Amebiasis Still Creates Diagnostic Challenge in Our Patients with Ulcerative Colitis: Do ANCA Provide Differentiation?

HALEN, AMEBİAZİS ÜLSERATİF KOLİT HASTALARINDA TEŞHİSDE GÜÇLÜK NEDENİDİR. ANCA AYIRICI TANIDA KULLANILABİLİR Mİ?

Mehmet YILMAZ*, Erdal ESKİOĞLU*, Tankut KÖSEOĞLU*, Yücel ÜSTÜNDAĞ*, Sebahat AKSARAY**, Başak DOKUZOĞLU*, Mehmet ARHAN*

* Dept. of Gastroenterology, Ankara Numune Hospital,
** Dept. of Microbiology and Immunology, Ankara Numune Hospital, ANKARA

Summary

Purpose: Anti-neutrophilic antibodies (ANCA) have been reported to help in making differentiation between ulcerative colitis (UC) and amebic colitis. To the best of our knowledge, the authors investigating this subject made this conclusion in small number of amebic colitis patients. We decided to test ANCA in differentiation between UC and AC in a larger series of patients in Ankara Numune Hospital, Gastroenterology clinics.

Materials and Methods: This study included 16 ulcerative colitis patients without any sign of amebiasis (Group 1) and 25 patients with pure amebic colitis (Group 2). Thirteen patients with UC with amebic cysts and trophosoites in their stool examinations were also enrolled into the study (Group 3). Twenty healthy persons were taken as the control group (Group 4). The other possible etiologies of colitis including lymphocytic colitis, pseudomembranous colitis and colitis cases due to other known infectious agents in Group 3 were excluded via stool cultures, Clostridium difficile toxin in stool and, histopathologic evaluation of colonic biopsy samples. Blood samples obtained from each patient were studied for the presence of anti-myeloperoxidase ANCA by the ELISA method.

Results: There were no statistically significant differences between groups in respect to age and sex distributions. Anti-myeloperoxidase antibodies were present in 4 patients (25%) in Group 1, 4 patients (30.8%) in Group 3. These antibodies were not present in none of patients in Group 2 and Group 4. In all UC patients (totally 29 patients), anti-myeloperoxidase antibodies were found in 8 (%27.8).

Conclusion: Anti-myeloperoxidase ANCA were positive in sera from Turkish patients with UC and, amebic colitis cases were tested as negative. These antibodies can help us not making misdiagnoses as amebic colitis in patients having UC and preventing long term unsuccessful anti-amebic therapies in such patients.

Key Words: Ulcerative colitis, Amebic colitis, Anti-neutrophilic antibodies

T Klin J Gastroenterohepatol 2001, 12:8-12

Özet

Amaç: Literatürde, ülseratif kolit (ÜK) olgularında serumda anti-nötrofil antikorların (ANCA) sıklıkla saptandığı, ancak, amibik kolit dahil diğer infeksiyöz kolitlerde, bu antikorların bulunmadığı ileri sürülmektedir. Biz Ankara Numune Hastanesi Gastroenteroloji kliniğinde, ÜK ve amibik kolit ayrımında serum ANCA varlığının rolünü araştırmayı planladık.

Materyel ve Metod: ÜK tanılı 16 hasta (Grup 1), amibik kolit tanılı 25 hasta (Grup 2), gaita örneklerinde amip trofozoit ve kistlerin saptandığı ÜK tanılı 13 hasta (Grup 3) ve sağlıklı 20 birey (Grup 4) çalışmaya dahil edilmiştir. Olası diğer inflamatuvar ve infeksiyöz kolit nedenleri gaita mikroskopisi, gaita kültürü, klostridium difisil ve kolonik biyopsi örneklerinin histopatolojik incelemeleri ile ekarte edilmiştir. Bu hasta gruplarında, serumda anti-myeloperoksidaz ANCA varlığı ELISA ile araştırılmıştır.

Bulgular: Anti-myeloperoxidaz ANCA pozitifliği Grup 1 ve 3'de 4'er hastada (%25 ve % 30.8) bulunurken, bu antikorlar Grup 2 ve 4'de saptanmamıştır ($p<0.05$). ÜK tanılı 29 hastanın 8'inde (%27.8) Anti-myeloperoxidase ANCA pozitifliği vardır.

Sonuç: Serum ANCA pozitifliğinin amibik ve ülseratif kolit ayrımında yardımcı bir gösterge olarak kullanılabileceği saptanmıştır.

