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RAPID COMMUNICATION

Perinuclear anti-neutrophil cytoplasmic antibodies (p-anca) in chronic ulcerative colitis: Experience in a Mexican institution

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Abstract

AIM: To assess the prevalence and clinical value of p-ANCA in a sample of Mexican ulcerative colitis (UC) patients.

METHODS: In a prospective, IRB-approved protocol, p-ANCA was determined in 80 patients with UC (mean age, 32 ± 12.9 years). The severity and extension of disease were determined by clinical methods, searching a statistical association with p-ANCA status.

RESULTS: p-ANCA were detected in 41 (51%) patients. Severity of disease was the only clinical variable statistically associated with their presence ($P < 0.0001$; OR = 9; CI 95% = 3.2-24.7).

CONCLUSION: The prevalence of p-ANCA was similar to that reported in other countries. Their presence was associated to UC severity, but offered no more information than the obtained by clinical methods.

Key words: Ulcerative colitis; Inflammatory bowel disease; Perinuclear anti-neutrophil cytoplasmic antibodies

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INTRODUCTION

Anti-neutrophil cytoplasmic antibodies (ANCA) have been described in subjects with different types of vasculitis, and they are essential to establish the diagnosis of Wegener´s granulomatosis, microscopic polyangiitis, Churg-Strauss Syndrome and renal-limited vasculitis[1-4]. Perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA), a subset of ANCA, have been described in patients with inflammatory bowel disease, mainly ulcerative colitis (UC). However, their role in pathogenesis and diagnostic value are still controversial[5]. Previous reports have suggested that p-ANCA might be a sensitive and specific test to diagnose UC[6], to distinguish it from Crohn's disease and other colitides[7], and to provide a prognosis regarding response to medical treatment and risk of pouchitis following the pelvic pouch[8,9].

The prevalence of p-ANCA in patients with UC varied from 40% to 88%[10-13]. High titers have been reported by some authors in subjects with active disease, but its clinical value is controversial because others have not found any correlation between their levels and the activity or extension of disease[14-18].

The value of a diagnostic test, such as p-ANCA, should be evaluated by several practical factors like costs, availability, and usefulness for clinical decisions before accepting them as a standard diagnostic tool.

The aim of this study was to assess the prevalence and clinical usefulness of p-ANCA in a sample of Mexican patients with UC.

MATERIALS AND METHODS

A prospective study was performed at the Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, which is a referral center for gastrointestinal diseases in Mexico City. The protocol was reviewed and approved by the Institutional Review Board. All patients invited to participate in the study signed an informed consent before inclusion. Eighty consecutive patients with diagnosis of UC were included. In all cases, the diagnosis of UC was based on clinical, endoscopic and histopathologic findings. The extension of disease was determined by total colonos-

copy, and categorized into pancolonic or left-sided colitis. The severity of disease was determined on clinical basis according to the Truelove and Witts criteria[19], and categorized into mild, moderate and severe. Clinical data, including severity and extension of disease were collected by an investigator who was blind to the results of p-ANCA.

p-ANCA determination

Detection of ANCA was done by Indirect Immunofluorescence (IFL), in accordance with the recommendation of the International Workshop[20]. Human neutrophils were prepared from peripheral blood of patients and normal healthy volunteers. The slides were fixed in 98% ethanol at 4℃ for five minutes and dried quickly in air. After using phosphate-buffered saline (PBS) in a dilution of 1:40, the elements where dissolved twofold until it reached 1:320. Following incubation for 1 h r at RT, the slides were washed three times with PBS and bound antibodies were incubated for 30 min and detected with FITC-conjugated rabbit anti-human IgG at RT. Subsequently, the slides were washed three times with PBS and covered with glycerin-phosphate-buffered saline. A titer of 1:20 or higher was considered positive.

The antigen-specific enzyme-linked immunosorbent assay (ELISA) method was used to test the sera for the presence of either mieloperoxidase (MPO) or anti-proteinase-3 antibodies. Human MPO or human proteinase-3 were diluted to 2 mg/L in carbonate buffer (pH 9.6) and 100 μL of each solution was placed in the wells of a 96-well microplate and left for 24 h at 4℃. After plate was washed with PBS three times, 100 μL of diluted sera (1:100 in PBS containing 0.1% tween 20 and 0.1% skim milk) was added to each well, and the plate incubated for 1 h at 37℃. After washing, the enzyme reaction was performed, and color development was measured with a microplate colorimeter. Optical density values > 2 SD more than the mean of the control subjects were considered positive.

Statistical analysis

The student t- -test was used for dimensional variables, and the association between categorical variables was studied by means of the χ^2 with Yates correction. The *P* values were 2-tailed and less than 0.05 was considered statistically significant.

