

Fecal polymerase chain reaction for *Mycobacterium tuberculosis* IS6110 to distinguish Crohn's disease from intestinal tuberculosis

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Abstract

Background We have previously shown that amplification of *Mycobacterium tuberculosis* specific DNA (TB PCR) from feces reliably diagnosed intestinal tuberculosis. This study was undertaken to determine how well this test would distinguish intestinal tuberculosis from Crohn's disease in a country endemic for tuberculosis.

Methods Consecutive patients with diagnoses of Crohn's disease and intestinal tuberculosis were enrolled, and the diagnoses confirmed by follow up. DNA was extracted from fecal samples and subjected to polymerase chain reaction TB PCR for IS6110 sequence which is specific for *M. tuberculosis*.

Results Twenty one of 24 patients with intestinal tuberculosis and 5 of 44 patients with Crohn's disease tested positive by TB PCR. The sensitivity, specificity, positive predictive and negative predictive values for TB PCR in distinguishing tuberculosis from Crohn's disease were 0.79 (95% confidence interval 0.57–0.92), 0.88 (0.75–0.96), 0.79 (0.57–0.92) and 0.88 (0.75–0.96), respectively. A combination of fecal TB PCR with mycobacterial culture of mucosal biopsy specimens identified 23 of 24 (96.2%) of patients with intestinal TB, with sensitivity, specificity, positive predictive and negative predictive values (95% CI)

of 0.95 (0.78–0.99), 0.88 (0.75–0.96), 0.82 (0.63–0.93) and 0.97 (0.86–0.99), respectively.

Conclusion Fecal TB PCR is a good screening test to distinguish intestinal tuberculosis from Crohn's disease.

Keywords India · Inflammatory bowel disease · Tropics

Introduction

Intestinal tuberculosis (ITB) and Crohn's disease (CD) are granulomatous diseases of the bowel that mimic one another. The ability to distinguish between the two is a significant need in tuberculosis-endemic countries where an increasing incidence of CD is set against a background of high prevalence of ITB [1, 2]. Both diseases share common pathogenic and clinical characteristics, which make the differentiation difficult, but at the same time misdiagnosis could result in serious repercussions. Concerns about the side effects of anti-tubercular therapy, and delay in diagnosis of potential CD, preclude advocating a trial of treatment as a diagnostic test. However nearly a half of patients with CD from the developing world are initially misdiagnosed and treated as ITB [2, 3].

Once considered rare in the developing world, the incidence of inflammatory bowel disease including CD is increasing in the Asia Pacific region and India [4]. TB is on the resurgence globally with 8.9 million cases and 1.7 million deaths in 2004 [5]. Clinical presentation, endoscopic features, histology and serological tests like anti-*Saccharomyces cerevisiae* antibody (ASCA) and anti-neutrophilic cytoplasmic antibodies (ANCA) have been used to differentiate CD from ITB [6–10]. Attempts to detect *Mycobacterium tuberculosis* in biopsy specimens have met with variable success, with positive PCR

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noted in 22–75% of ITB patients [11–14], and in up to 5% of patients with CD [11]. A study from India combined both conventional and in-situ PCR methods and found that 40% of patients with ITB and 10% of those with CD were positive by either method [15].

We have shown that fecal PCR, targeting the IS6110 sequence of the *M. tuberculosis* genome, can be useful in diagnosing ITB and active pulmonary tuberculosis where swallowed sputum contributes to mycobacterial DNA in the stool [16]. We undertook the current study to determine whether fecal TB PCR would discriminate between ITB and CD in India.

Methods

Consecutive patients seen in the Department of Gastrointestinal Sciences at Christian Medical College, Vellore over an 18 month period from June 2005–December 2006 with a provisional diagnosis of ITB or CD were requested to provide a sample of stool for the study. Patients who had received treatment for tuberculosis within three months prior to fecal collection were excluded. Patients were investigated following routine clinical protocols and followed up after therapy. The study protocol was approved by the Ethics Committee of the Christian Medical College, Vellore, and informed written consent was obtained from all patients.

