Diagnostic value of antineutrophil cytoplasmic antibodies and anti-*Saccharomyces cerevisiae* antibody in Iranian patients with inflammatory bowel disease

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Abstract

Background and study aims : **Perinuclear antineutrophil cytoplasmic autoantibodies (pANCA) and anti-*Saccharomyces Cerevisiae* antibody (ASCA) are potential markers for diagnosis of inflammatory bowel disease (IBD). The aim of the present study was to evaluate the diagnostic value of pANCA and ASCA in Iranian patients with IBD.**

Patients and Methods : **Serum samples were collected from 144 patients with IBD (113 ulcerative colitis and 31 Crohn's disease) and patients with non-IBD problems were assayed for ASCA by Enzyme-Linked Immunosorbent Assay (ELISA) and for pANCA by indirect immunofluorescence assay.**

Results : **Sensitivity and specificity of pANCA in UC were 39.8% and 82.1%, respectively. For CD, pASCA test provided the sensitivity of 58% and specificity of 70%. A combination of $pANCA^{+}/ASCA^{-}$ for diagnosis of UC showed a sensitivity of 31.9% and specificity of 89.1%. In addition the combination of $pANCA^{-}/ASCA^{+}$ showed a sensitivity of 35.5% and specificity of 79.8% for diagnosis of CD.**

Conclusion : **Due to low sensitivity of pANCA and ASCA alone or in combination, they are not valuable serological markers for diagnosis of UC or CD.** (Acta gastroenterol. belg., **2009**, 72, **301-305**).

Key words : inflammatory bowel disease, Antineutrophil antibodies, Anti-*Saccharomyces cerevisiae* antibody, Crohn's disease, ulcerative colitis.

Introduction

Inflammatory bowel diseases (IBD) are subdivided into ulcerative colitis (UC) and Crohn's disease (CD). Making an earlier and more accurate diagnosis of IBD is important as the management of CD and UC is different, especially when surgery is planned. A search for serological tests to differentiate CD from UC has been underway for a long time. Various autoantibodies have been recognized in patients with inflammatory bowel disease.

A subset of antineutrophil antibodies, commonly referred to as perinuclear antineutrophil cytoplasmic autoantibody (pANCA) has been observed in sera from patients with IBD. The prevalence of a positive pANCA varies from 40% to 80% in UC and from 0% to 20% in CD (1,2). Since 1988, systemic antibodies against the yeast *Saccharomyces cerevisiae* (ASCA) have been reported in sera from patients with CD. The prevalence of ASCA varies from 60-80% in CD and 10-15% in UC (1-4).

The use of pANCA and ASCA alone has low sensitivity and specificity for diagnosis of UC and CD. Combination of positive pANCA and negative ASCA as well as negative pANCA and positive ASCA increases the specificity of UC and CD diagnosis, respectively. The combined measurement of pANCA and ASCA has been advocated as a valuable diagnostic approach in IBD (2,5).

The aim of the present study was to investigate the diagnostic value of pANCA and ASCA alone and in combination in a sample of Iranian patients with UC and CD.

Materials and methods

Patients

One hundred thirteen Iranian patients with UC and 31 patients with CD were evaluated. Patients were treated at the department of Gastroenterology of Taleghani University Hospital during 2003 to 2004. This center is a well-known gastrointestinal (GI) center in Tehran (capital of Iran) which welcomes individuals with GI problems from all around the country.

The diagnosis of IBD was verified on the basis of well-established clinical, endoscopical, radiological, histological, and surgical criteria as described by Lennard-Jones (6).We excluded patients with indeterminate colitis and patients in whom a diagnosis of UC or CD had not been clearly established.

In patients with UC, disease was regarded as quiescent when quiescent or mildly active, active when moderately active and severe according to the Truelove and

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Witts index (7). Disease activity in CD patients was assessed using the Crohn's disease activity index (CDAI) (8).

Patients' information including smoking habit, oral contraceptive consumption, and history of appendectomy, family history of IBD, duration of disease, extra-GI manifestations, intestinal complications and taking medications were obtained from database of Research Center for Gastroenterology and Liver Disease.

Controls

Patients with miscellaneous non-IBD illnesses were taken as controls. This group consisted of 47 patients with dyspepsia, 42 with irritable bowel syndrome, 35 with rectorrhagia, 35 with flatulence complaint and 17 with GI cancers, for whom the diagnosis of IBD was excluded. These patients were referred to above clinics during 2003 to 2004 and their information was gathered through the same way as for the IBD patients. Determination of pANCA was done in 107 and ASCA in 133 control patients.

