Tissue Polymerase Chain Reaction in Diagnosis of Intestinal Tuberculosis and Crohn’s Disease

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Abstract
Aims and Objectives: 1) To evaluate the utility of PCR in differentiating intestinal tuberculosis from Crohn’s disease. 2) To compare histological features of tuberculosis and Crohn’s disease.

Material and Methods: A total of 60 cases of diagnosed intestinal tuberculosis and 20 Crohn’s disease were included in the study. Clinical data, radiological and endoscopic findings and response to treatment were taken into consideration. Endoscopic biopsies from affected areas were subjected to histopathological examination and polymerase chain reaction (PCR) assay. Acid fast staining on tissue and culture was done whenever possible.

Results: Clinical symptoms, radiological and endoscopic findings were almost similar between intestinal tuberculosis and Crohn’s disease. PCR was positive in 21.6% cases of intestinal tuberculosis and 5% Crohn’s disease. Nine out of 42 cases (21.4%) without granuloma were also positive by PCR. There was no statistical difference for PCR positivity between patients with intestinal tuberculosis with or without granuloma on histology and also between caseating and non-caseating granuloma.

Conclusion: PCR assay showed high specificity (95%) for the diagnosis of intestinal tuberculosis hence may be valuable method to differentiate intestinal tuberculosis from Crohn’s disease.

INTRODUCTION
Tuberculosis continues to be a major public health problem in developing country like India.1,2 Deaths due to tuberculosis account to 50 / 100,000 population.3 Forty percent of the cases in India contract tuberculosis by the age of 6 years and 80% by the age of 16 years.4 The incidence of tuberculosis is also rising in Western countries.5-7 Hence development of efficient tests for early diagnosis is the most important in diagnosing the disease. As compared to pulmonary tuberculosis, the diagnosis of extrapulmonary tuberculosis is not very easy. Intestinal tuberculosis is one such example where symptoms and signs at presentation may be non-specific. It resembles clinically, radiologically and histologically with Crohn’s disease.8-10 It was believed earlier that Crohn’s disease is rare in India but in last 5 years many reports on Crohn’s disease been published from various parts of the country.11-13 Since there are many similarities between two condition clinicians face problems in differentiating intestinal tuberculosis from Crohn’s disease. This is mainly due to current routine diagnostic methods for tuberculosis which are inefficient and non-invasive tests are not diagnostic. Staining for acid-fast bacilli (AFB) lack sensitivity and specificity and the biopsy culture is time consuming.8 There is also difficulty in obtaining adequate material for histological or microbiological studies from affected, stricturous sites of the bowel at endoscopy. Amplification of mycobacterial DNA sequences by polymerase chain reaction (PCR), which is rapid and accurate diagnostic method, has been shown more promising for mycobacterial detection in clinical specimens.14-16

The current study aims to evaluate the utility of PCR in diagnosis of intestinal tuberculosis and differentiating it from Crohn’s disease and to compare histological features of tuberculosis and Crohn’s disease on histology.

MATERIAL AND METHODS
This is retrospective analysis, which consists of total 80 cases, which were evaluated and treated at gastroenterology departments of two tertiary referral hospital during year 2001. These include 60 cases of intestinal tuberculosis and 20 cases of Crohn’s disease. Complete history with clinical and endoscopic findings was reviewed from patient’s records.
All patients had undergone ileocolonoscopy with biopsy. Multiple (5 to 10) biopsy bits were obtained from affected areas, which were processed routinely.

The diagnosis of intestinal tuberculosis was made using any of the following criteria: Presence of acid-fast bacilli (AFB) on histology or positive AFB culture, presence of caseating or non-caseating granulomas on biopsy, associated evidence of active tuberculosis at other sites which were confirmed on biopsy or on AFB culture along with a complete response in all patients to anti-tuberculosis treatment. The diagnosis of Crohn’s disease was based on clinical, endoscopic and radiological parameters with exclusion of intestinal tuberculosis, biopsy showing either non-specific inflammation or non-caseating granulomas with negative AFB smear or culture, evidence of extra-intestinal manifestations, good clinical response to steroids and failure of clinical improvement after anti-tuberculosis treatment.

