THE PREVALENCE AND CLINICAL SIGNIFICANCE OF PERINUCLEAR ANTINEUTROPHIL CYTOPLASMIC ANTIBODY IN PATIENTS WITH INDETERMINATE COLITIS

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ABSTRACT

Background: Indeterminate colitis (IC), a term originated by pathologists to characterize confounding histopathlogic appearance of resected mucosa, has become catch phrase for cases in which diagnostic criteria at all levels elude classification as Crohn's disease (CD) or ulcerative colitis (UC).

OBJECTIVES: evaluate the prevalence of pANCA expression in the sera and its isotypes.

Patients and methods: PATIENTS GROUP consisted of 60 patients (40 males and 20 females) with indeterminate colitis and their age range was (19-84 years). CONTROL GROUP consisted of 30 (15 males and 15 females) healthy volunteers and their age range was (20- 66 years).

Antineutrophil cytoplasmic (pANCA and cANCA) testing was performed by an IIF technique on ethanol fixed human EOH granulocytes as substrate (EUROIMMUNE- Germany). Sigmoidoscope and colonoscope examination were done for the patients group and biopsies were taken from the patients for histopathological examination.

Results:

Serological results of ANCA showed a significant increased frequency of pANCA (63.3%) in indeterminate colitis patients as compared to controls (p=0.000). The highest percentage of this pANCA titer was 1:10 (p=0.000) then 1:100 (p=0.008) and most of them was IgG (53.3%) (p=0.000). Sensitivity of pANCA was 60%, specificity of pANCA was 40%, positive predictive value of pANCA was 61.1% and negative predictive value of pANCA was 66.6%. cANCA did not demonstrated in both groups.

Conclusions : pANCA was more prevalent in indeterminate colitis and could be used as a predictive serological marker for the outcome of disease.

Key words: ANCA , auto antibodies , indeterminate colitis.

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Introduction:

The chronic inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC) are now common causes of gastrointestinal disease in the Western world [(1).] CD and UC may present with similar symptoms, but differentiation is based on clinical features, anatomical distribution and pathological findings [(2).] However, in approximately 10% -15% of cases of colonic IBD, indeterminate colitis (IC) is diagnosed [(3).]

Antibodies to several specific antigens have been reported in the sera of patients with IC. It was hoped that studies of such antibodies would provide insight into disease pathogenesis, heterogeneity and putative serological markers to adjunct / replace current diagnostic protocols [(4).] perinuclear antineutrophil cytoplasmic antibodies (pANCA) have been proposed as a marker for UC, with 60–80% prevalence compared with 10% in CD patients [(5).] In a prospective study, patients with pANCA positive developed pouchitis after ileal pouch-anal anastomosis (IPAA) [(6).] The site of pANCA production has been localized to the gastrointestinal mucosa, suggesting the importance of mucosal immune response to luminal antigen [(7).] In fact, absorption of enteric bacterial antigens in the lumen of the intestine greatly reduced or abolished pANCA, indicating that pANCA antibodies were developed as a cross-reactivity to enteric bacterial antigens [(8).] Family studies showed that 16–30% of healthy first degree relatives of patients were pANCA positive [(9,10)]. They indicated that pANCA may be a genetic marker[(11,12).]

We aimed to evaluate the prevalence of pANCA expression in the sera and its isotypes, titers, sensitivity , specificity, positive predictive value and negative predictive value in patients with indeterminate colitis.

Methods:

The patients group consisted of 60 patients. They were defined as indeterminate colitis (IC) by their physicians. They were admitted to Al-Kindi Teaching Hospital – Colonoscopic department from Jun -2007 to May- 2009. most of them (95%) from Baghdad and the rest from other province.

The control group consisted of 30 healthy volunteers, age and sex matched.

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.

Blood samples were collected from two groups and serum were separated, stored at $-10C^0$ till examination was done in the microbiology and immunology laboratory in Al-Kindi College of Medicine –Baghdad University, Diabetic and endocrinology Center- Immunological laboratory and Immunological laboratory in Teaching laboratories- Medical city -Baghdad .

Antineutrophil cytoplasmic (pANCA, cANCA) testing was performed by an IIF technique on ethanol fixed human EOH granulocyte as substrate (EUROIMMUNE- Germany), Briefly, samples were initially diluted to 1:10 in phosphate buffered saline. An FITC conjugated rabbit antihuman (mixed IgG IgM IgA) antibody (Dako, Copenhagen, Denmark) was used for detection of bound immunoglobulin , then isotype was determined by using FITC conjugated rabbit antihuman IgG, FITC conjugated rabbit antihuman IgM, and FITC conjugated rabbit antihuman IgA antibodies. The positive serum (1:10) was also diluted to 1:100 and 1:1000 and all above procedure was repeated using different isotypes. All slides were evaluated by two independent observers and in the event of a difference in opinion, a third observer was decisive. Staining patterns that were considered ANCA positive were perinuclear (pANCA) and cytoplasmic (cANCA) staining of neutrophils. Samples that were scored positive were further analyzed for antinuclear antibodies.

