Anti-*Saccharomyces cerevisiae* antibody is not useful to differentiate between Crohn's disease and intestinal tuberculosis in India

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ABSTRACT

Context: Clinical, endoscopic, radiological and histological parameters of intestinal tuberculosis (IT) and Crohn's disease (CD) are so similar that differentiation between these two diseases, which require different treatment, is difficult. Anti-*Saccharomyces cerevisiae* antibody (ASCA), which is often present in the sera of patients with CD, may be potentially useful to differentiate CD from IT. **Aim:** To evaluate the role of enzyme-linked immunosorbent assay test for ASCA in serum in differentiating CD from intestinal tuberculosis. **Settings and Design:** Prospective case-control study. **Materials and Methods:** Sixteen patients with IT, 16 CD, 36 UC diagnosed using standard parameters and 12 controls (11 healthy subjects and one with colonic carcinoma) were tested for IgG ASCA in serum. **Statistical Analysis Used:** Categorical variables were analyzed using Chi-square test with Yates' correction, as applicable. Continuous variables were analyzed using Mann-Whitney U test. **Results:** Eight of 16 (50%) patients with IT, 10 of 16 with CD (62%), nine of 35 with UC (26%) and one of 12 controls tested positive for ASCA in serum. Though the frequency of ASCA in serum was comparable among patients with IT and CD (8/16 vs. 10/16, P = ns), IT and UC (8/16 vs. 9/35, P =ns), CD and UC (10/16 vs. 9/35, P=ns), its frequency in CD or IT but not in UC was higher than healthy controls ($P < 0.01$). **Conclusions:** Serum ASCA is unlikely to be useful to differentiate between CD and IT in India.

KEY WORDS: Crohn's disease, gastrointestinal tuberculosis, tropical country, ulcerative colitis

Ulcerative colitis (UC) and Crohn's disease (CD) are inflammatory diseases of the bowel possibly resulting from exaggerated autoimmunity against intestinal luminal microbes.[1] CD was believed to be uncommon in India in the past.[2,3] However, CD has increasingly been reported from India recently.[4-6] One possible reason for the lower frequency of diagnosis of CD from India in the past could be related to misdiagnosis of CD as intestinal tuberculosis (IT).[7] In fact, clinical, radiological, endoscopic and operative features of CD closely mimic IT.[8] Moreover, histological features of CD are only marginally different from those of IT.[9,10] Acid-fast bacillus (AFB) on histology, smear and culture of intestinal biopsy, the absolute criteria to differentiate IT from CD, are found only in a small proportion of patients.[9] Therefore, a simple, cheap, sensitive and specific test to differentiate CD from IT is the need of the day.

Antibodies to oligomannosidic epitopes of baker's yeast *Saccharomyces cerevisiae*, have been shown to be strongly associated with inflammatory processes of the intestine.[11-13] Several studies from the developed countries reported the usefulness of an enzyme-linked immunosobent assay (ELISA)-based anti-*Saccharomyces cerevisiae* antibody (ASCA) test in differentiating CD from UC.[14] However, clinical, endoscopic and radiological features of CD, particularly the common variety that involves small as well as large bowel, are widely different from that of UC.[1,15] Moreover, treatment of UC and CD is somewhat similar.[1] Therefore, misdiagnosis of CD as UC and vise versa may not have as much clinical consequences as misdiagnosing CD as IT. One study, which included a small number of patients with IT, showed that only one of 14 (7%) patients with IT had a positive result to ASCA ELISA test in serum in contrast to 49% with CD.[16] Therefore, we hypothesize that ASCA may be a potentially useful test to differentiate CD from IT in areas of world with high prevalence of tuberculosis. Accordingly, we undertook a prospective study to evaluate the role of ELISA test for ASCA in serum in differentiating CD from IT.

Materials and Methods

Sixteen patients with IT, 16 with CD and controls (35 IBD controls [UC] and 12 non-IBD controls [11 healthy subjects and

one with colonic carcinoma]) were included in the study during a three-year period (from January 2001 to December 2003).