Paraffin embedded biopsy specimens were tested for detection of Mycobacterium tuberculosis complex by the Gen-Probe (USA) Amplified Mycobacterium tuberculosis direct test by PCR assay.7 All samples were processed by the N-acetyl-L-cysteine-sodium hydroxide decontamination procedure prior to DNA extraction. After two phenol-chloroform extractions, the DNA was precipitated by ethanol and finally resuspended in 40 µl of distilled water. Oligonucleotide primers for detection of Mycobacterium tuberculosis were selected to amplify a 123 base pair (bp) fragment of the 5‘ portion of IS 6100, which is specific for Mycobacterium tuberculosis and contains an internal endonuclease site that allows confirmation of the product by digestion of the endonuclease. The sequences of the primers (5’ to 3’) were CCTGCCAGGCTAGGCCTCGG and CTCGTCCAGCCGCTTCCGG. Amplifications were performed in 30 cycles using thermostable DNA polymerase. After PCR amplification the product was analyzed by electrophoresis on 1.8% agarose gels or 12% acrylamide gels satined with ethidium bromide and visualized by ultraviolet transilluminator.

PCR quality control was maintained by taking strict precautions to avoid contamination in laboratory, while performing each step including use of disposable equipments. Positive and negative PCR controls were included with each set of reactions. To demonstrate the presence of substance capable of inhibiting amplification, all negative samples were tested by PCR for the human β-globin gene containing 110 bp sequences.

Histopathological examination: Biopsy specimens were stained with hematoxylin eosin and reported by an experienced gastrointestinal pathologist, who was completely unaware of clinical diagnosis and PCR results. Histological parameters compared between intestinal tuberculosis and Crohn’s disease were presence of granulomas with or without caseation, ulceration of surface epithelium, presence of lymphoid aggregates and inflammatory infiltrate were evaluated by the criteria of Pulimood et al.16 In addition one section from the paraffin embedded block was stained with Ziehl-Neelsen method for the presence of acid-fast bacilli. Some of the biopsy specimens were also subjected to AFB culture wherever possible.

Statistical analysis

Students’ t test and χ² test was used for analysis of difference in proportions. A value of P < 0.05 was considered to be statistically significant.

Results

From total 80 cases male to female ratio was 3:2 with the age ranging from 16 to 48 years with the mean age of 32.7 ± 6.4 years. The clinical presentations, radiological and endoscopic findings were as shown in Table 1. Mean duration of symptoms was 7 ± 2.5 months and 42 ± 7.3 months for abdominal tuberculosis and Crohn’s disease respectively. The common presentation in abdominal tuberculosis were abdominal pain in 52 (86.6%), fever-54 (90%) and weight loss-50 (83.3%). Other symptoms were diarrhoea-20 (33.3%), abdominal lump-12 (20%) and intestinal obstruction-8 (13.3%) cases. In Crohn’s disease symptoms were abdominal pain-18 (90%), weight loss-14 (70%), diarrhoea-16 (80%), abdominal lump-five (25%), intestinal obstruction-two (10%), fistulas-three (15%) and perianal disease in two (10%) cases. On radiology bowel thickening were seen in 40 (66.6%) of intestinal tuberculosis and 12 (60%) cases of Crohn’s disease on barium meal follow through and CT scan. Other findings on CT scan were abdominal mass-five (8.3%), mesenteric thickening-36 (60%), ascites-25 (41.6%) and abdominal lymphadenopathy in 16 (26.6%) cases of intestinal tuberculosis while in Crohn’s disease abdominal mass was seen in two (10%) and mesenteric thickness in 10 (50%) cases. None of the Crohn’s disease patients showed evidence of ascites and abdominal lymphadenopathy on CT scan. On Colonoscopy, ulcers were found in 46 (76.6%) and 12 (60%), ulcerated mass-six (10%) and 0%, pseudopolyps-42 (70%), 16 (80%) and strictures in eight (13.3%) and 3 (15%) cases of intestinal tuberculosis and Crohn’s disease respectively. Considering the site of lesions both diseases showed ileocaecal junction followed by ileum as the common sites. Extra-intestinal evidence of tuberculosis were seen in 23 (38.3%) case of which majority were diagnosed on sputum AFB smears, bronchoalveolar levage stained for AFB and pleural biopsy, in six cases abdominal and cervical lymph nodes showing caseating granulomas and three cases of peritoneal tuberculosis diagnosed at the time of lapaproscopy with biopsy and single case of renal and hepatic tuberculosis each.