The final analysis included only those patients who fit the diagnostic criteria for ITB or CD. For ITB, these included any one of the following: [16, 17] (1) mucosal and/or surgical biopsies with caseating granulomas; (2) intestinal granulomatous inflammation accompanied by extra-intestinal confirmed tuberculosis with caseating granulomatous inflammation and/or acid fast bacilli; (3) endoscopic evidence of ileocecal ulceration, nodularity and/or stenosis, with non-caseating granulomas on biopsy and complete endoscopic resolution after a 9-month course of anti-tuberculous therapy; (4) culture of *M. tuberculosis* from mucosal or surgical biopsy of the intestine. Diagnostic criteria for CD included all the following: [18] (1) bowel symptoms and characteristic endoscopic and/or radiographic features of CD, including small and/or large bowel involvement, and skip lesions (at least two discontinuous discrete segments of ulceration or pseudopolyp formation with intervening normal mucosa); [19] (2) mucosal biopsy showing non-caseating granulomas of typical morphology as described earlier [9], or resection specimen showing transmural inflammation or non-caseating granulomas; (3) clinical and biochemical response to therapy with mesalazine, corticosteroids or immunosuppressive drugs over a two year period of follow up; (4) absence of AFB by histology or culture; and (5) absence of pulmonary infiltrates on chest radiographs.

A single sample of stool was collected from each patient, transported immediately to the laboratory, and stored at -20°C prior to processing. DNA was extracted from 250 mg samples of feces using the QiaAMP Stool DNA minikit (Qiagen, Germany) and the IS6110 sequence was amplified using oligonucleotide primers described earlier [16], along with appropriate positive and negative controls. The test was considered positive if a 123 bp fragment was amplified that was digested by *SalI* with two distinct bands of 66 and 57 bp.

Results

One hundred and four patients were initially recruited for the fecal collection. As shown in Fig. 1, 25 were excluded from the study after the stool sample was collected because the diagnosis was established to be other than ITB or CD. The remainder were initially classified as either ITB ($n=31$) or CD ($n=48$). Five patients with a provisional diagnosis of ITB who did not return for follow up and two who did not respond to anti-tubercular therapy were excluded from analysis. Two patients with CD who did not return for follow up assessment, and two who did not show response to therapy were also excluded (Fig. 1).

Twenty four patients (14 men) with ITB were finally included. They ranged in age from 15 to 57 years with a median age of 26 years. Fourteen of these patients had ileocecal, 4 ileal and 6 colonic disease. A single fecal sample was obtained from each patient at the time of diagnosis of probable granulomatous bowel disease, and prior to initiation of anti-tubercular therapy. In two patients, the diagnosis of tuberculosis was made following surgery either for ileal perforation or for intestinal obstruction due to an ileal stricture; fecal samples in these patients were obtained after recovery from surgery. AFB culture of the mucosal biopsy provided *M. tuberculosis* in 11 (45.8%) of the 24 patients. In the remaining patients, diagnosis of ITB was based on extraintestinal lymph node involvement with caseation necrosis and intestinal involvement in two patients, presence of granulomas with necrosis in three patients and by complete endoscopic resolution after nine months of anti-tubercular chemotherapy in six patients. No patient had active pulmonary infiltrates at the time of initial evaluation.

Forty-four patients (30 men; age 28 [10–63] years) with CD were included in the final analysis. Of these, 20 had colonic disease, 11 had ileocolonic disease, 11 had ileal disease, and two had disease limited to the jejunum. Two patients had fistulizing disease whereas seven had symptomatic strictures. Eight patients underwent surgery for their disease. All patients received aminosalicylates; in addition, 18 received azathioprine or mercaptopurine and 14 received corticosteroids. All these patients showed relief