Statistical analysis: Data were analyzed statistically using:

- Descriptive statistics: frequencies, mean and standard deviation.
- Inferential statistics: Fisher's Exact test was used.

P- Values <0.05 were considered statistically significant. Calculations were performed using MiniTab statistical software program 13.20.

Results:

The patient group consisted from 40 males and 20 females, their age rang was (19-84 years), mean 44.42 years, SD 18.16 , SEM 2.34 . Thirty –eight (63.3%) were smokers. , The control group consisted of 15 males and 15 females, their age rang was (20 - 66) mean 42.9 years , SD 16.63 , SEM 3.04 as shown in table -1-.

Table-1- Demographic data for IC patients and healthy control.

	Indeterminate colitis No.=60 No. %	Healthy control No.=30 No. %	P value
Sex			
Male	**40 66.66**	**15 50**	
female	**20 33.33**	**15 50**	
Age at sampling			
X ± SD	**44.42±18.16**	**42.9±16.63**	**NS**
Age range	**19-84**	**20-66**	

NS= not significant

Patients showed a significant increased percentage of pANCA + (63.3%), (p=0.000) in indeterminate colitis patients than control group(table-2-). In case of cANCA, it was not detected in both groups.

Table-2- Serologic results of ANCA (pANCA and cANCA) in indeterminate colitis and healthy control group using Fisher's Exact test with sensitivity, specificity, positive predictive value and negative predictive value.

	ANCA		ANA
	pANCA true + No. %	cANCA true + No. %	No. %
indeterminate colitis No.=60	**38 63.3** p=0.000	**0 0** Not significant	**1 3.3** Not significant
Control No.=30	**1 3.3**	**0 0**	**1 3.3**

	sensitivity	specificity	Positive predictive value	Negative predictive value
pANCA+	**60%**	**40%**	**61.1%**	**66.6%**

The highest percentage of this pANCA titer was 1:10 which is significantly difference from the control (63.3%) (p=0000) then 1:100 (25%) (p=0.008) and lastly 1:1000 which is not significantly difference from the control and most of them was IgG(53.3%) (p=0.000) (table-3-).

Table-3- Immunoglobulins titers and isotypes of pANCA in the indeterminate colitis patients and healthy control .

immunoglobulins	indeterminate colitis 1:10 titer No. %	indeterminate colitis 1:100 titer No. %	indeterminate colitis 1:1000 titer No. %	Healthy control 1:10 No. %
Mix(IgG. IgA, IgM)	38 63.3 P=0000	15 25 P=0.008	5 8.3 P=0.343	1 3.3
IgG	32 53.3 P=0.000	13 21.6 P=0.000	4 6.6 NS	1 3.3
IgA	4 6.6	2 3.3	1 1.6	0 0
IgM	2 3.3	0 0	0 0	0 0

Discussion:

The association between UC and ANCA has been widely studied. Investigators around the world have shown ANCA to be present in the sera of 23%–88% of patients with UC, with the prevalence in most series ranging between 50% and 80% [13]. The majority of UC associated ANCA, when examined using indirect immunofluorescent microscopy, stain with a diffuse non granucallar cytoplasmic pattern with perinuclear highlighting (pANCA) [14].

In our study, we found significant (63.3%) (p=0.000) higher level of pANCA in comparison with control group. This was in agreement with other results that found about 50% of this indeterminate colitis develop pANCAantibodies [15]. However, patients with pANCA+ predicts to develop ulcerative colitis (UC) in 64% of them [16]. Generation of pANCA antibodies is poorly understood. The pathogenic significance of these antibodies has not been established and it remains unclear whether they arise due to tissue damage, increased permeability or the mucosal immune perturbation seen, others reports found that loss of immune tolerance towards the resident bacterial flora is one of the major pathogenetic concepts for this disease. Possibly, pANCA are due to cross reactivity to bacterial antigens [17]. Bacterial and yeast antigens are ubiquitous, permanently present in the gastrointestinal tract. Therefore, it would be of great interest to evaluate when these antibodies are generated. In our study, we found most of them 52.6% developed pANCA during the period (13-40)years. Although the percent of patients expressing pANCA did not vary significantly with age of onset, there was a tendency towards higher levels of pANCA expression with increasing age of onset. This was in agreement with others [18]. Age dependent immune influences have been described in other immune mediated disorders [19].

The precise antigen was unknown and the isotype of it was 39% IgG, 3% IgA and 58% was mix type (EUROIMMUNE-Germany) and in our results, IgG was 53.3 %, IgA was 6.6 % and mix was 63.3%, other results showed that 87.8 % was IgG [20]. This discrepancy in this results may be due to severity of the diseases and genetic factors that control the immune response.

Lee *etal* 2009 who demonstrated that patients with pANCA + had more sever clinical finding, higher relapse rates and disease aggressiveness [21]. Thus pANCA proposed to be a predictive serological marker for the outcome of disease [22]. The specificity of pANCA tends to be higher than sensitivity, so it is more useful in differentiation of inflammatory bowel disease rather than population screening [23].

In case of cANCA did not detected in both groups. It was mostly detected in Wegener's granulomatosis [24].

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