24 (63.1%) are males and 14 (36.8%) are females. Out of 16 patients with CD; 12 (75%) are males and 4 (25%) are females.

According to Table 1, prevalence of UC is highest among the patients in the age group of ≥ 40 years and CD among patients in the age group of 30-39 year old. There was no significant difference regarding the mean age of onset in both forms of IBD.

Ethnicity, environment, food habits, antigenic exposure, and many other factors could play roles in triggering IBD. Accordingly, our findings regarding the age group and gender of the patients may differ from patients from other areas.

sHLA-G was a strong indicator of CD with 100% patients testing positive (Table 2). In contrast, only 47.4% of patients with UC were positive for sHLA-G and none of the controls. Therefore, sHLA-G may be a new and powerful tool to distinguish these two forms.

HLA-G, as anti-inflammatory factor has been studied by many authors with respect to different categories of autoimmune disease. The earlier relevant studies on the expression of HLA-G in relationship to immune tolerance have indicated the presence of this antigen on the surface of cytotrophoblast during fetal stages, preventing this cell from the killing action of uterine NK cells, implicating the cytotrophoblast in the immune tolerance [8-11]. Using immunohistochemistry, Torres et al. [12] have demonstrated a different expression of this antigen on the surface of intestinal epithelial cells from patients with UC than those from CD patients. They have suggested a potential tool distinguishing between these two forms of IBD.

Table 2 shows the significant differences in the expression of s.HLA-G among each of the three studied groups. Using different techniques, HLA-G has been investigated in many auto-immune diseases; in cutaneous diseases [13], in heart diseases [14], in rheumatologic diseases [15], and in many other systemic and organ specific auto-immune diseases, reviewed in [7]. Regarding IBD, similar studies have been carried-out using PCR.

The 14 bp INS/DEL genotype for HLA-G was found to be significantly decreased in UC patients compared to CD patients and healthy individuals [16]. Using ELISA, Rizzo et al. [17], it has reported a spontaneous secretion of sHLA-G in the supernatant of LPS-stimulated peripheral mononuclear cells from these patients with CD, but not from UC or healthy individuals. Soluble HLA-G is found in biopsies and serum from patients undergoing heart transplantation and associated with better prognosis of acceptance. To the best of our knowledge, there is no previous study on the elevation of the serum level of sHLA-G in IBD patients, and this is the first of its kind in this regard.

Similar prevalence and diagnostic values for Anti-Elastase has

	Total	Positive HLA-G		Positive Anti-Elastase		Positive Anti-Cathepsin-G		Positive Anti-lysozyme	
	N	N	%	N	%	N	%	N	%
Age group (years)									
<40	17	4	23.5	8	47.1	8	47.1	1	5.9
40+	21	14	66.7	16	76.2	13	61.9	1	4.8
P (Fisher's exact)		0.011		0.09 [NS]		0.51 [NS]		1 [NS]	
Duration of the disease (years)									
<5	28	9	32.1	19	67.9	18	64.3	2	7.1
5+	10	9	90.0	5	50.0	3	30.0	0	0.0
P (Fisher's exact)		0.003		0.45 [NS]		0.08 [NS]		1 [NS]	

[NS]: Not Significant.

Table 3: The effect of age and duration of the disease on the positivity of the four serological tests among UC patients.

	Total	Positive sHLA-G		Positive Anti-Elastase		Positive Anti-Cathepsin-G		Positive Anti-lysozyme	
	N	N	%	N	%	N	%	N	%
Age group (years)									
<40	11	11	100.0	11	100.0	4	36.4	6	54.5
40+	5	5	100.0	5	100.0	0	0.0	1	20.0
P (Fisher's exact)		**		**		0.24 [NS]		0.31 [NS]	
Duration of the disease (years)									
<5	14	14	100.0	14	100.0	4	28.6	6	42.9
5+	2	2	100.0	2	100.0	0	0.0	1	50.0
P (Fisher's exact)		**		**		1 [NS]		1 [NS]	

[NS]: Not Significant; **Incomparable

Table 4: The effect of age and duration of the disease on the positivity of the four serological tests among CD patients.

Extra-intestinal manifestations-Arthralgia	Total	Positive s.HLA-G		Positive Anti-lysozyme		Positive Anti-Elastase		Positive Anti-Cathepsin-G	
	N	N	%	N	%	N	%	N	%
Negative	24	7	29.2	13	54.2	10	41.7	1	4.2
Positive	14	11	78.6	11	78.6	11	78.6	1	7.1
P (Fishre's exact significance)		0.006		0.18 [NS]		0.043		1 [NS]	

[NS]: Not Significant.

Table 5: Association of Arthralgia as an extra-intestinal manifestation with different serological tests in patients with UC.

been obtained in the studied groups; 100% of CD patients compared to 63.2% UC patients have reported positive while, no positive result is seen in any of the control group members.

