PCR Amplification of the IS*6110* Insertion Element of *Mycobacterium tuberculosis* in Fecal Samples from Patients with Intestinal Tuberculosis

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PCR amplification of insertion element IS*6110* of *Mycobacterium tuberculosis* in fecal samples was evaluated in the diagnosis of intestinal tuberculosis (ITB). The numbers of samples that tested positive by PCR with SalI digestion were 16/18 untreated-ITB samples, 0/8 treated-ITB samples, 12/14 smear-positive pulmonary tuberculosis samples, and 0/30 control samples. The sensitivity, specificity, positive predictive value, and negative predictive value of fecal PCR were 88.8%, 100%, 100%, and 93.7%, respectively.

Tuberculosis (TB) infection affects a third of the world's population (4). Gastrointestinal TB mimics many conditions, including inflammatory bowel disease, malignancy, and infectious diarrhea, and is often difficult to diagnose (7, 17). With colonic disease, endoscopy and biopsy may confirm the diagnosis (13, 16), while surgical biopsy is necessary with disease confined to the small intestine. By use of mucosal biopsies, *Mycobacterium tuberculosis* is demonstrated by culture to occur in one-third (2) and by PCR in two-thirds (6) of patients with colonic TB. In countries with a high incidence of TB, gastrointestinal tuberculosis is often diagnosed and treated on empirical grounds and confirmed by resolution following therapy. This study evaluated the ability of PCR of feces to amplify the IS*6110* insertion element of *M. tuberculosis* in the diagnosis of gastrointestinal TB.

Consecutive patients with intestinal tuberculosis seen in the Department of Gastrointestinal Sciences between April 2003 and January 2005 were included. The diagnosis was made on the basis of one of the following criteria: (i) intestinal biopsies showing caseating granulomas, (ii) intestinal granulomas accompanied by confirmed extraintestinal tuberculosis, (iii) endoscopic findings of TB with noncaseating granulomas on biopsy and complete resolution after antituberculosis therapy, or (iv) culture of *M. tuberculosis* from intestinal biopsy specimens. All patients were negative for acid-fast bacilli in induced-sputum specimens. Twenty-four subjects with irritable bowel syndrome (all with normal hematology and colonoscopy) undergoing colonoscopy during this period and six healthy adult volunteers served as controls. Fourteen patients with pulmonary TB and acid-fast bacilli on Ziehl-Neelsen stain of sputum smears were recruited from the DOTS Clinic of Christian Medical College, Vellore, India. Eight patients with intestinal tuberculosis who completed 9 months of antituberculosis therapy and were clinically symptom free with normal hemoglobin, sedimentation rate, serum albumin, and globulin were in-

cluded. The protocol and consent forms were approved by the Research Committee of the Christian Medical College, Vellore, India.

Fresh feces were collected after consent and stored at −20°C. Feces (250 mg) were homogenized in 2 ml sterile phosphate-buffered saline and heated at 90°C for 15 min. After centrifugation (500 × *g*, 3 min), the supernatant was mixed with lysozyme (10 mg/ml) (Genei, India) and incubated at 37°C for 1 h; then, proteinase K (Genei, India) (30 mg/ml) and 20% sodium dodecyl sulfate (Sigma, India) were added, and the mixture was incubated at 55°C for 2 h. Following centrifugation (9,000 × *g*, 10 min) nucleic acid was extracted from the supernatant by using equal volumes of equilibrated phenol (pH 8.0; Ambion) and chloroform-isoamyl alcohol (25:24:1). DNA was precipitated with ethanol and 2.5 M ammonium acetate (2.0:0.1, −20°C, 1 h), pelleted (18,000 × *g*, 20 min), washed with 70% ethanol, air dried, resuspended in 50 μl sterile water, and stored at −20°C (12). A positive control was prepared from killed culture of *Mycobacterium tuberculosis* strain H37Rv. The primers used (MTB1, 5′ CCT GCG AGC GTA GGC GTC GG 3′, and MTB2, 5′ CTC GTC CAG CGC CGC TTC GG 3′) amplified a 123-bp fragment of IS*6110* (5), which is specific for *M. tuberculosis* and contains an internal endonuclease site that allows confirmation of the product by digestion with SalI. PCR amplification in 10 μl used titanium *Taq* polymerase (BD Biosciences Clontech) and 10 pmol of each primer (Sigma, Bangalore, India). The final PCR conditions consisted of an initial denaturation at 95°C for 30 s, followed by 40 cycles of denaturation (94°C, 30 s), annealing (68°C, 30 s), and extension (72°C, 30 s), with a final extension for 10 min. The PCR product was subjected to electrophoresis on 2% agarose, stained with ethidium bromide, and imaged (Bio-Rad). The PCR products were digested with 10 U/μl of SalI (MBI Fermentas) at 37°C overnight, followed by electrophoresis on 3% agarose. Specificity of the primers was tested by sequence searches against prokaryotic DNA databases, in silico PCRs, and PCR using DNA from atypical mycobacteria—*M. avium*, *M. kansasii*, and *M. fortuitum*. Serial 10-fold dilutions of *M. tuberculosis* H37Rv (3) were added to PCR-negative fecal samples to establish the detection limit of PCR.