RESULTS

Eighty patients with diagnosis of UC were included. There were 41 men and 39 women, with a mean age of 32 ± 12.9 years. The mean duration of disease was 7.2 ± 6.6 years. Pancolitis was present in 58% and left-sided colitis in 42%. Disease severity was categorized as severe in 20 (25%), moderate in 7 (10%), mild in 15 (18%), and there was no evidence of activity in the remaining 38 (47%) patients. Extraintestinal manifestations were present in 43%; and corresponded to arthritis (23%), primary sclerosing cholangitis (7.5%), erythema nodosum (5%), ankylosing spondylitis (2.5%), pyoderma gangrenosum (2.5%), anterior uveitis (2.5%), and aphthous ulcers (2.5%). Fourteen (25%) patients required surgical treatment due to failure of medical therapy.

Table 1 Clinical features in UC patients with positive and negative p-ANCA

Clinical feature	p-ANCA + *n* = 41	p-ANCA - *n* = 39	*P* value	OR	CI 95%
Pancolitis	22	24	0.62	0.72	(0.29-1.76)
Left-sided	19	15			
Colectomy	7	7	0.84	0.94	(0.29-2.98)
No colectomy	34	32			
EIMs present	14	17	0.52	0.67	(0.27-1.65)
EIMs absent	27	22			
Severe	31	10	< 0.0001	9	(3.2-24.7)
No Severe	10	29			

OR = odds ratio; CI 95% = Confidential interval 95%; *n* = Number of patients.

The p-ANCAs were detected by IFL in 41 (51%) and c-ANCA in 4 (5%) subjects. Titers ranged from 1:20 to 1:160. The antigenic specificity of p-ANCA tested by ELISA in 33 patients showed a positive reaction for myeloperoxidase in 29 and proteinase-3 in 4. Age, gender and age at diagnosis was similar between patients with or without p-ANCA .

A statistical association was observed between p-ANCA status and the severity of UC (*P* = < 0.0001; OR 9, CI 3.2-24.7). Twenty-two (48%) patients with pancolitis and 19 (56%) with left-sided colitis were positive for p-ANCA (*P* = 0.47; OR = 0.72; 95% CI: 0.27-1.94). Seven out of the 14 (50%) patients who underwent surgical treatment were positive as compared with 34 of 66 (52%) who were not operated upon [*P* = 0.91; OR = 0.94; 95% CI: 0.26-3.34].No association was found between the presence of extraintestinal manifestations and p-ANCA. See Table 1.

DISCUSSION

The determination of p-ANCA could give information in subjects with UC in three different clinical issues: (1) as a marker of genetic heterogeneity; (2) to assess inflammatory activity, and (3) for prognosis regarding response to medical treatment and postoperative outcome (pouchitis).

The prevalence of p-ANCA in our series was 51%, within the range published by other authors in different parts of the world[9-13]. It supports that not all patients with UC exhibit the same immunologic pattern, a fact against a pathogenic role of ANCA's in subjects with UC. On the other hand, the wide range of prevalence reported in the literature that goes from 40 to 88% could be rather explained by ethnic or genetic variations than by technical difficulties since both, IFL and ELISA, are simple, sensible, and reproducible assays, as observed in the present study[14,21].

Ethnic and genetic variability have been consistently reported in patients with IBD[22,23]. In a previous study, the authors found that Mexican patients with UC had an increased frequency of HLA-DR1 (DRB1*0102 and 0103) and HLA-DR2 (DRB1*15) when compared with healthy controls. HLA-DRB1*0103 and *0102 were associated

with more severe disease and necessity of surgical treatment[24]. Yang *et al* observed a linkage between p-ANCA positive UC patients and HLA-DR2[25]. The clinical usefulness of p-ANCA as a genetic marker was suggested many years ago by Shanahan who found a higher prevalence of p-ANCA in first-degree relatives of patients with UC[26]. This finding was confirmed by Lee[27] *et al*, in a different ethnic group. So, p-ANCA determination could be useful as a marker of genetic predisposition. However, today there is no information about predictive values of p-ANCA in this setting, and more research is necessary to answer this important question.

Several studies, although not all, have demonstrated a correlation between serum levels of p-ANCA and severity of UC[5,12,15,29-31]. In the present study, an association between the presence of p-ANCA and a subset of patients with severe UC was found but, as other studies, there was no correlation between levels of p-ANCA and the activity of disease evaluated by clinical and biochemical methods. From the point of view of the authors, a sophisticated laboratory exam such as the p-ANCA determination may not currently have a role in the evaluation of severity because it can be evaluated by simple and reproducible methods.

Fleshner *et al*, reported high pre-operative levels of p-ANCA in subjects who developed chronic pouchitis after the ileo-anal pouch[8]; however, it should be noted that some patients with acute and chronic pouchitis were p-ANCA negatives. The follow-up of patients in our series is still too short to analyze this aspect.

In conclusion, the prevalence of p-ANCA in this series was similar to that reported in other countries. Their presence was associated to severity of UC, but offered no more information than the obtained by clinical methods. Future research may clarify their role as a screening test for the first-degree relatives of UC patients, or for patients requiring an ileo-anal pouch.

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