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Original Article

Distinguishing Crohn's disease from intestinal tuberculosis – a prospective study

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ABSTRACT

Background: Distinguishing Crohn's disease (CD) from intestinal tuberculosis (ITB) is clinically challenging but important for prognostication and patient management.

Methods: Patients with diagnosis of CD and ITB were prospectively enrolled in the study from January 2006 to October 2007. The patients were followed up for further 15 months to ascertain that the diagnosis had not changed. Clinical, laboratory, serological [IgG anti Saccharomyces cerevisiae antibody (ASCA)], endoscopic and histologic features were compared between the ITB and CD patients. The ASCA titers were estimated in 100 healthy controls. Patients were diagnosed as ASCA positive when their ASCA titers were three standard deviations above mean of controls.

Results: Thirty patients with CD (age 33.9 + 15.2 years, 70% males) and thirty with ITB (age 35.1 + 12.2years, 53.3% males) were included in the study. Features commoner in CD were longer duration of symptoms ($p<0.001$), blood mixed stool ($p=0.006$), presence of longitudinal ulcers ($p=0.005$) and skip lesions ($p=0.008$) on colonoscopy and more number of colonic segments involved ($p=0.004$). Anorexia was commoner in ITB patients ($p=0.008$). Positive ASCA was commoner in CD (30%) than ITB (10%) but did not reach statistical significance ($p=0.1$).

Conclusions: A combined evaluation of clinical features, endoscopy, histology and response to treatment is the key to differentiate between CD and ITB.

KEYWORDS: Crohn's disease, intestinal tuberculosis, differentiating, endoscopy, histology, serology

Introduction

The last two decades has seen the emergence of Crohn's disease (CD) in developing countries like India where intestinal tuberculosis (ITB) is prevalent as well.[1] Distinguishing CD from ITB is often challenging as both diseases have similar radiological, endoscopic and histologic features.[2] Since treatment and prognosis of the two conditions are different, it is crucial to diagnose them correctly.

Tests for evaluation of patients with suspicion of CD or ITB include abdominal imaging, serological tests, endoscopy and histology. Results of individual investigations (except for presence of AFB or caseating granuloma for tuberculosis) are often insufficient for diagnosis. Diagnosis is often made after a combined evaluation of above investigations and response to therapy.[2] In this prospective study we report the role of

clinical features, serology, endoscopy and histology in distinguishing CD from ITB.

Methods

Patients diagnosed to have CD and ITB at our institute were prospectively enrolled in the study between January 2006 and October 2007. Thirty patients with CD and thirty with ITB were recruited. The diagnosis of Crohn's disease was made based on a combination of clinical, radiological, endoscopic and histological features suggested by European evidence based consensus on the diagnosis and management of Crohn's disease.[3] Intestinal tuberculosis was diagnosed in the presence of any of the following features: (I) Intestinal mucosal biopsy showing - a) AFB positive on histopathology or culture, and/or b) caseating granulomas, and/or c) large or confluent granulomas (II) Response to treatment.[4]

Clinical, investigation and treatment data were systematically recorded on a pre-designed proforma. Colonoscopy and terminal ileoscopy (if possible) (Olympus, CF 150L) was performed on the study subjects and gross findings were recorded. Multiple mucosal biopsy specimens were obtained from different segments of colon and terminal ileum during the scopy. Upper gastrointestinal endoscopy (Olympus, GIF 150) and biopsy from duodenum (D2) and stomach (antrum and incisura) were obtained in all CD patients irrespective of gross findings to assess extent of disease. Evaluation of anti saccharomyces cerevisae antibody (ASCA) was done using the IgG ASCA ELISA kits (AIDA diagnostics, Germany). ASCA recognizes specific mannan, a component of the outer wall of yeast.[5] Serum samples were incubated in the microplates coated with specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction was washed off in subsequent steps. Afterwards, anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) were incubated and reacted with the antigen-antibody complex of the samples in the microplates. Unbound conjugate was washed off. Addition of 3,5,3',5'-tetramethylbenzidine (TMB)-substrate generated an enzymatic colorimetric (blue) reaction and the absorbance was read at 450 nm within 30 minutes. The optical density (OD) of each calibrator (y axis) was plotted against the corresponding concentration values in U/ml (x axis). From this plot the antibody concentration of each sample was calculated by finding out the concentration corresponding to their respective OD values. ASCA levels were assessed in 100 healthy controls. The test was considered positive in patients if OD values were 3 standard deviation above mean value of controls.