Results of PCR and AFB positivity on endoscopic biopsy specimens are shown in Table 2. PCR was positive in 13 / 60 (21.6%) cases of intestinal tuberculosis and 1/20 (5%) of Crohn’s disease. The only case of Crohn’s disease positive for PCR did not show evidence of granuloma on histology. Amongst intestinal tuberculosis cases, PCR positivity was seen in 4/18 (22.2%) in granuloma positive and 9/42 (21.4%) granuloma negative cases. (P>0.05), while comparing granulomas with and without caseation there was no statistical
difference (P>0.05) observed in PCR positivity. On these results the sensitivity, specificity, positive and negative predictive values in this study were 21%, 95%, 92% and 28% respectively.

Histological features on biopsy (Table 3) showed presence of granulomas in 18 (30%) cases of intestinal tuberculosis of which five (8.4%) showed caseating necrosis in the centre and 13 (21.6 %) were non-caseating. As against this none of the Crohn’s disease cases showed granulomas and all these biopsies revealed non-specific inflammation. Other histological parameters compared were mucosal ulceration, lymphoid aggregates or follicles and presence of inflammation. However we have not found any significant difference in these parameters between intestinal tuberculosis and Crohn’s disease. Hence, differential diagnosis only on histological findings was very difficult unless caseating granuloma was present. There were eight cases, which showed AFB positivity, and all of them were positive for PCR. None of the Crohn’s disease cases were positive for AFB smears or culture.

**DISCUSSION**

In the developing countries like India with ever increasing incidence of tuberculosis there is also increase in the incidence of Crohn’s disease that makes diagnosis of both conditions most challenging.8 Since routine diagnostic methods for tuberculosis are inefficient it gives rise to difficulty in controlling disease, which becomes major public health problem and also compromises the treatment of patients.3 Appropriate anti-tuberculous treatment leads in most cases of intestinal tuberculosis to complete cure where as Crohn’s disease is a progressive, relapsing illness unaffected by antituberculous treatment and shows good response to steroids.

Clinical manifestations of both intestinal tuberculosis and Crohn’s disease are protean. Considering age and gender, no difference was found between two conditions in this study. Though female predominance in tuberculosis is reported, we have not found any significant difference in gender.10,19 Abdominal pain, weight loss, fever and diarrhoea were frequent symptoms in intestinal tuberculosis while abdominal pain, weight loss and diarrhoea were common in Crohn’s disease. Fever was common in tuberculosis patients while absent in Crohn’s disease (p=0.001). The mean duration of symptoms was much less (7 months) in patients with tuberculosis while 24 months in patients with Crohn’s disease (P<0.001). These symptoms were more or less similar to other Indian studies.20,21 Radiological methods such as plain X-ray abdomen, abdominal computed tomography (CT) and sonography often reveal non-specific findings.22-24 Thickening of bowel wall, abdominal mass and mesenteric thickening are not specific of any disease. They can be present in various other lesions such as infections, ischemia, carcinoma or lymphoma. Presence of ascites and abdominal lymphadenopathy was found in 41.6 % and 26.6 % respectively in patients with tuberculosis while 24 months in patients with Crohn’s disease (P<0.001). These symptoms were more or less similar to other Indian studies.20,21 Radiological methods such as plain X-ray abdomen, abdominal computed tomography (CT) and sonography often reveal non-specific findings.22-24 Thickening of bowel wall, abdominal mass and mesenteric thickening are not specific of any disease. They can be present in various other lesions such as infections, ischemia, carcinoma or lymphoma. Presence of ascites and abdominal lymphadenopathy was found in 41.6 % and 26.6 % respectively in patients with tuberculosis and was not seen in patients of Crohn’s disease. Although colonoscopic findings of ulceration, mass lesion, pseudopolyps and strictures are seen in both intestinal tuberculosis and Crohn’s disease, it provides an access to disease site and is helpful in suspecting the diagnosis and obtaining biopsies.25,26 From our 60 cases of intestinal tuberculosis, ileocecal involvement

