Diagnostic Value of ASCA and Atypical p-ANCA in Differential Diagnosis of Inflammatory Bowel Disease

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ABSTRACT

BACKGROUND

Worldwide, the incidence of inflammatory bowel disease (IBD) is increasing. This study aims to evaluate the diagnostic value of two serological markers, atypical perinuclear anti-neutrophil cytoplasmic antibodies (atypical-P-ANCA) and anti-Saccharomyces cerevisiae antibodies (ASCA), with the intent to determinetheir relationship to ulcerative colitis (UC) and Crohn's disease (CD), in addition to the location and extent of bowel involvement.

METHODS

There were 97 patients enrolled in this study, 72 diagnosed with UC and 25 with CD.The control group consisted of 40 healthy individuals. ASCA was determined by enzyme-linked immunosorbent assay (ELISA) and atypical-P-ANCA by indirect immunofluorescence assay (IIF). For data analyses, we used the chi-square and independent t-tests. Significance was considered to be $p<0.05$.

RESULTS

For CD, the sensitivityof ASCA was 16% and its specificity was 97%. ASCA had a specifity of 90% in UC patients. The atypical P-ANCA test had a sensitivity of 44% and specificity of 86% for UC. The positive predictive value (PPV) for atypical P-ANCA in UC patients was 78% and for the negative predictive value (NPV), it was 58%.There was no correlation between ASCA and atypical P-ANCA results and the location of gastrointestinal (GI) involvement in CD (p=0.61) and UC (p=0.28) patients.

CONCLUSION

According to the results, ASCA and atypical P-ANCA markers are not useful for IBD screening. Our study suggests that atypical P-ANCA is a useful parameter to differentiate UC from CD. However, ASCA is of limited value for screening and differentiating UC from CD.

KEYWORDS

Inflammatory bowel disease; Anti-Saccharomyces cerevisiae antibody; Atypical perinuclear anti-neutrophil cytoplasmic antibody

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INTRODUCTION

Inflammatory bowel disease (IBD) is identified by two major disorders, ulcerative colitis (UC) and Crohn's disease (CD). UC usually affects the colon whereas CD can involve the entire gastrointestinal (GI) tract, from the oral cavity to the anus. Although the etiology of IBD is not fully understood, it is considered an immunologically mediated disease in genetically susceptible patients.[1] Worldwide, the incidence of IBD is increasing.

UC and CD can be manifested by special histopathological patterns and colonoscopic features. Different treatments are warranted; in some cases it is difficult to differentiate between these two disorders by colonoscopy and clinical evaluation. Under these circumstances less invasive evaluations such as serological biomarkers can assist with both diagnosis and choosing the appropriate treatment. Two serological markers that have been reported to be valuable for differentiating UC from CD are anti-Saccharomyces cerevisiae antibodies (ASCA) and perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA).[2-4] Some studies conclude that these biomarkers are of limited value in the diagnosis of IBD and differentiation between UC and CD. These studies have noted that the sensitivity and specificity of the results vary markedly depending on the disease prevalence in the comparison population.[5,6]

Previously, most studies have used the enzyme-linked immunosorbent assay (ELISA) method for evaluating ASCA and p-ANCA, however recent studies have shown more reliable results using the indirect immunofluorescence assay (IIF) for p-ANCA. The current study intends to define the specificity and sensitivity of ASCA and atypical-p-ANCA (according to IIF) in the diagnosis of IBD and differentiation of UC from CD among patients in our region in Mashhad. an attempt is made to determine an association between the location of GI involvement and the result of serological markers.

MATERIALS AND METHODS

This case-control study enrolled 97 IBD patients, 72 diagnosed with UC and 25 CD patients. There were 40 healthy individuals in the control group. Diagnosis was made by colonoscopy, histopathological analyses and a barium transit study of the small bowel in UC and CD patients. Inclusion criteria were: all patients who referred for colonoscopic evaluation of chronic diarrhea, abdominal pain or screening. Patients diagnosed with IBD according to colonoscopy, imaging, and pathological results were considered as the case group. Those with normal colonoscopy and pathology and normal imaging were considered as the control group. Excluded were those with bowel infections, history of collagen vascular diseases and cancer.

The study protocol was approved by the Ethics and Science Committee of Mashhad University of Medical Sciences. Each patient signed an informed consent.

At the beginning of the study, 5cc of venous blood was taken from each patient and stored at-20°C until analysis. Samples were analyzed for ASCA (IgG) by the ELISA method using Euroimmun kits. An ASCA IgGlevel of greater than 24.0 EU/ml was considered positive. All sera were tested for atypical-p-ANCA by the IIF method (Euroimmun kits). Following fixation of sera by ethanol, the following immunofluorescence patterns were diagnosed: i) coarse diffuse cytoplasmic fluorescence of neutrophils (C-ANCA); ii) fine homogeneous rim pattern around the nucleus (typical p-ANCA); and iii) fine rim-like staining with intranuclear foci or broad nonhomogeneous rim-like staining of the nuclear periphery as a mixture of c-ANCA and p-ANCA (atypical-p-ANCA).

The sera were examined independently for their immunofluorescence patterns on fixed neutrophils by two pathologists who had no knowledge of the patients' diagnoses. We determined sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of ASCA and atypical-p-ANCA for UC and CD. For data analyses, we used the chi-square and independent-tests. Significance was considered to be $p < 0.05$.

