

Histopathology and TB-PCR kit analysis in differentiating the diagnosis of intestinal tuberculosis and Crohn's disease

Xian Ji Jin, Joon Mee Kim, Hyung Kil Kim, Lucia Kim, Suk Jin Choi, In Suh Park, Jee Young Han, Young Chae Chu, Ju Young Song, Kye Sook Kwon, Eun Joo Kim

Xian Ji Jin, Department of Pathology, Inha University Graduate School of Medicine, 7-206 Shinheung-Dong 3 Ga, Joong-Gu, Incheon 400-711, South Korea

Joon Mee Kim, Lucia Kim, Suk Jin Choi, In Suh Park, Jee Young Han, Young Chae Chu, Ju Young Song, Department of Pathology, College of Medicine Inha University, 7-206 Shinheung-Dong 3 Ga, Joong-Gu, Incheon 400-711, South Korea

Hyung Kil Kim, Kye Sook Kwon, Gastroenterology College of Medicine, Inha University, 7-206 Shinheung-Dong 3 Ga, Joong-Gu, Incheon 400-711, South Korea

Eun Joo Kim, Department of Nano and Bio Research, Daegu Gyeongbuk Institute of Science and Technology, Deocksandong 110, Daegu 700-742, South Korea

Author contributions: Kim JM designed the research; Jin XJ and Kim EJ performed the research; Kim HK and Kwon KS collected the clinical data; Kim L, Choi SJ, Park IS, Han JY, Chu YC and Song JY collected and analyzed pathologic data; Jin XJ wrote the paper.

Supported by Inha University Research Grant and Korean Food and Drug Administration

Correspondence to: Joon Mee Kim, MD, PhD, Professor, Department of Pathology, College of Medicine Inha University, 7-206 Shinheung-Dong 3 Ga, Joong-Gu, Incheon 400-711, South Korea. jmkpath@inha.ac.kr

Telephone: +82-32-8903983 Fax: +82-32-8903464

Received: January 27, 2010 Revised: March 19, 2010

Accepted: March 26, 2010

Published online: May 28, 2010

Abstract

AIM: To compare the histopathologic features of intestinal tuberculosis (ITB) and Crohn's disease (CD) and to identify whether polymerase chain reaction for *Mycobacterium tuberculosis* (TB-PCR) would be helpful for differential diagnosis between ITB and CD.

METHODS: We selected 97 patients with established diagnoses (55 cases of ITB and 42 cases of CD) who underwent colonoscopic biopsies. Microscopic features

of ITB and CD were reviewed, and eight pathologic parameters were evaluated. Nine cases of acid fast bacilli culture-positive specimens and 10 normal colonic tissue specimens were evaluated as the positive and negative control of the TB-PCR test, respectively. PCR assays were done using two commercial kits: kit <A> detected *IS6110* and *MPB64*, and kit detected *IS6110* only; a manual in-house PCR method was also performed on formalin-fixed, paraffin-embedded colonoscopic biopsy specimens.

RESULTS: Statistically significant differences were noted between ITB and CD with regard histopathologic criteria: size of granulomas ($P = 0.000$), giant cells ($P = 0.015$), caseation necrosis ($P = 0.003$), confluent granulomas ($P = 0.001$), discrete granulomas ($P = 0.000$), and granulomas with lymphoid cuffs ($P = 0.037$). However, 29 cases (52.7%) of ITB showed less than five kinds of pathologic parameters, resulting in confusion with CD. The sensitivities and specificities of the TB-PCR test by kit <A>, kit , and the in-house PCR method were 88.9% and 100%, 88.9% and 100%, and 66.7% and 100% in positive and negative controls, respectively. The PCR test done on endoscopic biopsy specimens of ITB and CD were significantly different with kit <A> ($P = 0.000$) and kit ($P = 0.000$). The sensitivities and specificities of TB-PCR were 45.5% and 88.1%, 36.4% and 100%, and 5.8% and 100%, for kit <A> and kit and in-house PCR method on endoscopic biopsy specimens. Among the 29 cases of histopathologically confusing CD, 10 cases assayed using kit <A> and 6 cases assayed using kit were TB-PCR positive. A combination of histologic findings and TB-PCR testing led to an increase of diagnostic sensitivity and the increase (from 47.3% to 58.2) was statistically significant with kit ($P = 0.000$).