### Table 1: Clinical, radiological and endoscopic profile of intestinal tuberculosis and Crohn’s disease

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intestinal tuberculosis</th>
<th>Crohn’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.2 ± 5.6</td>
<td>34.2 ± 7.3</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>3:2</td>
<td>3:1</td>
</tr>
<tr>
<td>Mean duration of symptoms (months)</td>
<td>7 ± 2.5</td>
<td>42 ± 7.3 months</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>52 (86.6%)</td>
<td>18 (90%)</td>
</tr>
<tr>
<td>Fever</td>
<td>54 (90%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>50 (83.3%)</td>
<td>14 (70%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>20 (33.3%)</td>
<td>16 (80%)</td>
</tr>
<tr>
<td>Abdominal lump</td>
<td>12 (20%)</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>Intestinal obstruction</td>
<td>8 (13.3%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Fistula</td>
<td>0 (0%)</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Perianal disease</td>
<td>0 (0%)</td>
<td>2 (10%)</td>
</tr>
</tbody>
</table>

### Table 2: PCR positivity in biopsy specimens from intestinal tuberculosis and Crohn’s disease

<table>
<thead>
<tr>
<th>Granuloma Type</th>
<th>Intestinal tuberculosis</th>
<th>Crohn’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Cases</td>
<td>1/5 (20%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Caseating</td>
<td>3/13 (23.07%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Non-caseating</td>
<td>9/42 (21.4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total</td>
<td>13/60 (21.6%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

### Table 3: Histological features between intestinal tuberculosis and Crohn’s disease

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Intestinal tuberculosis</th>
<th>Crohn’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granuloma</td>
<td>18 (30%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Caseation</td>
<td>5 (8.4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Non-caseating</td>
<td>13 (21.6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Ulceration of surface epithelium</td>
<td>2 (3.3%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Lymphoid aggregates or follicles</td>
<td>33 (55%)</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>Chronic inflammation</td>
<td>60 (100%)</td>
<td>20 (100%)</td>
</tr>
</tbody>
</table>
was seen in 42 (70%), only distal ileum in 16 (26.6%) and
distal colon in two (3.3%) cases. In Crohn’s disease, 11 (55%)
showed involvement of ileocecal junction, six (30%) distal ileal
disease and in three (15%) distal colonic involvement
was seen. This shows that ileocecal junction and distal ileum
are the common sites for both diseases. None of our patients
had discrete small bowel disease other than distal ileal lesions
contiguous with caecal involvement. The reason for this
distribution of pattern is not clear. Very often small bowel
disease is misdiagnosed as tuberculosis. Extra-intestinal
lesions of tuberculosis were seen involving pleura, lungs,
lymph nodes, peritoneum and a focus in liver and kidney.
Commonest extra-intestinal site for tuberculosis is pulmonary
tuberculosis.27 The relation between abdominal tuberculosis
and pulmonary tuberculosis is not clear. Sub-mucosal or deep
mucosal location of granulomas supports the hypothesis that
intestinal lesions come via lymphatic rather than an external
source.28 Extra-intestinal lesions found in Crohn’s disease
were in the form of pyoderma gangrenosum in one case and
a case of ankylosing spondylitis.