CD was diagnosed based on endoscopic, radiological, histological parameters and findings at laparotomy in some patients.[1] Most of these patients did not respond to one or more courses of antitubercular drugs before presenting to the study center or at the study center. Intestinal tuberculosis was diagnosed in most patients by demonstration of AFB either in intestinal biopsy or another site with or without granuloma at histology and response to treatment with antitubercular drugs. Ulcerative colitis was diagnosed on clinical, endoscopic and histological parameters.[1] The healthy controls included some of the authors and the staff members of the institute. All the patients and healthy subjects gave consent for inclusion into the study. The study was reviewed and approved by Institutional review committee.

Tests for ASCA

5 ml blood was collected from each patient by sterile venepuncture before starting any specific treatment at the study center. The serum was stored at -20° C till tested. IgG ASCA was tested by commercially available ELISA kit (Genesis diagnostics, Cambridge shire, UK) on the stored serum samples after thawing. Briefly, diluted serum samples were incubated with mannan immobilized on microtitre wells. After washing away unbound serum components, rabbit anti-human IgG conjugated to horseradish peroxidase was added to the wells and incubated again. Unbound conjugate was removed by washing. A solution containing 3, 3', 5, 5'-tetramethylbenzidine (TMB) and enzyme substrate was added to trace specific antibody binding. At this stage, stop solution was added to terminate the reaction and to provide the appropriate pH for development of color. The optical density of standard (10U/ml), controls and test samples was measured using a micro plate reader at 450 nm. Optical density is directly proportional to the antibody present in the serum. A sample considered positive was more than that of the 10U/ml standard as per manufacturer's instructions. The investigators (UG, HS) testing the samples were blinded to the clinical details of the patients including the final diagnosis.

Treatment and follow-up

Patients with IT were treated with anti-tubercular drugs that included isoniazide, rifampicin, ethambutol and pyrazinamide for two months followed by isoniazide and rifampicin for six to nine months. Response to treatment was determined by disappearance of symptoms and signs (e.g. abdominal lump, ascites, lymphadenopathy), general well-being, weight gain and no recurrence even after stopping treatment. Patients with CD were treated with azathioprine and 5-aminosalicylic acid. Response was defined as persistent reduction in the Harvey-Bradshaw score[17] to 3 or below at least over one month. Those who did not respond or had bone marrow toxicity of azathioprine were treated with infliximab.

Statistical analysis

Categorical variables were analyzed using Chi-square test with Yates' correction, as applicable. Continuous variables were analyzed using Mann-Whitney U test. *P* values below 0.05 were considered significant.

Results

Of 72 patients initially included, five were excluded from the final analysis as a definite diagnosis could not be made due to inadequate follow-up (n=2) or diagnosis of either IT or CD could not be finalized based on the available evidences (n=3). Demographic, clinical, endoscopic, radiological and histological parameters of patients with IT and CD are shown in Tables 1 and 2. Patients with IT (n= 16, median age 34 years, range 19 to 63; eight male), CD (n= 16, median age 44 years, range 24 to 63; 12 male), UC (n= 35, median age 39 years, range 17 to 68; 20 male) and 11 healthy subjects (median age 39 years, range 29 to 56; six male) were similar in age and gender distribution.

Clinical parameters

IT: Patients with IT presented with subacute intestinal obstruction (n = 11), tubercular peritonitis with intestinal involvement (n = 2), pyrexia of unknown origin (n = 1), constrictive pericarditis with right iliac fossa lump (n = 1) and colonic ulcers (n = 1); the latter patient also had active pulmonary tuberculosis. One other patient had intra-thoracic lesion in the form of hilar lymphadenopathy. Two other patients were treated for pulmonary tuberculosis in the past. All the patients responded to anti-tubercular drugs.

CD: Patients with CD presented with recurrent subacute intestinal obstruction (n = 5), bloody diarrhea due to colonic disease (n = 4, one of whom also reported history of subacute intestinal obstruction), chronic diarrhea without blood (n = 3), perianal disease (n = 1), acute abdomen due to intestinal perforation (n = 1), obscure gastrointestinal bleeding (n = 1), lump in right iliac fossa (n = 1). In four of these patients intestinal perforation was documented and three had intestinal fistula. All the patients responded to treatment with corticosteroids, azathioprine and 5-aminosalicylic acid except two patients, in whom infliximab was needed to control the disease [Table 2].