Anahtar Kelimeler: Ülseratif kolit, Amebik kolit, Anti-nötrofilik antikorlar

T Klin Gastroenterohepatol 2001, 12:8-12

Geliş Tarihi: 03.04.2000

Yazışma Adresi: Dr.Yücel ÜSTÜNDAĞ
Bağcılar 5.Sok 7/4, Ceren Apt
06700, Gaziosmanpaşa, Ankara/TURKEY

In Turkey, there is an overdiagnosis of amebic colitis in patients with bloody diarrhea and, these cases were exposed to long term antiamebic therapy which fails at most of the time (1). Then, the pa-

tients are reevaluated and found to have ulcerative colitis (UC). Although, there are some ways to differentiate these diaseases on clinical, endoscopic and other laboratory background, some cases are really challenging to make correct differential diagnosis between two diseases (2).

In literature, some authors reported the absence of anti-neutrophilic antibodies (ANCA) in sera of patients with amebic colitis and suggested that ANCA can be helpful in making differential diagnosis between UC and amebic colitis (3-5). We believe that the authors in these reports had investigated serum ANCA in very few number of amebic colitis cases to conclude the absence of these antibodies in amebic infection. Additionally, one of the reports in the literature showed ANCA to be present in most of patients with amebic liver abscess. To evaluate this subject, we investigated serum anti-myeloperoxidase ANCA in a large series of patients with amebic colitis and compared the results with anti-myeloperoxidase antibody positivity in our patients with UC with or without amebic coinfection.

Material and Methods

Sera were studied from patients with newly diagnosed UC and amebic colitis cases. The diagnosis of UC was based on accepted clinical and endoscopic criteria supported by histopathology. Amebic colitis was diagnosed in cases with positive stool samples for Entameoba hystolytica cysts and trophosoites together with colonic endoscopic signs. Moreover, colonic tissue biopsy samples were investigated for the presence of amebic microorganisms. All the patients in the study group were undergone stool cultures for various bacteria (Salmonella, Shigella, E coli, Campylobacter jejuni). Clostridium difficile toxin in feces was also investigated in all patients. The possible other etiologies of colitis like UC in Group 3 were excluded via stool cultures, histopathologic evaluation of multiple colonic biopsy samples by experienced pathologists. The diagnosis of amebic colitis was further supported by demonstration of amebic microorganisms in histopathologic evaluation of colonic biopsy samples.

According to investigational results, the patients were grouped into 4. Group 1 consisted of 16 pure UC patients, Group 2; 25 pure amebic colitis patients and Group 3; 13 UC patients with concurrent amebic infection. Twenty healthy persons were taken as the control group in the study (Group 4). UC patients were also classified according to the extent of colonic inflammation on colonoscopic examination as pancolitis, left sided colitis and distal colitis. The patients with UC with or without amebic co-infection were also investigated for the presence of associated sclerosing cholangitis. The liver function tests and abdominal ultrasonography were undertaken in all 29 UC cases.

Anti-myeloperoxidase ANCA were studied in all patients' serum samples. Myeloperoxidase antibodies directed against myeloperoxidase (the Clark Laboratories, Inc.). Myeloperoxidase Ig G, A, M were used.

ELISA test

Purified myeloperoxidase antigens were attached to a sold phase microassay well. Diluted test sera was added to each well. If antibodies are present that recognize the antigen, antigen-antibody complexes are formed. After incubation, the wells were washed to remove unbound antibody. An enzyme labeled anti-human Ig G, A, M was added to each well. If antibody is present, the conjugate will bind to the antigen-antibody complexes. After incubation, the wells were washed to remove unbound conjugate. A substrate solution was added to each well. If enzyme is present, the substrate will undergo a color change. After an incubation period, the reaction was stopped and the color intensity was measured photometrically, producing an indirect measurement of spesific antibody in the patient specimen. The positive results were compared with high positive and low positive standart serum samples.

Statistics

The statistical evaluation of sex and age distributions and anti-myeloperoxidase antibodies positivity was performed by using Chi square and Fischer's exact tests.

Results

We included 29 patients with a known diagnosis of UC. Out of 29, 16 had negative stool and

Table 1. The demographic characteristics and ANCA positivity in the groups are demonstrated

Subjects	UC n:16 (Group 1)	UC with amebic coinfection n:13 (Group 2)	Amebic colitis n:20 (Group 3)	Controls n:25 (Group 4)
Male/female	10/6	9/4	11/9	19/6
Age (years)	40.4+15.5 (34-56)	41.8+13.2 (27-55)	38.5.1+19.1 (21-57)	50.2+16.8 (33-66)
p ANCA positive	4 (25%)	4 (30.8%)	0 (0%)	0 (0%)

colonic biopsy samples for amebic infection which were evaluated as pure UC cases (Group 1). The 13 of 29 patients with UC also had amebic cysts and trophosoites in their stool examinations (Group 3). Twenty-five patients with pure amebic colitis were also enrolled into the study (Group 2).