CONCLUSION: The TB-PCR test combined with histopathologic factors appears to be a helpful technique in

formulating the differential diagnosis of ITB and CD in endoscopic biopsy samples.

© 2010 Baishideng. All rights reserved.

Key words: Intestine; Tuberculosis; Crohn's disease; Histopathology; Polymerase chain reaction; Kit; *IS6110*; *MPB64*

Peer reviewer: Lisa J Herrinton, PhD, Division of Research, Kaiser Permanente, 2000 Broadway Avenue, Oakland, CA 94612, United States

Jin XJ, Kim JM, Kim HK, Kim L, Choi SJ, Park IS, Han JY, Chu YC, Song JY, Kwon KS, Kim EJ. Histopathology and TB-PCR kit analysis in differentiating the diagnosis of intestinal tuberculosis and Crohn's disease. *World J Gastroenterol* 2010; 16(20): 2496-2503 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v16/i20/2496.htm> DOI: <http://dx.doi.org/10.3748/wjg.v16.i20.2496>

INTRODUCTION

Tuberculosis (TB) is one of the most prevalent diseases in the world and a cause of significant mortality and morbidity in developing countries^[1]. In Korea, the total number of newly developed TB patients was 19 652 in 2000, but the number was markedly increased to 34 123 in 2001 and 34 157 in 2008^[2]. The resurgence of TB is, in part, due to the pandemic of human immunodeficiency virus infection^[1,3,4]. The incidence of intestinal TB (ITB), one of the most common forms of extra-pulmonary TB, has been increasing in parallel with the overall resurgence of TB. Sometimes the diagnosis of ITB is very challenging to make, because of a close resemblance to Crohn's disease (CD) in clinical, radiological, endoscopic, and histological appearance^[5,6]. This is especially true in Asia, where the incidence and prevalence of CD are also rising^[7]. For this reason, definitive diagnosis of ITB and CD is increasingly important. In the case of ITB misdiagnosis, unnecessary anti-tuberculosis therapy poses a risk of toxicity and delays the treatment of CD. Conversely, treatment of ITB with steroids alone can lead to severe deterioration, or even death.

Pathologic features such as size and number of granulomas, giant cells, caseation necrosis, confluent granulomas, discrete granulomas, ulcers with bands of epithelioid histiocytes and granulomas with lymphoid cuffs are known to be important criteria for differentiating ITB and CD^[8,9]. Several molecular techniques have been introduced in addition to histopathologic criteria for the detection of mycobacteria in histopathological specimens and for differentiating these two conditions^[10-12]. Studies have shown that detection of the *IS6110* gene in *Mycobacterium tuberculosis* (*M. tuberculosis*) through an in-house method has high sensitivity and specificity^[13,14], but the results are still controversial. Recently, many kinds

of commercial kits have been developed using different targets to detect the *M. tuberculosis* genome, but their diagnostic accuracy has not been sufficiently examined in the literature. In this study, we compared the in-house PCR test, which detects the *IS6110* gene sequence, with two commercially available kits (represented as <A> and) that have been used widely in Korea. Kit <A> detects both the *MPB64* and *IS6110* genes simultaneously, and kit detects the *IS6110* gene sequence only. We reviewed the histopathological features of ITB and CD and performed PCR for *M. tuberculosis* (TB-PCR) in the endoscopic biopsy specimens to evaluate whether the histopathologic features and ITB-PCR would improve the differential diagnosis of these two diseases.

MATERIALS AND METHODS

Patient selection

We reviewed the records of patients who underwent endoscopic biopsies at Inha University Hospital, Incheon, South Korea, between 1996 and 2007 and determined their ITB and CD status. Before the study, an ethical committee approved the study protocol. A total of 334 cases which were pathologically suspicious for chronic colitis were selected. All the clinical data and hematoxylin- and eosin-stained sections were retrieved and reviewed. The definitive diagnosis of ITB or CD was based on clinical symptoms, colonoscopic findings, radiological investigation, pathological evaluation, response to antituberculous treatment, and follow-up data for more than 3 mo. A diagnosis of ITB was made when at least two of the following criteria were met: (1) demonstration of acid-fast bacilli on histologic examination of Ziehl-Neelsen stained sections; (2) positive *M. tuberculosis* culture; (3) radiologic, colonoscopic, and/or operative evidence of ITB with proven tuberculosis elsewhere; and (4) response to antituberculous therapy without subsequent recurrence in patients with radiologic, colonoscopic, and/or operative evidence of ITB. A diagnosis of CD was made when at least two of the following criteria were met: (1) clinical history of abdominal pain, malaise, diarrhea, and/or rectal bleeding; (2) endoscopic findings of mucosal cobblestoning, linear ulceration, skip areas, or perianal disease; (3) presence of entero-cutaneous or entero-enteric fistulae and/or chronic perianal disease; and (4) resolution of symptoms and morphological (endoscopic and histological) features after corticosteroid and 5-ASA therapy. Ultimately, a total of 55 cases of ITB and 42 cases of CD were selected and the endoscopic biopsy specimens before medical therapy were used for this study.

