ANTINEUTROPHIL CYTOPLASMIC AUTOANTIBODIES IN ULCERATIVE COLITIS

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Abstract - Antineutrophil cytoplasmic autoantibodies (ANCA) were detected in patients with certain autoimmune and vascular diseases such as Wegner's granulomatosis, Polyarthritis nodosa and systemic lupus erythematous. Indirect immunofluorescence (IIF) technique was employed to detect these autoantibodies. ANCA have been recently detected in some forms of inflammatory bowel disease (IBD), ulcerative colitis (UC), Crohn's disease (CD,) and primary sclerosing cholangitis (PSC). By IIF method, two general patterns of ANCA were seen: a cytoplasmic (C-ANCA) and perinuclear form (P-ANCA). In this study we evaluated the presence of ANCA in 52 UC patients and 69 matched normal control group by IIF technique, and it's relationship with disease activity, site of colon involvement, and lesion extent. The results showed that all control group were ANCA negative, but 58% of patients had ANCA, and most cases (76%) had C-ANCA. The obtained results also revealed that there was no relationship between ANCA and disease activity.

Acta Medica Iranica 38 (3): 187-189; 2000

Key Words: Antineutrophil cytoplsmic autoantibodies, ulcerative colitis

INTRODUCTION

Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed against endosomal enzymes of human neutrophils and monocytes. These autoantibodies have been detected in various forms of vasculitis including segmental necrotizing glomerulonephritis, Wegener's granulomatosis (WG), and microscopic polyarthritis (1,2). ANCA have been recently detected in some forms of inflammatory bowel disease (IBD), ulcerative colitis (UC), Crohn's disease (CD) and primary sclerosing cholangitis (PSC) using indirect immunofluorescence and fixed granulocyte ELISA (3,4,5). Two major staining patterns can be distinguished (on indirect immunofluoresecence (IIF), a cytoplasmic pattern (C-ANCA), and a perinuclear one (P-ANCA), (6). The main target antigen associated with C-ANCA is proteinase-3 and for P-ANCA is myeloperoxidase (7,8). In the present study, we examined the presence of ANCA in 52 UC patients and 69 normal control group by IIF technique. Relationship between ANCA and disease activity, site of colon involvement and lesion extent were the other goals of this study.

MATERIALS AND METHODS

52 UC patients (31 female and 21 male) from different provinces referred to gastroenterology section of Shariati Hospital - Tehran were selected. 69 healthy adult volunteers were studied as control group. The subjects' sera were screened for antineutrophil cytoplasmic antibody (ANCA) and antinuclear antibody (ANA) by indirect immunofluorescence technique (IIF).

Presence of ANCA in undiluted serum and detection of ANA in serum dilution greater than 1:40 serum were our criteria to consider a patient's serum as positive. P-ANCA positive subjects with positive ANA were excluded and considered as ANCA negative.

Statistical analysis (Chi-square and T test) was done to determine the correlation of ANCA positivity and disease activity and the significance of different types of ANCA.

RESULTS

In this study the presence of ANCA in 52 patients with ulcerative colitis (31 female 14-55 years old and 21 male 16-66 years old) was evaluated; 69 healthy matched volunteers were considered as control group. The patients were classified to 4 groups (inactive, mild, moderate and severe) according to the clinical symptoms (frequency of diarrhea) in the patients (table 1). 40 out of 52 UC cases (76%) were in the inactive form and the 12 remainders (24%) had active form of the disease.

Table 1. Presence of ANCA in ulcerative colitis patients relevant to disease activity

Disactivity	ANCA+	P-ANCA	C-ANCA	Total
Inactive	23	8	15	40
Mild	3	-	3	5
Moderate	3	-	3	5
Severe	1	1	-	2
Total	30	9	21	52

According to the site of colon involvement and lesion extent the patients were also classified into 6 groups (table 2). The majority of cases had pancolitis (16 cases) and left sided colitis (15 cases). Table 1 and 2 show that 30 (58%) UC cases have ANCA out of whom which 21 of them (70%) are C- ANCA type and 9 remainders (30%) P-ANCA positive. 23 (76 %) out of 30 ANCA positive cases were in the inactive form and 7 (24%) were in the active form of the disease. As indicated in table 2, 11 out of 16 pancolitis cases and 9 out of 15 left sided colitis were ANCA positive.

