SHORT PAPER

Auto-antibodies in Patients with Inflammatory Bowel Disease Unclassified

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ABSTRACT

Background: Inflammatory bowel disease unclassified (IBDU) is considered to be an aberrant immune response with loss of tolerance to many antigens. **Objective:** This paper tries to address whether there is any value to test for auto-antibodies in such patients. **Methods:** 60 patients with inflammatory bowel disease unclassified participated in the study. Auto-antibodies to nuclear antigen, intestinal goblet cell, exocrine part of pancreatic acinar cells, perinuclear antineutrophil cytoplasmic, cytoplasmic antineutrophil cytoplasmic and *Saccharomyces cerevisiae* were tested and compared to 20 ulcerative colitis (UC) patients and 30 healthy controls matched for age and sex. **Results:** There was a significant difference (p=0.000) between patients and control group in anti-exocrine part of pancreatic acinar cells, perinuclear antineutrophil cytoplasmic and *Saccharomyces cerevisiae* auto-antibodies. There was also a significant difference between IBDU and UC patients in the auto-antibodies directed against intestinal goblet cells, (p=0.000) exocrine part of pancreas (p=0.000) and anti *Saccharomyces cerevisiae* antibody (p=0.000). **Conclusions:** Due to the autoimmune nature of indeterminate colitis, involvement of some antigens from gastrointestinal tract or the bile system in the initiation of this disease is likely.

Keywords: Acinar Cells, Antineutrophil Cytoplasmic Antibodies, Colitis, Pancreatic, *Saccharomyces cerevisiae*

INTRODUCTION

The term indeterminate colitis (IC) was first used by Kent et al. (1) when evaluation rendered a diagnosis of ulcerative colitis (UC) or Crohn's disease (CD) indefinite. Thus, some pathologists suggest that IC is a temporary diagnosis (2). In 2004, Burakoff supported the premise that IC may be a separate entity (3). This favors the broader use of IC, which is based on endoscopic, histologic, and radiologic tools. The pathologist

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Geboes et al. (4,5) used the term inflammatory bowel disease unclassified only when endoscopic biopsies were available for the study and the surgeons used the term indeterminate colitis for the same condition on surgical specimens.
This disease results from an aberrant immune response and loss of tolerance to many self-antigens, leading to chronic inflammation of the gut. This idea is supported by the occurrence of antibodies directed to microbial and self antigens. In case of CD, NOD2/CARD15 gene conferred susceptibility to this disease (6). Peeters et al. (7) evaluated patients with IC prospectively to determine if the serological autoantibody tests might be helpful in improving the diagnostic accuracy of categorizing IC. Auto-immune disorders form part of a broad spectrum of diseases which are often associated with impaired Treg production or maintenance (8). Joosens et al. in a multicenter prospective study, had found that ASCA + /pANCA- predicts CD in 80% of patients with IC and ASCA- /pANCA + predicts UC in 63.6%. Patients who remain IC do not show antibodies against ASCA or pANCA (9). There are a series of integrated genetic and serological markers and clinical phenotypes in the classification of inflammatory bowel disease (10). Serum autoantibodies, which appear long before onset of clinical disease, are the characteristic feature of autoimmune diseases (11). The seroprevalence of these Auto-antibodies among different studies has been quite variable, likely in part due to lack of standardization of the techniques used in these assays (12). These Auto-antibodies (serological markers) are non-invasive and may be helpful in the identification of this group of inflammatory bowel disease unclassified.

