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Antineutrophil cytoplasmic autoantibodies and anti-Saccharomyces cerevisiae antibodies in inflammatory bowel diseases

İnflamatuvar barsak hastalıklarında antinötrofil sitoplazmik antikor ve "anti-Saccharomyces cerevisiae" antikorları

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Background/aims: *Perinuclear antineutrophil cytoplasmic autoantibody is a marker for ulcerative colitis, and anti-Saccharomyces cerevisiae antibody is known to be associated with Crohn's disease. The purpose of this study was to search the value of detecting perinuclear antineutrophil cytoplasmic autoantibody and anti-Saccharomyces cerevisiae antibody for the diagnosis of Turkish inflammatory bowel disease patients.* ***Methods:*** *Serum samples were obtained from 80 patients with ulcerative colitis, 61 patients with Crohn's disease and 40 healthy controls. Determination of both anti-Saccharomyces cerevisiae antibody and antineutrophil cytoplasmic autoantibody was performed with the standardized enzyme-linked immunosorbent assay.* ***Results:*** *In cases with ulcerative colitis, 65% tested seropositive for antineutrophil cytoplasmic autoantibody, whereas the controls showed 2.5% positivity. In cases with Crohn's disease, 63.9% tested seropositive for anti-Saccharomyces cerevisiae antibody, whereas the controls showed 2.5% seropositivity. The combination of a positive anti-Saccharomyces cerevisiae antibody test and a negative antineutrophil cytoplasmic autoantibody yielded a sensitivity and specificity of 32.0% and 97.5%, respectively. The combination of a positive perinuclear antineutrophil cytoplasmic autoantibody and a negative anti-Saccharomyces cerevisiae antibody test yielded a sensitivity and specificity of 44.2% and 97.5%, respectively.* ***Conclusions:*** *Both serologic tests may aid in the differential diagnosis of inflammatory bowel disease.*

Key words: Antineutrophil cytoplasmic autoantibody, anti-Saccharomyces cerevisiae antibody, Crohn's disease, ulcerative colitis

Amaç: *Perinükleer antinötrofilik sitoplazmik antikor ülseratif kolit için bir gösterge, anti-Saccharomyces cerevisiae ise Crohn hastalığına eşlik eden bir antikordur. Bu çalışmanın amacı, perinükleer antinötrofilik sitoplazmik antikor ve anti-Saccharomyces cerevisiae antikounun Türk inflamatuvar barsak hastalarındaki önemini ve yerini ortaya koymaktır.* ***Yöntem:*** *80 ülseratif kolit, 61 Crohn hastası ve 40 sağlıklı bireyden serum örnekleri alınmıştır. Perinükleer antinötrofil sitoplazmik antikor ve "anti-Saccharomyces cerevisiae" antikorları standardize edilmiş ELİZA yöntemi ile ölçülmüştür.* ***Bulgular:*** *Perinükleer antinötrofil sitoplazmik antikor, ülseratif kolitti olguların %65'inde, sağlıklı bireylerin %2.5'inde seropozitif bulunmuştur. Anti-Saccharomyces cerevisiae antikorları ise Crohn hastalarının %63.9'unda, sağlıklı bireylerin %2.5'inde seropozitif bulunmuştur. Perinükleer antinötrofil sitoplazmik antikor seronegatifliği ve "anti-Saccharomyces cerevisiae" antikorunun seropozitifliğinin birlikte tanı duyarlılığı ve özgüllüğü sırasıyla %32.0 ve %97.5'tir. Perinükleer antinötrofil sitoplazmik antikor seropozitifliği ve "anti-Saccharomyces cerevisiae" antikorunun seronegatifliğinin birlikte tanı duyarlılığı ve özgüllüğü sırasıyla %44.2 ve %97.5'tir. Sonuç: Her iki serolojik test, inflamatuvar barsak hastalığı tanısında yardımcı testler olabilir.*

Anahtar kelimeler: ANCA, ASCA, Crohn hastalığı, ülseratif kolit

INTODUCTION

The inflammatory bowel diseases (IBD) are a heterogeneous group of disorders of unknown etiology. The group is primarily subdivided into ulcerative colitis (UC) and Crohn's disease (CD). Although CD and UC are generally considered distinctive forms of IBD, their clinical presentations may overlap. Despite several clinical, endoscopic, radiologic and histopathologic tools, in approxima-

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tely 1% of cases no differentiation can be made. The disease in these patients is called indeterminate colitis (IC). Indeed, the clinical overlaps and presence of IC support the concept that IBD represents a spectrum of diseases rather than two entities, CD and UC (1-4).