Statistical analysis of the results revealed that there was no significant correlation between ANCA presence, disease activity and site of colon involvement.

Table 2. Prevalence of antineutrophil cytoplasmic antibodies (ANCA) in patients with different kinds of ulcerative colitis

Location	Total	ANCA$^+$	ANCA$^-$	P-ANCA	C-ANCA
Pancolitis	16	11	5	2	9
Subtotal	5	2	3	-	2
Rectrosigmodi	6	2	4	1	1
Left sided	15	9	6	2	7
Proctitis	8	5	3	3	2
Pouch	2	1	1	1	-
Total	52	30	22	9	21

DISCUSSION

In this study ANCA presence in patients with UC was evaluated. The results showed that 30 out of 52 UC sera (58%) contained ANCA, while all control samples were ANCA negative. Similar findings concerning ANCA frequency in UC subjects were reported by others. Rump JA. and colleagues (1990) have shown that 20 of 34 (59%) sera from patients with ulcerative colitis had P-ANCA (9). Broekroelofs (1994) found P-ANCA in 33 of 67 (49%) ulcerative colitis patients (10). Sobajima and colleagues (1996) analyzed the clinical significance of ANCA in ulcerative colitis patients with either an indirect immunofluoreseence assay or an ELISA with fixed neurtrophils. 71% (25/35) of the patients were positive for ANCA (11). The most commonly observed pattern of ANCA in UC patients in other studies were P-ANCA (9,10,12,13) but Sung and colleagues (1994) in a study on Chinese patients with ulcerative colitis (N=19) indicated that 73% of subjects exhibited either P-ANCA (31%) or C- ANCA (42%). Also, our study showed that 21 out of 30 ANCA positive subjects (70%) had C-ANCA and 9 remainders (30%) had P-ANCA. In this regard, the genetic background of population, and environmental agents may be the suspected causative factors.

Abad and colleagues (1997) in a study showed that most ANCA positive sera from IBD patients were negative for antibodies to protienase 3 and myeloperoxidase by ELISA (3). They suggested that the autoantigens recognized by ANCA were different in patients with IBD from those with necrotizing vasculitis.

In the study conducted by Rosa and collegues none of the ANCA positive patients had antibodies to myeloperoxidase or to alpha granules which are usually found in sera of patients with ANCA - associated vasculitis (15). As reported in literature it seems that the P-ANCA staining pattern of granulocytes is not restricted to anti-myeloperoxidase antibodies. More recently a study on the antigen spicificity and new antigen of ANCA positive UC patients demonstrated that high mobility group (HMG) non-histone chromosomal proteins (HMG1and HMG2) are novel target antigens of PANCA. HMG1 and HMG2 are distributed in the nuclei and cytoplasm of eukaryotic cells and act as transcription factors (16,17).

As it has been shown in tables 1 and 2 , there is not any significant correlation between ANCA positivity and variables, such as disease activity, site of colon involvement and lesion extent.

Treatment of the disease also had no correlation with ANCA positivity (unpublished data). The study performed by Sobajima (1996) also revealed that there was no significant correlation between the ANCA positivity and various variables, i.e. disease activity, extent of lesion , or treatment of the disease. In contrast, Hertervig (1995) in a study on 155 patient with ulcerative colitis showed that the presence of ANCA was correlated to disease activity, extent, and age of onset of the disease (13).

Our results also revealed that there was no correlation between ANCA and ANA in these patients which indicates that the immune response against neutrophil cytoplasmic antigens is independent of the response against nuclear antigens.

Future investigations must be conducted to precisely evaluate the importance of these autoantibodies and their correlation to disease activity in inflammatory bowel disease. The type of ANCA, the target antigen of these autoantibodies and the importance of newly recognized autoantigens in UC, are the other subjects that must be considered in future studies.

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