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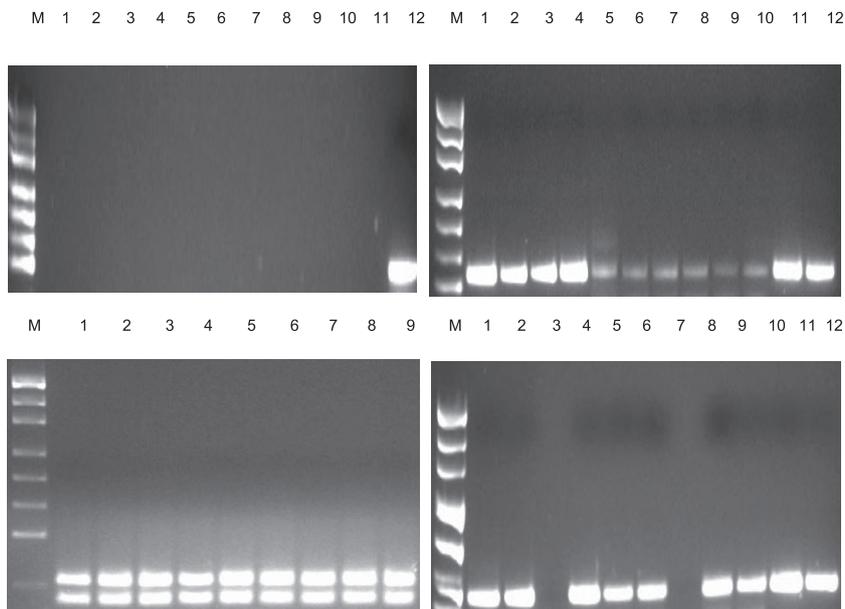
FIG. 1. Electrophoresis of PCR products on a 2% agarose gel (top panels and bottom right panel) or PCR product digest on a 3% agarose gel (bottom left panel). In each panel, lane M is the molecular weight marker. (Top left) Lanes 1 to 11 are DNA isolated from feces of control subjects. Lane 12 shows amplification of a 123-bp fragment from the positive-control DNA. (Bottom left) Digested PCR products, showing 66- and 57-bp fragments. Lanes 1 to 6, results from intestinal TB patients; lanes 7 and 8, results from pulmonary TB patients. Lane 9 is the positive control. (Top right) Lanes 1 to 11 show results from various intestinal TB patients, while lane 12 is a positive control. (Bottom right) Lanes 1 to 11 show results from patients with smear-positive pulmonary TB, while lane 12 shows the positive control.

All samples that tested positive were reconfirmed using two additional DNA extracts.

PCR using *M. tuberculosis* H37Rv, but not atypical mycobacteria, produced a 123-bp amplicon (Fig. 1, top left). With serial dilutions, PCR was able to detect >10 copies of *M. tuberculosis*. SalI digestion of the PCR product from positive cases and the positive control produced two bands, one of 66 bp and one of 57 bp, respectively (Fig. 1, bottom left). The specific PCR product was noted for 16 of 18 patients with untreated intestinal tuberculosis (Fig. 1, top right). Samples were negative by PCR for all 30 control subjects (Fig. 1, top left) and all eight treated gastrointestinal TB patients, five of whom were tested prior to therapy and were positive and three of whom were not tested prior to therapy. Twelve of 14 patients with smear-positive pulmonary tuberculosis tested positive (Fig. 1, bottom right), indicating fecal excretion of swallowed organisms. The sensitivity of fecal PCR for the diagnosis of intestinal TB was 88.8%, and the specificity was 100% (Table 1). In comparison to results with fecal PCR, 9/18 and 6/18 patients with intestinal TB tested positive by histopathology and culture, indicating sensitivities of 50% and 33.3%, respectively.

PCR is used increasingly in the diagnosis of tuberculosis (1). For smear-positive pulmonary TB, PCR confirms or excludes the diagnosis in 48 h, compared to culture, which takes 2 to 8 weeks (8). PCR of mucosal biopsy specimens diagnoses colonic TB in 45 to 64% of cases (6, 9). Unlike endoscopic biopsy, fecal PCR is noninvasive, and it is also less subject to sampling error. Stool PCR for atypical mycobacteria has been used for patients with human immunodeficiency virus (10). The IS*6110* sequence is present in most clinical isolates of *M. tuberculosis*;

however, 4/378 (1%) isolates and 19/80 (24%) isolates in two studies from southern India showed an absence of even a single copy of IS*6110* (11, 14). As with intestinal TB samples, untreated-pulmonary-TB samples were positive by fecal PCR, likely due to swallowed *M. tuberculosis*. Gastric lavage is used widely to diagnose infection in adults and children with pulmonary TB who do not expectorate sputum (15, 18). This study suggests that fecal PCR will achieve the same end. Fecal PCR for the IS*6110* sequence is thus an attractive test for the diagnosis and follow-up of intestinal TB. Its utility in distinguishing patients with Crohn's disease from those with TB and in identifying patients with active pulmonary TB needs further study.

TABLE 1. Outcome of fecal PCR for IS*6110* of *M. tuberculosis* in the various groups studied

Parameter	Value for group			
	Intestinal TB	Control	Sputum-positive pulmonary TB	Treated intestinal TB
Fecal PCR positive (no. of samples)	16	0	12	0
Fecal PCR negative (no. of samples)	2	30	2	8
Sensitivity (%)	88.8	88.8		
Specificity (%)	100	100		
Negative predictive value (%)	93.7	93.7		
Positive predictive value (%)	100	100		

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