Accurate diagnosis of IBD is very important. Because the treatment strategies in CD and UC differ, especially when surgery is required, much effort has been expended over the years to distinguish the cases. Non-invasive tests are expected to display a crucial role in the differential diagnosis. In IBD, investigators have searched for several years for antibodies that correlate with various diseases. Two antibodies for use in the clinical diagnosis of IBD have been identified, i.e. perinuclear antineutrophilic cytoplasmic antibody (ANCA) and anti-Saccharomyces cerevisiae antibody (ASCA) (5-7).

p-ANCAs are a subset of ANCA with perinuclear staining by indirect immunofluoresence, and they have been found in about 60-70% of patients with UC, in 5-10% of patients with CD and in 0-5% of healthy controls. ASCAs are the antibodies directed against the cell wall oligomannosidic epitope of Saccharomyces cerevisiae. ASCAs have been identified in raised titers in 6-70% of patients with CD, in 10-15% of UC patients and 0-5% of healthy controls. The measurement of p-ANCA alone has been found to be of limited clinical diagnostic value in IBD because of insufficient sensitivity to diagnose UC and CD. Thus, the combined measurement of p-ANCA and ASCA has been proposed as a valuable diagnostic approach in IBD. The p-ANCA and ASCA tests are non-invasive and may be helpful in the identification of subgroups of patients with IBD and in prediction of activity (7-10). The possible usefulness of ASCA and p-ANCA in IBD has been considered previously, but prospective studies in different geographic regions are necessary regarding the disease subgroups and activity.

The seropositivity of p-ANCA and ASCA in IBD patients in different ethnic backgrounds may help to understand the pathogenesis of the heterogeneous IBD spectrum. The p-ANCA seropositivity was between 0%-80% in UC and ASCA seropositivity was between ll%-79% in CD in recent studies (10-15).

In the present study, the association between p-ANCA and ASCA and CD and UC was evaluated, by single use or combined use of these tests. The relation between serological test results and clinical features of both diseases was also studied. We compared the marker status with healthy controls.

MATERIALS AND METHODS

Our study population consisted of 141 consecutive IBD patients. According to their concordant diagnosis established by standard clinical criteria and with radiologic, endoscopic and histopathological confirmation, the patients were classified as CD (n=61) or UC (n=80). The cases with IC whose initial endoscopic and histopathological diagnosis was inconclusive were not included in the study. Clinical activity in CD was measured by Crohn disease activity index (CDAI) and in UC by Rachmilewitz endoscopic index. CDAI higher than 150 was predicted as active disease in CD. Rachmilewitz index equal to or higher than 4 was predicted as active disease in UC.

Sera from 40 healthy persons without gastrointestinal complaints or a familial history of IBD served as controls. Demographic data of the study population are given in Table 1.

Table 1. Demographic data in CD, UC patients and healthy controls (*some patients received more than one drug)

	CD patients	**UC patients**	**Healthy controls**
	n=61	**n=80**	**n=40**
Female/Male	21/40	35/45	17/23
Mean age, yr	37.6	41.6	44.8
Range, yr	(18-68)	(16-79)	(21-68)
Mean Disease Duration, months	46.8	56.1	
Range, months	(2-120)	(1-240)	
Location (n)			
Small bowel	24 (39%)		
Colon	16 (26%)		
Small bowel+colon	21 (35%)		
Proctitis		15 (18%)	
Left-sided		28 (35%)	
Pancolitis		37 (47%)	
Type			
Fibrostenotic	27 (44%)		
Penetrating	13 (21%)		
Inflammatory	21 (34%)		
Surgery (n)	25 (41%)	8 (10%)	
Treatment*			
Salasopryine	4	17	
Mesalazine	40	60	
Oral steroid	7	14	
Ciprofloxacin	6	7	
Azathioprine	27	3	
Metronidazole	7	9	
Methotrexate	2	0	
None	3	5	

CD: Crohn's disease; UC: ulcerative colitis

All serum samples were stored at -20^{0}C until analyzed. Antibody screening was performed using coded serum samples. Investigators had no knowledge of patient diagnosis or clinical features at the time the serological tests were conducted.

Determination of p-ANCA was performed by enzyme linked immunosorbent assay (ELISA) using myeloperoxidase IgG (Euroimmune, Germany). Sera were incubated at a 1/101 dilution and washed. According to the manufacturer's instructions, the cut-off value is 20 RU/ml; values higher than 20 RU/ml were interpreted as positive.