For Anti-Cathepsin G and Anti-Lysozyme Auto-antibodies, there was a significant increase among CD and UC patients when compared to healthy individuals that are part of control group.

Moreover, in the sera of UC patients, Anti-elastase frequency was significantly higher than it among the healthy control group members.

While the other test shows a significant difference compared with CD patients (Table 2).

According to Table 3, Production of s.HLA-G is more likely raised among the UC patients, as they are more than 40 years old. Duration of the disease also plays a prominent role. It is most likely to take place among the patients living with this disease for five years. Hence, both the age and duration of the disease were significantly affecting the prevalence of this antigen among the subgroup of patients (Table 3).

Whereas, no such effect for these two factors have been traced in CD patients (Table 4). Previous data regarding the auto-antibodies to atypical p.ANCAs in the two forms of IBD are of controversy, whether they aid in the diagnosis or not. These studies are either used in different methods or different ways to calculate the cut-off values for the same assays. For example, there were 53%, 46%, 46%, positive results for Lysozyme, Elastase, and Cathepsin G, respectively in the sera of UC patients compared with 39%, 18%, and 12%, respectively in CD patients using ELISA [18]. Whereas, such auto-antibodies were undetectable in both forms of the disease [19]. Different methods and different results are reviewed in [6]. As IBD is a multi-factorial idiopathic disease, the genetics, the environment, and even the ethnicity may interfere with the development and the outcome of the disease; this may explain the extreme positive values in our population compared with others.

We extended our results to investigate whether these serologic markers were also associated with the development of extra-intestinal manifestation of IBD. The only significant association was between s.HLA-G and Anti-Elastase and arthralgia among UC patients as shown in Table 5. As mentioned previously, many authors have reported the presence of HLA-G in many cutaneous and rheumatologic diseases [7], the same thing is true for Elastase. Whether the presences of these antigens have a direct correlation with these manifestations or it is an overlapping of auto-immune profile with other diseases do exist, it is to be elucidated. The validity parameters of the tests used in this study have demonstrated considerable data utilized to diagnose and differentiate the cases in question. In differentiating UC from healthy control, Table 6 shows the highest sensitivity, specificity, accuracy, positive and negative predictive values (PPV and NPV, respectively) for Anti-Elastase examination followed by Anti-Lysozyme and s.HLA-G. All three tests have offered 100% PPV at pretest probability of 50%, 90%, and 100% specificities were recorded for the three tests.

In the diagnosis and differentiation of CD patients when compared to healthy control group, equal and complete validity parameters for

Test in differentiating UC&healthy	Sensitivity	Specificity	Accuracy	PPV at pretest probability=		NPV at pretest probability=10%
				50%	90%	
s.HLA-G	47.4	100.0	66.1	100.0	100.0	94.5
Anti-Elastase	63.2	100.0	76.3	100.0	100.0	96.1
Anti-lysozyme	55.3	100.0	71.2	100.0	100.0	95.3
Anti-Cathep.-G	5.3	90.5	35.6	35.6	83.3	89.6
Test in differentiating CD and Healthy						
s.HLA-G	100.0	100.0	100.0	100.0	100.0	100.0
Anti-Elastase	100.0	100.0	100.0	100.0	100.0	100.0
Anti-lysozyme	25.0	100.0	67.6	100.0	100.0	92.3
Anti-Cathepsin-G	43.8	90.5	70.3	82.1	97.6	93.5

Table 6: Validity parameters for selected serum antibodies when used as test to diagnose UC and CD differentiating them to healthy controls.

The positive test	Sensitivity	Specificity	Accuracy	PPV at pretest probability=		NPV at pretest probability=10%
				50%	90%	
s.HLA-G	100.0	52.6	66.7	67.9	95.0	100.0
Anti-Elastase	100.0	36.8	55.6	61.3	93.4	100.0
Anti-Cathepsin-G	43.8	94.7	79.6	89.3	98.7	93.8

Table 7: Validity parameters for selected serum antibodies when used as test to diagnose CD differentiating it to UC.

each of s.HLA-G and Anti-Elastase have been recorded, followed by Anti-Lysozyme with lower sensitivity (Table 6).

Furthermore, Table 7 shows interesting validity parameters between the CD and UC patients. The higher two tests were s.HLA-G and Anti-Elastase, as each had 100% sensitivity, with lower specificity, accuracy and PPV. A high (100%) NPV for each of the two tests were calculated at pretest probability of 10%, strengthening the exclusion potential of these two tests in the diagnosis of CD.

From the present study, we may conclude that the serum level of sHLA-G as well as Anti-Elastase may aid in the diagnosis of UC and CD patients.

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