After the diagnosis of CD or ITB was made, the patients were followed up for a further 15 months to assess response to therapy and to confirm that the diagnosis had not changed. Clinical profile, serology, endoscopy and histopathology were compared between the two groups of patients to assess the ability of the above to differentiate Crohn's disease from intestinal tuberculosis. The study was approved by the institutional review board.

Data is presented as mean with standard deviation for normally distributed continuous variables and as median with range for non-normally distributed continuous variables. Categorical data is presented as proportions. For comparing categorical variables (including ASCA) chi square test was used. For comparing continuous variables with normal distribution, t test was used. For continuous variable with non-normal distribution, Mann-Whitney's U test was used. A p value of <0.05 was considered significant. The statistical analysis was done using SPSS software for windows version 11.0.

Results

We selected 67 probable patients for our study of whom 60 (30 CD and 30 ITB) fulfilled the inclusion criteria. Four patients with suspected ITB and 3 with suspected CD were excluded due to insufficient diagnostic criteria. At baseline, 5 patients with CD (included in the study) had history of receiving ATT in past. Demographic and clinical features in patients with CD and ITB are shown in (**Table 1**). There was no significant difference in age, sex and geographical location in patients with CD and ITB. The median duration of symptoms in patients with ITB was 3 months (range 1 month to 2 years) while it was 2 years (range 6 months to 15 years) in patients with CD, the difference being statistically significant ($p<0.001$). Dull aching and poorly localized abdominal pain was the commonest symptom in patients with CD (60%) and ITB (70%). Blood mixed with stool was seen more often in patients with CD ($p=0.006$). Anorexia ($p=0.008$) and weight loss ($p=0.067$) were seen more often in ITB. Diarrhoea and fever were similar in both groups. Clinical examination of abdomen showed right iliac fossa mass in two patients with CD. In patients with ITB right iliac fossa mass was revealed in two patients, hepatosplenomegaly in

one, hepatomegaly in one and cervical lymphadenopathy in two patients.

According to the Vienna classification, the disease location in CD patients was L1 (ileal) in 13.3%, L2 (colonic) in 23.3%, L3 (ileocolonic) in 60% and L4 (upper gastrointestinal) in 3.3%. Although upper gastrointestinal lesion was seen in 5 patients (on endoscopy and/or histology), 4 of them had major lesions in ileum and/or colon and hence were not classified as L4. Two patients with CD had internal fistula (jejunocolic and colovesical), 4 had fistula-in-ano and two had anal fissure. Extraintestinal manifestations were present in 4 patients – 3 had arthralgia, 1 had pyoderma gangrenosum and 3 had oral ulcers. Thirteen patients underwent surgery for their disease – 5 had small bowel surgery for strictures, 7 had right hemicolectomy for ileocolonic disease and 1 had fistulectomy for perianal fistula. The disease location in ITB patients was ileocolonic in 53.3%, ileal in 3.3%, colonic in 33.3% and upper gastrointestinal in 10%. Among patients with ITB two patients had internal fistula (duodenocolic and jejunocolic) and 1 had fistula-in-ano. Extraintestinal manifestation was present in only 1 patient who had arthralgia. Three patients underwent surgery for their disease – 1 had small bowel surgery for strictures and other two had partial colonic resections as treatment of internal fistulas. Patients with CD underwent surgery significantly more often compared to ITB patients (p=0.007). Lab investigations revealed low albumin (<3.5 g/dl in 38 patients), high ESR (>30 in 40 patients) and borderline low hemoglobin (<11.0 g% in 32 patients) in most patients. ESR was significantly higher in ITB group compared to CD (p=0.05).

All patients with CD and 24 patients with ITB underwent colonoscopy. The other 6 patients with ITB had radiological evidence of intestinal involvement with evidence of tuberculosis at extraintestinal sites. The colonoscopy and mucosal biopsy findings are shown in (**Table 2**). Colonoscopy showed lesions in twenty five (83%) patients with CD and twenty three patients with ITB (95.8%). Presence of skip lesions (p=0.008), longitudinal ulcers (p=0.005), and multiple colonic segment involvement (p=0.004) were commoner in CD than in ITB. Other colonoscopic findings were not helpful in differentiating CD from ITB. Three patients with CD had UGI involvement on endoscopy (gastric erosions in 2 and duodenal nodularity in 1) and five had noncaseating granulomas on histology (4 in stomach and 1 in duodenum).