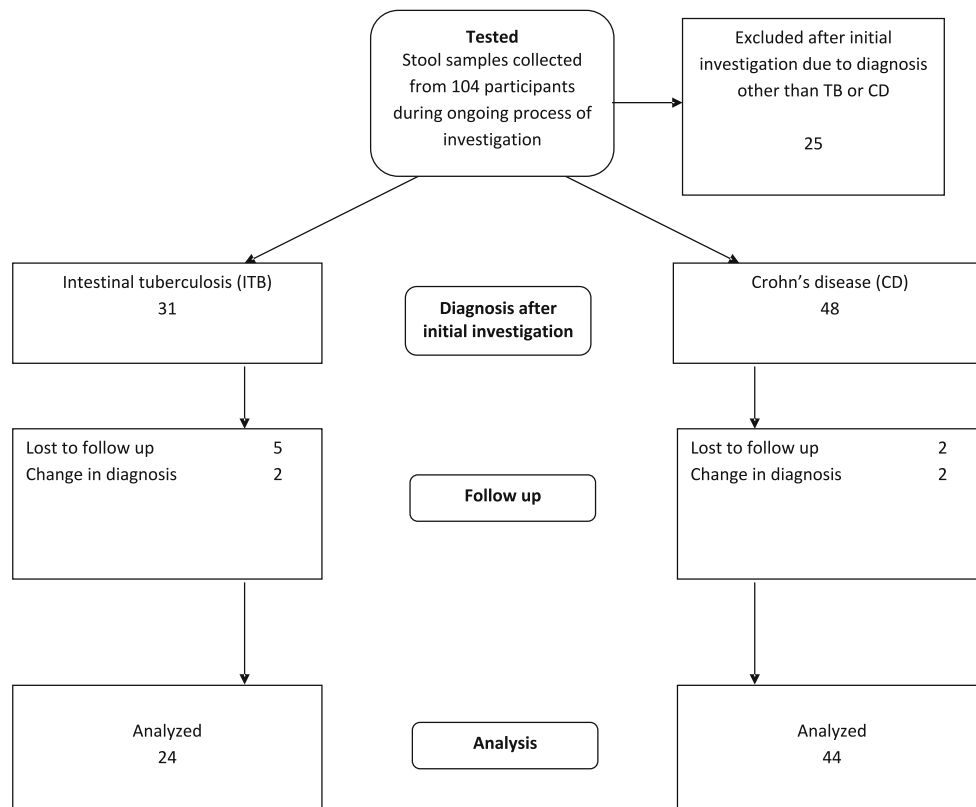


Fig. 1 Flow diagram of patients recruited for the study

of symptoms, reversal of weight loss, and increase in hemoglobin and albumin levels after initiation of therapy.

Table 1 lists the results of fecal PCR and tissue AFB culture in patients with ITB and CD. Nineteen of 24 patients with ITB and five of 44 patients with CD tested positive by PCR. This difference was highly significant ($P < 0.0001$, Fisher's exact test). AFB culture of the biopsy in 4 of 5 PCR negative ITB patients grew single or two colonies of AFB with characteristics of *M. tuberculosis*.

AFB culture of the biopsy was negative in all 5 CD patients with positive fecal TB PCR. All five CD patients with positive TB PCR had received steroids for variable periods of time and were on therapy for more than one year with azathioprine. None of the patients in this study received infliximab or any other biological therapy. Table 1 also shows the sensitivity, specificity, positive predictive value and negative predictive value of culture alone, PCR alone and culture+PCR in the diagnosis of tuberculosis in

Table 1 Outcome of fecal PCR for IS6110 of *M. tuberculosis* in the patient groups

	Intestinal tuberculosis (%)		Crohn's disease (%)	
Total number of patients	24		44	
AFB culture positive	11 (45.8)		0 (0)	
Fecal PCR positive	19 (79.1)		5 (11.3)	
Either AFB culture or fecal PCR positive	23 (95.8)		5 (11.3)	
Test	Sensitivity	Specificity	PPV	NPV
AFB culture	0.20 (0.07–0.42)	1.00 (0.91–1.00)	1.00 (0.47–1.00)	0.69 (0.56–0.80)
Fecal TB PCR	0.79 (0.57–0.92)	0.88 (0.75–0.96)	0.79 (0.57–0.92)	0.88 (0.75–0.96)
AFB culture+Fecal TB PCR	0.95 (0.78–0.99)	0.88 (0.75–0.96)	0.82 (0.63–0.93)	0.97 (0.86–0.99)

95% confidence intervals shown in brackets for sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV)

comparison to Crohn's disease. The combination of culture + PCR performed very well with 82% positive predictive value and 97% negative predictive value.