The mean age and sex distributions were similar in Group 1, Group 2, Group 3 and Group 4 (Table 1). There was no statistical differentiation between groups in respect to age and sex distributions ($p>0.05$).

Anti-myeloperoxidase antibodies (mostly Ig G, but also IgA and IgM) were present in 4 patients (25%) in Group 1, 4 patients (30.8%) in Group 3 ($p>0.05$). These antibodies were not present in none of patients in Group 2 and Group 4. In all UC patients (totally 29 patients), Anti-myeloperoxidase antibodies were found in 8 (27.8 %). There was no statistically significant difference between Group 1 and Group 3 in respect to anti-myeloperoxidase antibodies positivity ($p>0.05$). However, anti-myeloperoxidase ANCA positivity was significantly different between patients wiith UC (with or without amebic coinfection) and pure amebic colitis cases or controls ($p<0.05$). The positive predictivity of ANCA for UC cases in the present study was 100%. Its negative predictivity was found to be 68% (Table 2).

Out of 29, there were 8 patients with pancolitis (27.6%), 11 patients with left sided colitis (37.9%) and 10 with distal type colitis (34.5%) (Table 3). Anti-myeloperoxidase ANCA were found in 5, 1 and 2 patients in pancolitis, left sided colitis and distal colitis patients, respectively. There was statistical significant difference for anti-myeloperoxidase antibodies positivity in patients with pancolitis and the patients with left sided or distal coltis ($p>0.05$).

Table 2. Positive and negative predictivity of ANCA in our cases with and without UC are shown

ANCA status	UC present	UC absent	Total
ANCA positive	8	0	8
ANCA negative	21	45	66
Total	29	45	74

Positive predectivity: 100%
Negative predictivity: 68%

Table 3. ANCA positivity in respect to the extent of colonic inflammation is shown

UC cases	ANCA positivity (number of patients)
Pancolitis (n:8)	5 (62.5%)
Left sided colitis (n:11)	1 (9.1%)
Distal colitis (n:10)	2 (20%)

None of the patients with UC (including cases with amebic coinfection) had no laboratory signs of an associated sclerosing cholangitis.

Discussion

This is the fourth report in literature which underlines that a serum marker with an unknown pathogenic significance in UC, anti-myeloperoxidase ANCA, can be used at least for differentiaton between UC and amebic colitis. This is especially important for places wherein amebiasis is nearly an endemic infection like our country. In Turkey, the prevalance of amebic colitis is between 0.4% and 18.4% (6).

In the present study we detected anti-myeloperoxidase ANCA in 27.6% of patients with UC (8 out of 29) and none in pure amebic colitis cases.

The presence of anti-myeloperoxidase ANCA in UC patients with concurrent amebic infection can be attributed to UC. The presence of anti-myeloperoxidase ANCA confirmed a diagnosis of UC (with or without amebic infection) as the statistical analysis showed very high positive predictive rate (100%) of ANCA in our study. The negative predictivity was somehow lower as 68%. The prevalence of anti-myeloperoxidase ANCA in our group of UC patients (27.6%) was somewhat lower than that reported in some other studies (up to 84%) (7). This may be due to the differences in selected patient's groups (genetic heterogenity in inflammatory bowel disease). In addition; testing the sera from UC patients for different antigenic specificies (proteinase 3, elastyase, lactoferrin, myeloperoxidase and cathepsin G) in the different studies may lead to different percentages of ANCA positivity in these studies. (8). If we look at our 8 cases with pancolitis, anti-myeloperoxidase ANCA seropositivity was 62.5%, that reflected statistically significant difference in other patients with more limited colonic inflammation. In literature, the investigators did not aggree on the association between the anti-myeloperoxidase antibodies and extent of colonic inflammation (9,10) Our study results show that pancolitis cases had higher seroprevalance of anti-myeloperoxidase ANCA positivity. This may mean a direct pathogenic relation with anti-myeloperoxidase ANCA and colonic inflammation in UC. However, we could not insist on our study results which had been done on a limited number of patients with UC.