MATERIALS AND METHODS

Subjects. The patient group consisted of 60 patients with inflammatory disease unclassified. They were defined and diagnosed by their specialist physicians according to the clinical, endoscopic, radiological and histopathological examinations. The duration of the disease was more than one month. They had received no definite treatment and had no concomittant liver disease. They consulted Al-Kindi Teaching Hospital in Baghdad from January 2007 to January 2010. The second group consisted of 20 ulcerative colitis patients. Out of these, nineteen had left sided colitis and one patient had pan colitis.
Histopathology Study. The histopathological readings were done in triplicate on the specimens taken from either one or multiple biopsies from ileum or the different segments of colon depending on the case. The slides were read by more than one pathologist who did not know about the project. Patients with the clinical features of chronic inflammatory bowel disease unclassified, without small bowel involvement, in which endoscopy was non-conclusive, microscopy indicated active and patchy transmucosal chronic inflammation with a minimal to moderate architectural distortion. Small bowel involvement was excluded by macroscopic and microscopic evaluation of the terminal ileum at ileocolonoscopy and by small bowel x-ray barium follow-through studies.
Patients with malignant, hematomatous, hyperplastic and inflammatory polyp, diverticular disease, hemorrhoids and drug-induced colitis were excluded from the study. Infectious colitis and other types of colitis were excluded by stool cultures and parasitic examinations. The control groups consisted of 30 healthy volunteers, age- and sex-matched with the first group and were chosen from staff employees.

Sera prepared from the blood samples of each group were stored at -70°C until analyzed. Auto-antibodies against nuclear antigen, neutrophils (perinuclear antineutrophil cytoplasmic antibodies (pANCA) and cytoplasmic antineutrophil cytoplasmic antibodies (cANCA), intestinal goblet cells, *Saccharomyces cerevisiae* and the exocrine part of antipancreatic acinar cells were detected in the diluted serum (1:100) of both groups by indirect immunofluorescence using EURO IMMUNE test-(GERMANY). Assays were done in triplicate; the plates were read by more than one observer who was unaware of the origin of the sera.

The study was approved by the Ethical Committee of the Al-Kindi College of Medicine, Baghdad University and Al-Kindi Teaching Hospital, and all samples were obtained with informed consent in accordance with the Al-Kindi Teaching Hospital Declaration.

Statistical Analysis. Data were analyzed statistically using descriptive statistics (Frequencies for Tables, mean and standard deviation) and inferential statistics (Chi-square test). All of these were done using MiniTab statistical software program 13.20. A p value ≤ 0.05 was considered significant.

RESULTS

The results of this study revealed that males constituted 66.6% of the studying group. There were no significant difference between the healthy group and the UC group as shown in Table 1.

Table 1. Sex and age disparity among inflammatory bowel disease unclassified, ulcerative colitis patients and control group.

		IC patients n=60(%)	UC group n=20(%)	Control group n=30(%)	P value
Sex					
	Male	40 (66.6)	15 (75)	15 (50)	0.152[&]
	Female	20 (33.3)	5 (25)	15 (50)	
Age (Mean ± SD yrs)					
		45.67 ± 15.54	41.25 ± 20.5	44 ± 15.22	0.757[&]

[&]Not significant

There was no significant difference in mean age and state of smoking among inflammatory bowel disease unclassified, UC patients and control group (Tables 1 and 2). To best of our Knowledge there were no other patients diagnosed with CD in our country.

Auto-antibodies detected against perinuclear antineutrophil cytoplasmic antibodies (66.6%), *Saccharomyces cerevisiae* (58.3%) and exocrine part of pancreatic acinar cells (53.3%) were detected in inflammatory bowel disease unclassified patients, and not in the control group.

Table 2. State of smoking among inflammatory bowel disease unclassified, ulcerative colitis patients and the control group.

Smoker	IC patients n=60 No.(%)	UC group n=20 No.(%)	Control group n=30 No.(%)	P-value
Yes	25(41.6)	8(40)	19(63.3)	
No	20(33.3)	8(40)	8(26.6)	0.445
Ex-smoker	15(25.0)	4(20)	3(10.0)	

Table 3 shows significant differences among inflammatory bowel disease unclassified, UC and control group in Auto-antibodies directed against intestinal goblet cells, exocrine part of pancreatic acinar cells, perinuclear antineutrophil cytoplasmic antibodies and Saccharomyces cerevisiae.