Histological features suggestive of CD were seen in 90% of CD patients and these included crypt architecture abnormalities, mononuclear infiltration, granulomas, transmural inflammation, segmental distribution of the lesion and patchy and focal inflammation. Remaining three patients had non-specific histological features and in them, clinical and radiological features along with response to therapy formed the basis of diagnosis. All patients with CD showed response to treatment but 6 of them had a relapse during follow up. Histological features suggestive of ITB were seen in 20 of the

Table 1: Demographic, clinical and laboratory profile of patients

	CD (n=30)	ITB (n=30)	p value
Age (yrs.)	33.9±15.2	35.1±12.2	0.72
Sex (M/F)	21/9	16/14	0.29
Symptom duration [Median (range)]	24months (6-180months)	3 months (1-24months)	<0.001
Abdominal pain	18 (60%)	21 (70%)	0.59
Diarrhea	14 (46.7%)	12 (40%)	0.8
Weight loss	13 (43.3%)	21 (70%)	0.067
Anorexia	12 (40%)	23 (76.7%)	0.008
Blood in stool	11 (36.7%)	1 (3.3%)	0.001
Fever	8 (26.7%)	9 (30%)	1.0
Fistula	6 (20%)	3 (10%)	0.47
Extraintestinal manifestations	4 (13.3%)	1 (3.3%)	0.35
CRP (%Positive)	71.4%	75%	1.0
Hb(g%)	11.0±2.4	10.5±2	0.33
ESR (mm/hr.)	44.1(3-108)	62.8 (6-140)	0.05
Albumin (g/dl)	3.3±0.8	3.0±0.9	0.19

IC – Ileocolonic, I- Ileal, C- Colonic, O- Other site
M – Male, F-Female

Table 2: Colonoscopic and mucosal biopsy findings in patients with ITB and CD

Findings	CD (n=30)	ITB (n=24)	p value
Aphthous ulcer	11 (36.7%)	4 (16.7%)	0.13
Longitudinal ulcer	10 (33.3%)	1 (4.2%)	0.005
Transverse ulcer	2 (6.7%)	6 (25%)	0.14
Skip lesions	11 (36.7%)	2 (8.3%)	0.008
Perianal lesions	6 (20%)	1 (4.2%)	0.1
Mean number of segments involved	3±1.4	1.9±1	0.004
Cobblestone pattern	3 (10%)	0 (0%)	0.25
Pseudopolyp	9 (30%)	2 (8.3%)	0.09
Stricture	8 (26.7%)	4 (16.7%)	0.51
Narrowed IC valve	3 (10%)	5 (20.8%)	0.44
Patulous IC valve	2 (6.7%)	2 (8.3%)	1.00
Confluent granuloma	0 (0%)	9 (37.5%)	NA
Microgranuloma	3 (10%)	0 (0%)	0.25
Caseous necrosis	0 (0%)	2 (8.3%)	NA
AFB positive*	0 (0%)	9 (37.5%)	NA

*AFB – Acid Fast Bacilli *Does not include 2 patients with confluent granulomas and AFB together*

24 ITB patients undergoing colonoscopy and these included large or confluent granulomas (n=9, 37.5%), caseating granulomas (n=2, 8.3%), and presence of AFB in tissue section or culture of tissue (n=9, 37.5%). The ITB patients with caseation (n=2) or AFB (n=9) had discrete (non-confluent) granulomas seen on histology. Among the 9 patients with confluent granulomas, 2 had AFB. Of the 30 ITB patients, 27 responded to ATT. Among the remaining 3 (all had AFB on histology), 1 expired after 1 month of therapy and 2 had no response to 9 months of ATT.

ASCA ELISA was tested on 100 controls to establish the normal "cut-off" antibody levels in the population. The mean optical density value in controls plus 3 standard deviation was taken as "cut-off" value and patients with values above this level (equivalent to 7 U/ml) were considered to have a positive ASCA test. 30% of patients with CD had ASCA positive as compared to 10% of patients with ITB (p=0.1) (**Table 3**). The specificity of ASCA was 90% in differentiating CD from ITB.

The occurrence of stricturing and fistulising disease, small bowel disease, history of surgery, marker of disease activity (CRP, ESR, Harvey Bradshaw Score >4) and clinical course were not significantly different between the CD patients who were ASCA positive and those who were ASCA negative. However, the small number of patients makes meaningful comparison difficult.