Comparing PCR assay in this study, PCR positivity was
significantly higher in cases of tuberculosis as compared to
Crohn’s disease p<0.05. The only case of Crohn’s disease
positive for PCR but without granuloma did not show any
focus of intestinal tuberculosis elsewhere hence was
considered as false-positive report. Though false-positive
results are reported, the false-positive rate was low in our
case.39,40 Interestingly, nine cases (21.4%) without granuloma
were positive by PCR. This result is very encouraging for
differential diagnosis of two disorders, which did not show
granuloma. Absence of granulomas in these cases may be
due to deep location of granulomas which are not picked up
on mucosal biopsy, which is the frequent observation of
pathologists when resected specimen of the same case is
studied.18 Another possibility of false-positive results in the
application of PCR is generally caused by contamination.
However we had taken strict precautions to avoid
contamination and also these nine cases were diagnosed as
intestinal tuberculosis as per criteria mentioned earlier. The
presence of PCR inhibitors may give false-negative result, a
major problem in application of PCR.41 In this study 47 (78.3%)
cases of TB were PCR negative. To avoid this we have used
primers for human ß-globin gene to assess the efficacy of
amplification reaction and ensure that the specimens were
free of interfering substances. Our results did not show any
inhibitors of amplification in reaction. The false-negative
results in this study might be due to either non-homogenous
distribution of bacteria in the specimen or scarcity of bacilli
in the specimen. We have neither found significant difference
in PCR positivity in cases of tuberculosis with or without
granulomas (P>0.05) nor between caseating and non-
caseating granulomas (P>0.05). These results are similar to
the study from China.42

On histology, serpiginous or longitudinal ulceration of
mucosa, longer stricturous segment (>3 cm), presence of
fissures, transmural inflammation, non-caseating and discrete
granulomas, dilated submucosal lymphatics, prominent
lymphoid follicles and mesenteric lymph nodes without
granulomas are the features of Crohn’s disease. Tuberculosis
is characterized by transverse ulcers in the ileum, ulcero-
hyperplastic lesion at ileocecal junction, short stricturous
segment (<3 cm), presence of serosal tuberculosis, caseating or
non-caseating granulomas or mesenteric lymph nodes
showing similar tubercular granulomas.31 These features are
evident only on surgically resected specimen. British Society
of Gastroenterology has given guidelines for initial biopsy
diagnosis of suspected cases of inflammatory bowel disease
however there are only few reports that compare the
histological changes of intestinal tuberculosis and Crohn’s
disease on mucosal biopsies.5,11,18 Amongst those all of them
have emphasized the diagnostic difficulties in distinguishing
these two conditions. Out of 60 cases of intestinal
tuberculosis, caseating granulomas were seen in five (8.4%)
and non-caseating 13 (21.6%) cases, while in Crohn’s disease
there was not a single case showing granuloma. Incidence of
granuloma in CD ranges from 25 to 60 %,11,18 Since this study
includes only 20 cases of Crohn’s disease, a chance of finding
granuloma on biopsy is also low. Considering other
histological features there was no statistically significant
difference seen between tuberculosis and Crohn’s disease.
Thus differential diagnosis based on histological findings
alone is very difficult.

In conclusion, due to similar clinical, pathological,
radiological and endoscopic findings it is difficult to
differentiate intestinal tuberculosis from Crohn’s disease
in some of the cases. In this study, fever was predominant
symptom in patients of tuberculosis. CT scan revealing ascites
and mesenteric nodes favored diagnosis. Biopsy is of limited
diagnostic value in differentiating these two conditions.
Amplification of mycobacterial tuberculosis DNA by PCR
assay though not found to be highly sensitive but was very
specific for diagnosis of tuberculosis. With increasing
refinement in PCR technique sensitivity of the test can be
increased for the diagnosis of tuberculosis.

Acknowledgement

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helping us in carrying out PCR assay.

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