In literature, Arslan S et al evaluated the role of ANCA in diagnosing the co-existent amebic colitis with UC (3). ANCA was detected in 83.3% of patients whom had been treated as refractory amebic colitis for a long period of time. These cases were then rediagnosed as coexisting UC and amebic infection. Sera from their 7 patients suffering from pure amebic colitis did not show ANCA positivity. They suggested that the results indicate ANCA as a reliable serological marker to distinguish coexisting UC in amebic colitis patients. Sung JY et al studied ANCA in 3 patients with non-invasive amebic infection also revealed unexistence of these antibodies in sera from these patients (4). Duerr RH et al studied ANCA in sera of only one patient with active amebic colitis that gave a negative result (5). However, interestingly the situation changes in invasive amebiasis as ANCA have been reported to be present in 97.4% of the sera of cases with confirmed amebic liver abscess (11). This association was explained as either a cross reacting antibody formation to a component of Entameoba histolytica or antibody to polymorphonuclear leukocytes components released and modified by the action of E. histolytica on polymorphonuclear leukocytes. The absence of ANCA in colitic cases due to amebic infection but their presence in amebic sera of patients with invasive amebiasis is rather a conflict to make a legitimate explanation.

The target antigen of UC associated ANCA is already unknown (8). However, antibodies against MPO, a lysosomal enzyme that can be found in neutrophils have been reported to be present in the sera of UC patients (5). We could not answer whether these anti-myeloperoxidase ANCA which were detected in sera of our patients reflected UC associated ANCA or not. However, we believe that ANCA were not present in amebic colitis patients's sera, and they confirmed the diagnosis as UC.

The diagnosis of UC has been reported to be more difficult in Asian countries including Turkey, where infective colitis is more prevalent (4). ANCA as a confirmative marker may help us to differentiate amebic colitis cases from UC. Thus, we hope that unnecessary long term antiamebic therapy becomes unfashionable.

REFERENCES

1. Doganci L, Tanyuksel M, Gun H. Overdiagnosis of intestinal amoebosis in Turkey Lancet 1997 Oct 4; 350(9083): 1034.

2. Tedesco FJ, Hardin RD, Harper RN, Edwards BH. Infectious colitis endoscopically simulating inflammatory bowel disease: a prospective evaluation. Gastrointest Endosc 1983 Aug; 29(3):195-7.

3. Arslan S, Bakkaloğlu A, Öksüzoğlu G, Kadayıfcı A, Kansu E, Uzunalimoğlu B, et al. The value of p-ANCA as a serological marker in diagnosing the coextence of chronic active invasive amebic colitis andd ulcerative colitis. American J Gastroenterol 1995; 90:2265-66.

4. Sung JY, Chan KL, Jsu R, Liew CT, Lawton JW. Ulcerative colitis and antineutrophil cytoplasmic antibodies in Hong Kong Chinese. Am J Gastroenterol 1993; 88:864-69.

5. Duerr RH, Targan ST, Landers CJ, Sutherland LR, Shanahan F. Anti-neutrophil cytoplasmic antibodies in ulcerative coltis. Gastroenterology 1991; 100:1590-96.

6. Ak M, Kirağı D. Amebiasis. In: Özcel MA, ed. GAP ve paraziter hastaliklari.Türkiye parazitoloji Derneği yayini. No:11. Ege Üniversitesi Basimevi, İzmir. 1993: 71-88.

7. Saxon A, Shanahan F, Landers C, Ganz T, Targan S. A distinct subset of anti-neutrophil cytoplasmic antibodies is associated with inflammatory bowel disease. J Allergy Clin Immunol 1990; 86:202-10.

8. Shanahan F. Neutrophil autoantibodies in inflammatory bowel disease. Are they important. Gastroenterology 1994; 107:586-9.

9. Seibold F, Weber P, Klein R, Berg PA, Wiedmann KH. Clinical significance of antibodies against neutrophils in patients with inflammatory bowel disease and primary sclerosing cholangitis. Gut 1992; 33:657-62.

10. Rump JA, Scholmerich J, Gross V, Roth M, Helfesrieder R, Rautmann A, et al. A new type of perinuclear nti-neutrophil cytoplasmic antibody (p-ANCA) in active ulcerative colitis but not in Crohn's disease. Immunobiology 1990; 181:406-13.

11. Pudifin DJ, Dursma J, Gathiram V, Jackson TF. Invasive amoebiasis is associated with the development of anti-neutrophil cytoplasmic antibody. Clin Exp Immunol 1994; 97:48-51.