This project was approved by ethical committee of Shaheed Beheshti University of Medical Sciences and informed consent was taken from all cases and control groups.

ASCA

The determination of ASCA IgG and IgA were performed using commercially available enzyme-linked immunosorbent assay (ELISA) kit (GENESIS diagnostics Co, UK). Briefly, 100 µl of diluted serum samples (1:50), diluents, standard, positive and negative controls were added into the microplate wells and incubated 30 min at room temperature. After washing 3 times, 100 µL rabbit anti human IgG and IgA, conjugated to horseradish peroxidase in protein stabilizing solution and antimicrobial agent, were added to the wells and incubated 30 min at room temperature. Washed the wells four times then 100 µL of aqueous solution of tetramethylbenzidine and hydrogen peroxide were added to each well and incubated 10 min at room temperature. Then 100 µL of stop solution (0.25 M sulphuric acid) was added to each well and optical densities were measured using a microplate reader at 450 nm.

pANCA

To detect p-ANCA, an indirect immunofluorescence method was performed using commercially available kit (IMMCO Diagnostics, Inc. Canada). Briefly, Sera were diluted 1:20 with the buffered diluents. 50 µl of diluted sera, positive and negative control were placed on each slide well and incubated for 30 min in a humid chamber at room temperature. Slides were rinsed by dipping into beaker with PBS then transfered into Coplin jar and washed 10 minutes. FITC-conjugated anti-human IgG antibody (50 µL) was added to each well and incubated for 30 min. After washing slides were mounted and read independently under fluorescent microscope by two observers.

Statistical analysis

Commercial software (SPSS for Windows, V 11.5) was used for statistical analysis. Characteristics of all subjects were compared between groups using Chi-Square and Fisher's exact test. A p value less than 0.05 was considered statistically significant. The sensitivity and specificity of the tests were determined.

Results

One hundred forty four IBD patients were investigated of which 113 (78.5%) had UC and 31 (21.5%) had CD, confirmed by endoscopy and pathology (Table 1). Controls were 47 patients with dyspepsia, 42 with Irritable bowel Syndrome, 35 with non-IBD rectorrhagia, 35 with flatulence complaint and 17 with GI cancer (Fig. 1).

Of 113 UC patients, 23 had pancolitis, 25 left colitis, 11 distal colitis, 18 proctitis, whereas extension was not applicable in 36 patients due to severe acute colitis in colonoscopy or patients intolerance which the endoscopist unable to assess the extension. Fourty six percent of UC patients had mild colitis.

In CD patients, disease extension was restricted to the small intestine in 12 patients, large intestine in 10 patients, both small and large intestines in two patients, perianal region in four patients and extension was not applicable in three patients. In most of CD patients, disease severity was mild (71.4%).

pANCA and ASCA

As shown in Table 2, the frequency of pANCA positivity was significantly higher in IBD patients 40.3% (58/144) than non-IBD (controls) patients 17.8% (19/107) ($p = 0.0002$). The frequency of pANCA positivity was 39.8% (45/113) in UC and 41.9% (13/31) in CD. The frequency of pANCA positivity was significantly higher in UC or CD than control group ($p < 0.01$), While there was no significant differences between UC (45/113 (39.8%)) and CD 13/31 (41.9%) ($p = 0.83$) regarding pANCA positivity.

The frequency of ASCA positivity was not statistically significant differences ($p = 0.66$) between IBD patients 32.6% (47/144) and controls 30.1% (40/133). Positive ASCA was found in 18/31(58%) of CD and 29/113 (25.7%) of UC. There was a significant difference between CD and UC regarding ASCA positivity ($p = 0.001$). In addition, the frequency of ASCA positivity was significantly higher in CD than control group ($p = 0.003$), while there was found no significant differences between UC and control group regarding ASCA positivity ($p = 0.44$).