RESULTS

The ASCA test was performed on 97 IBD (25 CD and 72 UC) patients and 40 controls. In this study, 4 out of 25 (16%) CD patients had positive ASCA test results compared with 1 out of 72 (1%) UC patients. Specificity was 97% with a sensitivity of 16% for ASCA in diagnosing CD. The ASCA test had a NPV of 83% and a PPV of 57% for CD (Table 1). For UC, however this test had low sensitivity (1%) and high specificity (90%) for UC (Table 2).

The atypical-p-ANCA test analyzed 25 CD and 72 UC patients in addition to 40 healthy controls to determineits specificity and sensitivity for in diagnosing UC and CD. This test had a sensitivity of 44%, specificity of 86%, NPV of 58% and PPV of 78% for diagnosing UC (Table 3). The sensitivity of atypical-p-ANCA for CD was 16% and its specificity was 66% (Table 4).

Among all ASCA-positive patients, 33% had rectosigmoid involvement, 33% suffered from small intestine involvement and 33% had pancolitis. None had involvement of the left colon or transverse colon. There was no relationship between the site of GI involvement and the ASCA test results. Rectosigmoid involvement was observed in 44% of the patients who had positive atypical p-ANCA test results, left colitis and transverse colitis were seen in 48% and 8% had pancolitis. There was no significant relationship between the atypical p-ANCA results and the site of GI involvement.

DISCUSSION

About 10% of patients with IBD cannot be categorized as having CD or UC;the final diagnosis in these patients, even when the entire colon is removed, is "undetermined colitis".[7]

ANCAs and ASCA serologic studies in patients with indeterminate colitis can be helpful in establishing a diagnosis and it is important in the management and prognosis. A prospective study of 97 patients with indeterminate colitis tested patients for ASCA and ANCAs. After a one-year follow-up,31 were diagnosed with UC or CD. The ASCA+/ANCA- results predicted CD in 80% of cases(sensitivity:67%, specificity:78%). ASCA-/

ANCA+results predicted UC in 64% of patients (sensitivity:78%, specificity:67%). In approximately 49% of patients whose test results were negative, after a follow-up of 9.9 years, there was no definite diagnosis.[8]

According to our results, the atypical p-ANCA test might be a good test for the diagnosis of UC and differentiating UC from CD. The specificity of atypical p-ANCA for diagnosing UC was 86%.In agreement with the current research, one study that assessed atypical p-ANCA in IBD patients reported a sensitivity of 42% and specificity of 97% in the diagnosis of UC.[9] According to numerous studies

Table 1: ASCA test results in Crohn's disease (CD) and non-CD patients.

CD/ASCA	Yes (%)	No (%)	Total
Positive	4 (16)	3 (2.6)	7
Negative	21 (84)	109 (97)	130
Total	25	112	137

Table 2: ASCA test results in ulcerative colitis (UC) and non-UC patients.

UC/ASCA	Yes (%)	No (%)	Total
Positive	1 (1)	6 (9)	7
Negative	71 (99)	59 (90)	130
Total	72	65	137

Table 3: Atypical-P-ANCA results in ulcerative colitis (UC) and non-UC patients.

UC/Atypical-P-ANCA	Yes (%)	No (%)	Total
Positive	32 (44)	9 (13)	41
Negative	40 (55)	56 (86)	96
Total	72	65	137

Table4: Atypical-P-ANCA results in Crohn's disease (CD) and non-CD patients

CD/Atypical-P-ANCA	Yes (%)	No (%)	Total
Positive	4 (16)	37 (49)	41
Negative	21 (84)	75 (67)	96
Total	25	112	137

the patterns of nuclear and cytoplasmic ANCA were shown to be a useful tool for the diagnosis of IBD and to differentiate between CD and UC.[2-4] Other studies reported no diagnostic value for ANCA in the diagnosis of IBD.[5,6]

The ASCA examination according to a number of studies was shown to be helpful in distinguishing UC from CD.[2-4] In a Polish study, blood samples of 31 patients with CD were analyzed for ASCA by ELISA, which resulted in a sensitivity of 81% and specificityof 78% for CD. The authors concluded that this test had high specificity in differentiating CD from UC.[3] In the current study our results showed low sensitivity and high specificity of ASCA for both diseases, thus the ASCA test could not differentiate UC from CD. Another Iranian study's results were similar to the current study's findings.The ASCA test had low sensitivity for diagnosing IBD.[5] We presumed this finding could be an effect of genetics, race or laboratory precision in Iran.Because of the high NPV of the ASCA test in the current study,CD could be ruled out with high probability in those patients whose ASCA test results were negative.

There was no relationship between the site of colon involvement and atypical p-ANCA results. However due to the lack of enough studies in this field, it would be helpful to conduct additional studies. Previous studies have shown that ANCA is not related to a particular phenotype of UC,[10] although some studies have stated that phenotypic involvement of the colon which is similar to UC may be present in patients with CD who have positive ANCA test results.[11] Previous studies have demonstrated associations between ASCA and a particular phenotype of CD such as strictures, ileum involvement and perforation.[12-15] The current study has found no relationship between CD and ASCA examination results.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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