The ASCA was measured by a standardized ELISA using the crude mannan from S. cerevisiae as the antigen. Sera were incubated at a 1/50 dilution. The evaluation of ASCA was done using the ASCA IgA ELISA kits (Medipan Diagnostics, Germany). After incubation for 1 hour at -37^{0}C, the coated plates were washed and then anti-human IgA was added. After rewashing, the reaction was stopped and the absorbance was read at 450 nm in microplate reader. Samples were interpreted as positive if ASCA was higher than 20 RU/ml.

Statistical analysis

Sensitivity is defined as the probability of a diagnositc test being positive for a patient with the disease under investigation. Specificity is defined as the probability of a test being negative for a patient without the disease under investigation.

Positive predictive value (PPV) of a test is the probability of a patient being affected by the disease under investigation given that the test is positive. Similarly, negative predictive value (NPV) is the posterior probability of a patient not being affected by the disease under investigation given that the test is negative.

The relationship between serological markers and clinical parameters was studied using the x^2 test or Fisher's exact test where appropriate. A p value <0.05 was considered to be significant.

RESULTS

p-ANCA was detected by ELISA in 19 of 61 (31.1%) samples from CD patients and in 52 of 80 (65.0%) samples from UC patients (Tables 3 and 4). Only one of 40 (2.5%) samples from healthy controls was found positive for p-ANCA. The difference between the prevalences of p-ANCA positivity in UC and CD and control groups was statistically significant.

ASCA was detected by ELISA in 35 of 80 (43.7%) samples from UC patients and in 39 of 61 (63.9%) samples from CD patients (Table 2). Only one of 40 (2.5%) samples from healthy controls was found positive for ASCA. The difference between the prevalences of ASCA positivity in UC and CD and control groups was statistically significant.

A total of 33 UC patients had active disease, whereas 47 patients had inactive disease: 78.8% of the first group and 47% of the second group were p-ANCA positive. The difference was statistically significant (Table 2). However, no significant differences were found among the three groups of UC classified according to disease localization or regarding the surgery (Table 3).

In addition, no significant differences were found among CD patients regarding disease localization, activity and surgery, in relation to p-ANCA positivity (Table 4).

Raised titers of ASCA were found in 35 of 80 UC patients (43.7%), in 39 of 61 (63.9%) patients with CD and in one of 40 healthy controls. Among UC patients, no relation was observed between ASCA positivity and various clinical features (Table 3). In addition, among CD patients, no significant relation was observed among CD patients regarding disease localization, activity and surgery, in relation to ASCA positivity (Table 4).

Sensitivity, specificity, PPV and NPV values of p-ANCA and ASCA, either alone or in combination, for our patients are shown in Table 5. In patients with UC, the sensitivity, specificity, PPV and NPV of p-ANCA were as follows: 65%, 97.5%, 98.5%, 58.2%, respectively. These values were lowered slightly when the combination of p-ANCA-positive and ASCA-negative was used.

The use of ASCA test in diagnosing CD yielded a sensitivity, specificity, PPV and NPV as follows: 63.9%, 97.5%, 97.5%, 63.9%, respectively. The combination of ASCA-positive and p-ANCA-negative slightly lowered these values (Table 5).

DISCUSSION

In this study, we assessed the value of p-ANCA and ASCA, alone and in combination, in differentiating between UC and CD in the Turkish population. It seems that the p-ANCA test may have significant diagnostic value for UC with results (prevalence 65%) comparable to previous studies. The prevalence of p-ANCA in our UC patients was nearly equal to that of our neighbor country, Gre-

Table 2. ASCA and p-ANCA test results in patients with CD and UC (* for UC, f for CD)

Test Results	UC (n=80)	CD (n=61)
p-ANCA (+)	52 (65.0%)	19 (31.1%)
p-ANCA (+) and ASCA (-)	26 (32.5%)	6 (9.8%)
ASCA (+)	35 (43.7%)	39 (63.9%)
ASCA (+) and p-ANCA (-)	9 (11.3%)	27 (44.2%)

Table 3. Ulcerative colitis and p-ANCA positivity

Patient characteristics	n	p-ANCAC+)	ASCA(+)	P
Total	80	52 (65.0%)	35 (43.7%)	
Rachmilewitz index >4	33	**26 (78.8%)**	15 (45.5%)	**<0.05**
Rachmilewitz index <4	47	26 (55.3%)	20 (42.6%)	
Disease Location				>0.05
Proctitis	15	8 (53.3%)	4 (26.7%)	
Left-sided	28	19 (67.9%)	16 (57.1%)	
Pancolitis	37	25 (67.6%)	15 (40.5%)	
Surgery	8	5<[62.5%)	4 (50.0%)	>0.05