Discussion

PCR is increasingly used in the diagnosis of TB. For pulmonary TB, PCR confirmed or excluded the diagnosis in 48 hours compared to culture, which took 2 to 8 weeks [20]. PCR of mucosal biopsy specimens is commonly used to diagnose ileocolonic TB [11–14]. Unlike endoscopic biopsies, fecal PCR is non-invasive. It is also less subject to sampling errors than endoscopic biopsies as it can theoretically detect mycobacterial DNA shed from anywhere along the length of the gastrointestinal tract. The present study shows that fecal PCR combined with AFB culture is a good test to distinguish between intestinal tuberculosis and Crohn's disease, with a sensitivity of 95%, and specificity of 88%.

The primers used for the PCR detect a specific insertion sequence (IS6110) that is very specific for *Mycobacterium tuberculosis*, not being found in other mycobacteria or other organisms [16]. Therefore its detection confers great specificity. However, sensitivity using this PCR alone was only 79%. The negative PCR in approximately 20% of patients with intestinal tuberculosis thus remains a matter of concern. In four of the five patients with a negative TB PCR, the organism was grown from biopsy culture. Multiple copies of the IS6110 sequence are normally considered to be present in *M. tuberculosis* making it an attractive target sequence for PCR-based diagnosis [21]. However, in a recent study, 11% of mycobacterial isolates from different parts of India lacked the IS6110 element [22]. Future fecal PCR testing for ITB may therefore have to include a second gene specific for *M. tuberculosis* to cover the IS6110 negative strains.

The positive TB PCR in 5 of 44 patients with CD poses another problem. The IS6110 sequence is specific to *M. tuberculosis* and is not found in common non-tuberculous mycobacteria or other organisms [16]. The presence of *M. tuberculosis* may thus be an epiphenomenon, owing to its endemicity in the general population and incidental entry into the inflamed bowel from contaminated food or fomites. Two studies from India that evaluated *M. tuberculosis* PCR of mucosal biopsies reported PCR positivity in 5% of patients with CD [11, 15]. A third recent study from India reported a positive PCR of mucosal biopsy in 67.9% of patients with ITB [14]. In that study, the PCR targeted the mpt64 gene that is present in several mycobacteria other than *M. tuberculosis*. Although that study also examined patients with CD, the authors did not report whether any of the patients with CD had a positive PCR. In a study from

China, none of the patients with CD had a positive TB PCR in colonic biopsies, whereas in a study from Taiwan, 18% of resected intestine specimens from patients with CD were positive for TB PCR [13, 23]. It is well known that patients with CD may harbor latent tuberculosis infection, which may be unmasked on therapy with infliximab [24, 25]. Infliximab reduces the number of *M. tuberculosis*-reactive CD8⁺CCR7⁻CD45RA⁺ effector memory T cells (TEMRA cells) and triggers a reactivation of tuberculosis [26]. The use of infliximab in patients with CD is associated with a black box warning to exclude tuberculosis prior to infliximab use [27], and tuberculin skin tests and the interferon-gamma release assays are used in this context. However, while the latter tests perform well for diagnosis of latent tuberculosis in healthy individuals, they are not adequate to diagnose latent tuberculosis infection in patients with CD and immune-mediated inflammatory diseases [28, 29]. Under such circumstances, it is possible that fecal TB PCR can be used as an additional modality to diagnose latent tuberculosis infection in patients with CD.

In summary, fecal TB PCR for IS6110 sequence appears to be a good screening test to distinguish ITB from CD in a population endemic for tuberculosis with a rising incidence of CD. Its sensitivity may possibly be increased by adding a second PCR for additional sequences unique to *M. tuberculosis*. Its potential utility in identifying patients needing tuberculosis chemoprophylaxis prior to initiating treatment with biological agents needs to be examined.

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Competing Interest None to declare.

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