Result of tests for ASCA

Tables 1 and 2 show the results of ASCA test among patients with IT and CD. Eight of 16 (50%) patients with IT, 10 of 16 with CD (62%), nine of 35 with UC (26%) and one of the 12 non-IBD controls tested positive for ASCA in serum. Though frequency of ASCA in serum was comparable among patients with IT and CD (8/16 vs. 10/16, P = ns), IT and UC (8/16 vs. 9/35, P = ns), CD and UC (10/16 vs. 9/35, P = ns), its frequency in CD or IT but not in UC was higher than non-IBD controls ($P < 0.01$).

Discussion

The present study shows that ASCA is not useful to differentiate between CD and IT or UC. Inferior performance of this test to differentiate between IT and CD resulted from high frequency of positive test result in patients with IT.

Two other studies that attempted to evaluate the usefulness of ASCA to differentiate IT from CD showed contradictory results.[16,18] In one study, only one of 14 patients with IT was

Table 1: Demographic and clinical data of patients with intestinal tuberculosis

Age (y), sex	Presentation	Major parameters and basis for diagnosis	ASCA
40, F	SAIO, ascites	Small bowel stricture, enterolith on barium, ascites, surgical findings, no AFB	Negative
63, M	Fever, SAIO, melena	AFB in FNA from colonic wall and LN	Negative
43, F	SAIO, ascites	Jejunal stricture on barium, caseating granuloma in resected jejunum and adjoining lymph node	Negative
26, F	SAIO, ascites, hilar LN enlargement	Clinical presentation, hilar LN enlargement, surgical findings, AFB negative	Negative
31, M	Fever, epigastric lump, ascites	Low SAAG high lymphocyte multi-loculated ascites, purulent discharge from duodenum, no AFB; response to ATT	Positive
28, F	Fever, dyspepsia	AFB on colonoscopic biopsy	Positive
46, M	Chronic diarrhea	AFB in FNA of cervical lymph node	Positive
49, M	Ascites, RIF lump	Chronic calcific constrictive pericarditis, RIF lump, low SAAG ascites	Positive
26, F	SAIO, fever, RIF lump, transverse colon stricture on colonoscopy	No AFB in colonic biopsy Response to ATT	Positive
23, M	SAIO, pulmonary tuberculosis	Response to ATT	Positive
19, M	SAIO, fever	AFB in FNA from abdominal LN	Positive
26, F	SAIO, fever	AFB in FNA from abdominal LN	Positive
46, F	SAIO, fever	AFB in FNA from abdominal LN	Negative
21, M	SAIO	Multiple jejunal stricture, calcified mesenteric LN	Negative
37, F	SAIO	Stricture, ulcers, cobblestones in ascending colon, no AFB; response to ATT	Negative
37, M	Pulmonary tuberculosis, ascites, nephritic syndrome	Pulmonary tuberculosis; lymphocytosis, Langhans giant cell and high protein ascites. Colonic lesion. Response to ATT	Negative

ASCA: Anti-*Saccharomyces cerevisiae* antibody; SAIO: Subacute intestinal obstruction; FNA: Fine needle aspiration; AFB: Acid-fast bacillus; LN: Lymph node; SAAG: Serum ascitic fluid albumin gradient; RIF: Right iliac fossa; ATT: Anti-tubercular therapy.

Table 2: Demographic and clinical data of patients with Crohn's disease

Age (y), sex	Presentation	Basis for diagnosis	ASCA
56, F	Bloody diarrhea	Skip lesion, rectal sparing, suggestive histology, AFB negative, response to azathioprine, perforation	Positive
45, M	Perianal disease	Anal fissure, perianal fistula, perforation, surgical findings, response to infliximab	Negative
44, M	Obscure GI bleeding	Surgical findings, transmural inflammation, fissure, response to infliximab	Negative
31, M	Intestinal perforation	Ileal perforation, granuloma in rectal biopsy, response to azathioprine	Negative
31, M	SAIO	Small intestinal stricture on barium, enterovesical fistula, surgical findings	Positive
41, M	Chronic diarrhea	Linear ulcers in proximal colon, malabsorption, response to azathioprine and corticosteroid	Positive
24, M	Bloody diarrhea, SAIO	Ileal thickening on barium radiograph, no response to ATT, suggestive histology, response to specific therapy	Positive
55, M	RIF lump	Localized ileal perforation on barium small bowel series, fissuring ulcer with transmural inflammation on resected ileum	Positive
28, F	SAIO	Small bowel stricture with perforation, surgical findings, granuloma without AFB and transmural inflammation	Negative
46, F	SAIO	CT: ileocecal disease, barium small bowel series: multiple small bowel stricture with fistula despite ATT given in past	Positive
50, M	SAIO	Ileocolic disease on colonoscopy, suggestive histology, response to specific treatment	Positive
63, M	Chronic diarrhea, abdominal pain	Multiple colonic stricture, granuloma without AFB in colonic biopsy	Negative
50, M	Chronic diarrhea	Ulcerated ileocecal valve, no AFB, abnormal D-xylose test, no response to ATT but to immunosupressive	Positive
25, F	Bloody diarrhea	Suggestive colonoscopy and histology, response to specific treatment	Negative
36, M	Bloody diarrhea	Pancolonic disease, AFB negative, no response to ATT but to immunosupressive therapy	Positive
54, M	SAIO	Suggestive colonoscopy, no response to ATT, histology of resected ileocecal lesion	Positive