The sensitivity and specificity of pANCA and ASCA for distinguishing IBD fron non-IBD patients were

Table 1. — Demographic Characteristics of IBD Patients

		Ulcerative colitis	Crohn's disease	All patients
Number of patients (%)		113 (78.5)	31 (21.5)	144 (100)
Gender (male) (%)		49 (43.4)	12 (38.7)	61 (41.6)
Age (years), Mean (95% CI*)		39 (36 to 42)	35 (30 to 39)	38 (36 to 42)
Age of onset (years), Mean (95% CI)		33 (30 to 35)	30 (25 to 34)	32 (30 to 34)
Disease duration (months), Mean (95% CI)		69 (56 to 81)	57 (36 to 78)	66 (55 to 77)
Current cigarette smokers (%)		13 (11.5)	4 (13)	17 (12)
OCP consumers among females (%)		22 (19.5)	5 (16)	27 (23.6)
History of appendectomy (%)		8 (7)	3 (10)	11 (7.6)
History of IBD in first relatives (%)		7 (6)	4 (21)	11 (7.6)
Skin lesion (%)		14 (12.4)	2 (10)	16 (13)
Oral aphtus (%)		9 (8)	1 (3.2)	10 (7)
Musculoskeletal manifestations (%)		12 (10.6)	3 (9.7)	15 (10.4)
Arthritis and Sacroileitis (%)		9 (8)	2 (6.5)	11 (9)
Eye manifestations (%)		5 (4.5)	0	5 (3)
Liver manifestations (%)		8 (7)	2 (6.5)	10 (7)
Sclerosing cholangitis (%)		6 (5.3)	1 (3.2)	7 (4.8)
Intestinal complications	Dysplasia (%)	6 (5.3)	1 (3.2)	7 (4.8)
	Stenosis (%)	39 (34.5)	0	39 (27)
	Severe bleeding (%)	20 (17.7)	0	20 (17)
	Abdominal abscess (%)	0	2 (6.5)	2 (1.4)
	Pouchitis (%)	1 (0.9)	0	1 (0.7)

IBD : inflammatory bowel disease ; CI : Confidence Interval ; OCP : oral contraceptive preparations.

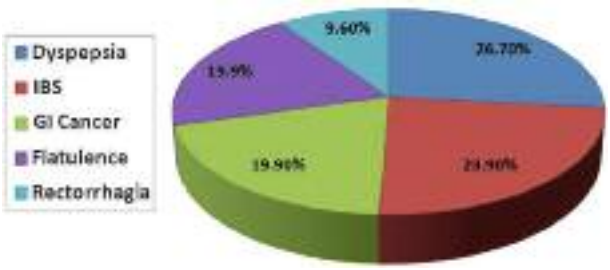

Fig. 1. — Types of diseases among patients in control group (non-IBD).

Table 2. — **Frequency of pANCA and ASCA positive in IBD, UC, CD and non-IBD patients**

	pANCA+	ASCA+
IBD	58/144 (40.3%)	47/144 (32.6%)
UC	45/113 (39.8%)	29/113 (25.7%)
CD	13/31 (41.9%)	18/31 (58%)
Controls (Non-IBD)	19/107 (17.8%)	40/133 (30.1%)

40.8%, 82.8%, and 32.2%, 69.9, respectively (Table 3). Sensitivity and specificity of pANCA and ASCA and both tests together for distinguishing UC and CD from non-IBD disease was shown in Table 4. The results showed that the sensitivity and specificity of pANCA$^+$ for distinguishing UC or CD from non-IBD were 39.8%, 82.1%, and 41.9%, 82.2%, respectively. Similarly, the sensitivity and specificity of ASCA$^+$ for distinguishing UC or CD from non-IBD were 25.6%, 69.9%, and 58.1%, 69.9%, respectively. A combination of pANCA$^+$, ASCA$^-$ showed a sensitivity of 31.9% and specificity of

Table 3. — **Sensitivity and specificity values of pANCA$^+$ and ASCA$^+$ for distinguishing IBD from non-IBD disease (control)**

Specificity (%)	Sensitivity (%)	
82.2	40.8	**pANCA$^+$**
69.9	32.6	**ASCA$^+$**

89.1% for distinguishing of UC from non-IBD. Similarly, the combination of pANCA$^-$, ASCA$^+$ result in a sensitivity and specificity of 35.5% and 79.9% for the diagnosis of CD, respectively

As shown in Table 5, for distinguishing UC from CD the pANCA$^+$ showed a sensitivity of 39.8% and specificity of 58.0%. In the same way, ASCA positivity showed a sensitivity of 25.7% and specificity of 42.0%.

pANCA had a sensitivity of 39.8% and specificity of 89.0% and ASCA had a sensitivity of 25.7% and specificity of 64.6% for distinguishing UC from control (CD + non-IBD) (Table 6).