Table 3. Auto antibodies of patients with inflammatory bowel disease unclassified, ulcerative colitis and the healthy control groups.

Positive Autoantibods* (IgG,IgM and IgA)	IC patients n=60 No (%)	UC group n=20 No (%)	Control group n=30 No (%)	P-value
Nuclear antigen	3 (5)	1 (5)	2 (6.6)	0.943
Intestinal goblet cell	2 (3.3)	10 (50)	1 (3.3)	0.000
Exocrine part of pancreatic acinar cells	32 (53.3)	2 (10)	1 (3.3)	0.000
perinuclear antineutrophil cytoplasmic antibodies (pANCA)	40 (66.6)	17 (85)	2 (6.6)	0.000
cytoplasmic antineutrophil cytoplasmic antibodies (cANCA)	0 (0)	0 (0)	0 (0)	–
Saccharomyces cerevisiae (ASCA)	35 (58.3)	1 (5)	3 (10)	0.000

*The isotypes of Auto-antibodies were mixed (IgG, IgA and IgM).

In this study, the patients with inflammatory bowel disease unclassified could not be followed-up to see if they progress to CD or UC or inflammatory bowel disease unclassified or recover.

DISSCUSION

The results reported in this study demonstrate that sera of patients suffering from inflammatory bowel disease unclassified contain Auto-antibodies reacting with one or more antigens (pANCA 66.6%, ASCA 58.3%, exocrine part of pancreatic acinar cells 53.3% and intestinal goblet cells 3.33%) and are significantly different (p=0.000) from other UC and control groups. The Auto-antibodies were of mixed isotypes (IgG, IgM and IgA). There was no age and sex limitation among inflammatory bowel disease unclassified, UC patients and the control group.

It seems that we are dealing with Auto-antibodies that contribute to the pathogenesis of bowel disease unclassified, because they are present in a considerable amount in intestinal goblet cells (3.33%) and the highest percentage acts against pANCA + ASCA- (66.6%). Circulating Auto-antibodies against these antigens have been reported by other authors. Joossens et al. (9) could demonstrate 61.5% of Auto-antibodies against ASCA and 63% against pANCA in IC patients and pANCA + predicts UC in 63.6%. This mild difference may be due to methods used and severity of disease in selected patients. It was reported that a combination of pANCA and ASCA measurments is better than estimating pANCA alone (13). ASCA develops due to an increased permeability of yeast antigens in the small bowel which activates immune cells (14-15-16). In case of pANCAs, they are directed against antigens in the nuclei, the granules and the cytosol (17-18) these serological markers have high specificities and low sensitivities; therefore they have therapeutic functions. a role in the treatment (19).

Other Auto-antibodies such as exocrine pancreas which is found in 30% of CD patients (20) is directed against normal pancreatic juice (21) and is found in a low titer in UC (22) especially in the first degree relatives of the patients (23). In our study we also demonstrated the exocrine pancreas autoantibody in inflammatory bowel disease unclassified (53.3%). Another Antibody observed is anti goblet cell autoantibody, present in 3.3% of the inflammatory bowel disease unclassified patients. This anti goblet cell autoantibody is specific to ulcerative colitis (69%) (24) and 3.33 % of our inflammatory bowel disease unclassified patients and 50% of our UC cases demonstrated it. These variations in the development of Auto-antibodies may be due to genetic factors (6), geographical (25), and ethnic factors [(26)], socioeconomic status (27) and human leukocyte antigen (HLA) typing (28).

These serological markers are important in the diagnosis, management, disease monitoring, severity and prognosis of disease in ulcerative colitis and Crohn's disease or inflammatory bowel disease unclassified, while Tremaine (29) has found the diagnosis of inflammatory bowel disease unclassified by exclusion.

We conclude the auto-immune nature of this disease. There are some antigens originating from gastrointestinal tract or the bile system that may be involved in the initiation of the inflammatory bowel disease unclassified.

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