Table 3: ASCA results of CD patients compared with ITB

ASCA	CD	ITB	p-value
Positive	9 (30%)	3 (10%)	0.10
Negative	21 (70%)	27 (90%)	
Total	30	30	

Discussion

The study was aimed at assessing distinguishing features between CD and ITB in a prospectively recruited cohort. About 35-45% of patients with CD in India are initially diagnosed to have ITB which aptly highlights the difficulty in distinguishing between the two diseases.[6,7] The rising incidence of CD in India further compounds this problem and calls for development of effective tools to differentiate the two conditions.[1] Both the diseases are common in the fourth decade of life and abdominal pain and weight loss are seen in up to two-third of the cases.[4,6] A large case series of ITB (n=173) and another one on CD (n=182) from India suggests that ITB patients have more frequent fever and shorter duration of symptoms (<1 year).[4,6] CD patients are usually symptomatic for years and diarrhea and bleeding per rectum are more common.[6,7] Symptom duration was shorter and anorexia was more common in our ITB patients. Blood in stool was commoner in CD patients while rest of the clinical features were comparable. Amarapurkar et al have reported anemia to be frequent in CD patients and ESR similar in patients with CD and ITB.[7] Our ITB patients had higher ESR than CD patients. In our study, lab features were not helpful in differentiating the two diseases.

Serological tests including ASCA and pANCA are commonly used in western countries to distinguish CD from ulcerative colitis.[8] ASCA is commoner in CD and pANCA in ulcerative colitis.[8] Since in India we are more often faced with problems of distinguishing CD from ITB, it is logical to study the role of serological tests to differentiate between them. Three studies have been done in India on utility of ASCA but none showed any significant difference in prevalence between CD and ITB patients.[7, 9,10] However, these reports have not commented on establishing local positive cut-offs of ASCA. We analyzed the serum of 100 healthy controls to establish the cut-off value of ASCA in our population. Using this cut-off, 30% of our patients with CD and 10% of patients with ITB were ASCA positive but the difference failed to reach statistical significance. Interestingly, the ASCA prevalence in CD in other Indian studies has ranged from 40-60% as compared to 30% in our series.[7,9,10] The use of cut-off value of ASCA established for our own population in this study may account for this difference. However, ASCA did have a good specificity in distinguishing CD from ITB. Eight of our nine CD patients who had ASCA positive had ileal involvement suggesting that ASCA positivity may point to small bowel involvement in CD.

Radiological investigations provide useful clues to diagnosis but their major role is in assessing the extent of the disease.[2] Since we used both barium studies and CT abdomen for this purpose, comparison of any single modality to differentiate the two diseases was not performed due to small number of patients. Studies comparing imaging findings in CD and ITB are few[11,12] and most describe findings in either a group of ITB patients or CD patients.[13,14] Asymmetric thickening of colonic wall, larger lymph nodes (>1cm), necrotic lymph nodes and ascites on CT are suggestive of ITB.[2] Symmetrical bowel wall thickening, smaller (<1cm) lymph nodes and bowel loop displacement by fibrofatty changes are suggestive of CD.[2]

Endoscopy plays an important role in diagnosis.[7,15] Apart from visual inspection, mucosal biopsy can be obtained for histopathology, culture and molecular tests. Presence of mucosal ulcers and involvement of ileocecal region are common in both diseases. Colonoscopy studies show that CD patients

usually have longitudinal ulcers, cobblestone appearance of mucosa and anorectal involvement.[16] A patulous ileocecal valve, transverse ulcer and involvement of lesser number of colonic segments are commoner in ITB.[7,16] None of these were discriminatory in our patients. Presence of AFB in biopsy samples on histology or on culture is diagnostic of tuberculosis but was present only in a small number of our cases. Granulomas in endoscopic biopsy samples are seen in 20-30% of CD and 50-70% of ITB patients.[6,7,17,18] Previous studies done at our center by Pulimood et al showed that granulomas in ITB and CD have different morphology.[19,20] Granulomas in ITB are multiple (mean number of granulomas per section: 5.35), large (mean widest diameter: 193 micron), confluent and often with caseating necrosis. In CD granulomas were infrequent, small and poorly organized. In addition to microscopic examination, tissue samples can be subjected to PCR tests. Conventional PCR is done after extracting nucleic acids from tissue followed by amplification reactions. In a report on 60 patients with ITB and 20 with CD, PCR was positive in 21.6% of ITB patients vs. 5% CD patients.[21] Another PCR technique called the 'in-situ PCR' does not require nucleic acid extraction from tissues. The targeted DNA sequence is amplified in intact cells. A study done at our center on 20 patients with ITB and CD each showed it to be positive in 30% and 5% patients respectively.[22] What is apparent from these data is that PCR has a good specificity but poor sensitivity. Apart from tissue PCR, fecal sample PCR testing has also been studied at our center in ITB patients and controls and the results are encouraging.[23]

In conclusion, differentiating CD from ITB continues to be a challenging problem. Presently, a combination of clinical features, endoscopy, histology, radiology and response to treatment continues to be the key to differentiate these two conditions. We need to continue to develop new tests to help clinicians differentiate between the two conditions.

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