Table 4. Crohn's disease and p-ANCA positivity

Patient characteristics	n	p-ANCA (+)	pASCA (-)	P
Total	61	19 (31.1%)	39 (63.9%)	
CDAI>150	23	4 (17.4%)	15 (65.2%)	>0.05
CDAI<150	38	15 (39.5%)	24 (63.2%)	
Disease Location				>0.05
Small bowel	24	5 (20.8%)	14 (58.3%)	
Colon	16	6 (37.5%)	9 (56.3%)	
Small bowel+colon	21	8 (38.1%)	16 (76.2%)	
Disease Type				>0.05
Fibrostenotic	27	8 (29.6%)	18 (70.4%)	
Penetrating	9	2 (22.2%)	7 (77.8%)	
Inflammatory	25	9 (36.0%)	14 (64.0%)	
Surgery	25	8 (32.0%)	17 (68.0%)	>0.05

CDAI: Crohn's disease activity index

Table 5. Diagnostic accuracy of the serological tests to distinguish between UC and CD (* for UC, f for CD)

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
P-ANCA+	65.0	97.5	98.5	58.2*
ASCA+	63.9	97.5	97.5	63.9f
P-ANCA+, ASCA-	32.0	97.5	96.2	41.9*
p-ANCA-, ASCA+	44.2	97.5	96.4	53.4f

ece (10). In our UC patients, p-ANCA was related to the activity of the disease - a finding contrary to most of the studies. In contrast to most of the previous reports, the prevalence of p-ANCA in the CD patients in our study was significantly higher in patients with ileo-colonic involvement than in those with only colonic or small intestinal disease (11, 16).

The prevalence of ASCA in CD patients was 63.9% in our population, which was in accordance with the reported prevalence of 60-70% in other studies (10-15). The prevalence of ASCA was weakly associated with ileocolonic involvement in CD patients, but this relationship was of no significance. No other relationship between ASCA and the clinical features of both diseases was found.

Combination of both tests (p-ANCA-positive and ASCA-negative) did lower the sensitivity, PPV, and NPV for UC compared to p-ANCA alone. The use of p-ANCA-negative and ASCA-positive combination also reduced the sensitivity, PPV and NPV (Table 5).

Similar results were found in the studies of Koutrobakis et al. (11), Quinton et al. (10) and Hoffenberg et al. (15). Testing for both antibodies did not achieve the same degree of discriminatinpower, which is in accordance with our results. The combination of both tests yielded a relatively high specificity for the diagnosis of either UC or CD, which could make the tests usefully for application to individual patients. This high specificity of both diagnostic panels may assist in making critical treatment decisions, such as in patients with severe colitis.

IBD is a clinically and genetically heterogeneous group of disorders. Both p-ANCA and ASCA have been proposed as subclinical genetic markers, and a familial expression of them is known (17,18). Even though p-ANCA positivity and titers are known to be unrelated with the extent of disease activity, we did find a significant relation between p-ANCA positivity and UC activity. We believe that p-ANCA positivity is an epiphenomenon in UC and that our finding is incidental. The presence of ASCA also seems to be independent of disease activity.

There is some evidence that p-ANCA and ASCA may be useful in identification of subgroups within CD and UC. In CD patients, ASCA positivity has been linked to small bowel disease, and our data are in agreement in some part with this observation because we found high positivity in cases with ileocolitis. However, we believe this remains to be elucidated with new clinical studies.

Positive p-ANCA in CD has been proposed as a predictor for UC-like phenotype (10, 16, 17). Koutrobakis et al. reported p-ANCA positivity as 30% in colonic involvement of CD patients (11). We found 37.5% (6/16) in colonic involvement and 38.1% (8/21) in ileocolonic involvement. Rates were high in CD cases with colonic involvement regardless of

whether small intestinal involvement was present. Our finding is in accordance with the literature.

The ASCA positivity in UC patients was 43.7% (35/80). This value is higher than that of the previous reports. It could be explained by the different geographic backgrounds.

It is known that there is 0-5% seropositivity in the normal population. We found 2.5% positivity of p-ANCA and ASCA in healthy controls, confirming the reliability of our ELISA technique.

In conclusion, while p-ANCA and ASCA are not sensitive enough to screen the population, they can be used for identifying disease subgroups.

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