In UC patients, pANCA was more positive in smokers than non-smokers (Fisher's exact test, $p = 0.01$). UC patients with sclerosing cholangitis (Fisher's exact test, $p = 0.003$) and severe bleeding ($p = 0.004$) were significantly more positive for pANCA than other UC patients. pANCA positive results were less observed in patients with mild colitis, in contrast with patients with severe colitis who had more positive pANCA, respectively ($p = 0.02$).

Discussion

In this sample of Iranian IBD patients, the sensitivity of pANCA for UC and ASCA for CD was low (39.8%

Table 4. — **Sensitivity and specificity values of pANCA, ASCA and both tests together for distinguishing UC and CD from non-IBD disease (control)**

Specificity (%)	Sensitivity (%)	
82.1	39.8	**pANCA$^+$ for UC**
82.2	41.9	**pANCA$^+$ for CD**
69.9	25.6	**ASCA$^+$ for UC**
69.9	58.1	**ASCA$^+$ for CD**
89.1	31.9	**pANCA$^+$, ASCA$^-$ for UC**
79.7	35.5	**pANCA$^-$, ASCA$^+$ for CD**

Table 5. — **Sensitivity and specificity of pANCA$^+$ and ASCA$^+$ for distinguishing UC from CD**

Specificity (%)	Sensitivity (%)	
58.0	39.8	**pANCA$^+$**
42.0	25.7	**ASCA$^+$**

Table 6. — **Sensitivity and specificity of pANCA$^+$ and ASCA$^+$ for distinguishing UC from control (CD + non-IBD)**

Specificity (%)	Sensitivity (%)	
89.0	39.8	**pANCA$^+$**
64.6	25.7	**ASCA$^+$**

vs. 58.1%) ; while the specificity of pANCA for UC and ASCA for CD was fairly high (82.1% *vs.* 69.9%). On the other hand, the combination of both markers increased the specificity, but decreased the sensitivity. Our findings showed that the incidence of pANCA positivity was low and similar in UC and CD. There are few reports that support this finding (9-11). Kazunori Sugi *et al.* reported that pANCA was detected in 63.5% of the Japanese patients with UC and 72.1% of the patients with CD (9). Saibeni *et al.* observed low prevalence of pANCA in ulcerative colitis patients from the Mediterranean area (12).

The present study showed that combination of pANCA and ASCA is specific but not sensitive for IBD diagnosis, which confirms most of previous studies (1,13-19). This finding could be explained by a low proportion of CD patients in our study. In addition, wide range of sensitivities and specificities for ASCA is observed in CD patients, using different ASCA assays. This is mainly a consequence of the cutoff value chosen for each individual assay (20).

Anand *et al.* (21) reported that a positive pANCA test alone provided a sensitivity of 50% and a specificity of 82% for UC. Our results are in agreement with their finding. Contrary to our results, they found that a positive ASCA test alone provided a sensitivity of 40% and a specificity of 100% for CD. They also found that a combination of positive pANCA and negative ASCA showed a sensitivity of 50% and specificity of 90% for the diagnosis of UC and the combination of ASCA-positive and pANCA-negative for the diagnosis of CD provided a sensitivity and specificity of 32% and 100%, respectively.

In agreement with our finding, Mokrowiecka *et al.* (22) had found that pANCA$^+$, ASCA$^-$ pattern had a sensitivity of 36% and specificity of 98% for diagnosis of UC. In addition they reported that the pANCA$^-$, ASCA$^+$ pattern for diagnosis of CD had a sensitivity of 35% and specificity of 88%. While in contrary to our results Mainardi *et al.* (23) had found that the association of ASCA$^-$/ ANCA$^+$ had a sensitivity of 70%, and specificity of 86% for the diagnosis of UC. They found that ASCA$^+$/ANCA$^-$ had 86% sensitivity and 93% specificity for CD, with a positive predictive value of 75%.

In our study, positive pANCA was related to UC complications and severity. In UC patients, positive pANCA was more observed in smokers, as well as in 6 UC patients with sclerosing cholangitis, severe bleeding and severe colitis. Previous studies have generally shown that the presence of pANCA in UC does not correlate with disease activity, complications, type of medical therapy, anatomical extension of disease and risk of pouchitis following ileal pouch-anal anastomosis (1,5,12,16,24-27). On the other hand, there are some studies that have correlated pANCA titer with disease activity (28), risk for pouchitis following ileal pouch-anal anastomosis (29) and treatment-resistant left-sided UC (30).

In conclusion, due to the low sensitivity of pANCA and ASCA alone or in combination, they are not valuable serological markers for diagnosis of UC or CD. It appears that Iranian patients with IBD differ in some characteristics with other populations.

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