ASCA: Anti-*Saccharomyces cerevisiae* antibody; SAIO: Subacute intestinal obstruction; CT: Computerized tomography scan; AFB: Acid-fast bacillus; RIF: Right iliac fossa; ATT: Anti-tubercular therapy; GI: Gastrointestinal.

positive to ASCA test.[16] Thus, the authors recommended that this test might be useful to differentiate IT from IBD.[16] However, that study included only a small number of patients. In the other study published recently from India including 59 patients with CD and 30 with IT, frequency of positive result to serum IgG ASCA was 51% and 47%, respectively.[18] Differences in the results between the two studies might also relate to differences in patient population. In fact, increased small intestinal permeability has been documented in healthy children in the tropics.[19] Non-specific changes in intestinal mucosa have been shown in healthy British Indian and Afro-Caribbean adults[20] similarly, abnormal urinary excretion of D-xylose is reported[21] and has been termed as tropical enteropathy. Since none of the 11 healthy controls had positive results, a possibility of non-specific positive result is unlikely. Though small sample size is a limitation of our study, looking at the high frequency of positive result to ASCA among patients with IT, it seems unlikely that even a large sample size would alter the conclusions. We used IgG ASCA as one previous study[16] has used this and the other study[18] showed IgG ASCA was comparable, perhaps somewhat better, than IgA ASCA.

Anti-*Saccharomyces cerevisiae* antibody is a non-specific antibody resulting from macromolecular transport of food antigens (including antigens contained in the baker's yeast), partly resulting from increase in intestinal permeability[22,23] as evidenced by its non-specific positive result in several other conditions in which gut epithelium is injured.[24-30] Therefore, any condition that increases macromolecular transport of food antigens across the intestinal mucosa may result in positive result to this test. It has been shown that 64% patients with CD are positive to this antibody;[31] in contrast, it is less frequently positive in patients with UC.[32] Whereas CD often affects the small bowel, UC does not.[1] Therefore, it is logical to believe that damage to small intestinal mucosa with consequent increase in its permeability leads to transport of macromolecular antigens including antigens contained in baker's yeast in patients with CD. Several authors suggested that ASCA is not a non-specific antibody in CD; such suggestions are based on the fact that it is often positive in family members of patients with CD,[33] it is unlikely to be secondary to chronic inflammatory lesions in the intestine. However, increased intestinal permeability in family members of patients with CD is well known.[34,35] Distribution of the lesion in IT is similar to that in CD including involvement of small intestine. Therefore, frequent positive test result to ASCA in patients with IT is not entirely unexpected.

We used very strict criteria to differentiate between IT and CD. Though all the patients with IT did not have AFB on histology, tissue smear or culture [Table 1], other parameters were quite substantial to suggest that they had tuberculosis. Therefore, the results of the present study showing frequent positive ASCA test has clinical implications as one of the two other available studies in the literature recommended its use to differentiate between IT and CD; this might prompt clinicians in developing countries including India, where IT is still a common gastrointestinal disease, to rely on this test for this purpose. However, the present study clearly shows that ASCA test is often positive in patients with IT in the tropical countries and therefore, is not of any use to differentiate between IT and CD. This is in accordance with the other recent study published from India[18] that showed that ASCA does not differentiate between IT and CD in India.

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