Histopathological examination

Retrieved hematoxylin- and eosin-stained sections from ITB and CD patients were reviewed by two pathologists (Jin XJ and Kim JM). They evaluated the eight histopathologic parameters suggested by Pulimood *et al*^[8,9]: size

of granuloma (small: < 200 μm , medium: 200-400 μm , large: > 400 μm), numbers of granulomas per biopsy piece, Langerhans giant cells, caseation necrosis (acellular pink areas of necrosis with karyorrhectic debris), confluent granulomas (merging of the adjacent boundaries of granulomas), discrete granulomas (well defined, majority of epithelioid histiocytes conforming to the shape of the granuloma), ulcers with bands of epithelioid histiocytes (conglomerate bands of epithelioid histiocytes lining the ulcer or ulcer slough), and perigranulomatous lymphoid cuffs (lymphoid aggregates surrounding granuloma). We divided the cases into four groups according to histopathologic parameters: group I, showing caseation necrosis; group II, showing more than four kinds of histopathologic parameters without caseation necrosis; group III showing one to four kinds of histopathologic parameters without caseation necrosis; group IV, without any histopathologic parameters.

Positive and negative control samples for TB-PCR

Nine acid fast bacilli (AFB) culture-positive samples (3 from colon, 3 from bone, 2 from vertebrae, and 1 from a joint) and ten normal colonic mucosa samples obtained from disease-free resection margins of colectomy specimens resected due to colon cancer were used as the positive and negative controls for the TB-PCR test. All samples were open biopsy or resection specimens, enough in sample size and formalin-fixed and paraffin-embedded. DNA extraction and PCR tests were done according to the manual in-house PCR method and instructions of kits <A> and , respectively.

Polymerase chain reaction

DNA extraction: The paraffin-embedded blocks for all samples used in this study were cut at 50 μm thickness and placed in a microcentrifuge tube. For the clinical sample, we used only one block containing one to eight pieces of intestinal mucosa revealing the most representative features of the disease. To prevent carry-over of DNA contamination, the microtome blade was changed after cutting each sample. For deparaffinizing, we added 1 mL xylene, vortexed vigorously, and centrifuged at 14000 r/min for 3 min at room temperature. The pellet was then washed with 100% ethanol twice and air-dried for 15 min at 37°C. Genomic DNA was extracted according to the manufacturer's protocol.

DNA amplification by PCR and detection: For each commercial kit, PCR was accomplished according to the manufacturer's protocols. Briefly, kit <A> was designed to detect the *IS6110* and *MPB64* genes of *M. tuberculosis* simultaneously. The first round of PCR was performed using 3 μL of genomic DNA in a total volume of 20 μL , in the presence of 1 \times TB2 first primer, 3 μL of 8-MOP solution, and 1 \times multiple master mix. PCR conditions included 35 cycles of 30 s at 95°C, 30 s at 60°C, and 30 s at 72°C. Nested PCR was performed using 3 μL of the first amplification mixture as the template in the same

composition of PCR mixture without primer (TB2 second primer instead of TB2 first primer). PCR conditions included 25 cycles of 30 s at 95°C, 30 s at 62°C, and 30 s at 72°C. PCR product size was 520 base pairs (bp) for the internal control, 260 bp for the first PCR product, and 200 bp for the second PCR product. An internal control was used as a standard for success or failure. Kit was designed to detect only the *IS6110* region of *M. tuberculosis*. The first round of PCR was performed using 4.5 μL of genomic DNA with 15.5 μL of first PCR master mix. PCR conditions included 35 cycles of 45 s at 95°C, 45 s at 68°C, and 30 s at 72°C. Nested PCR was performed using 1.5 μL of the first amplification mixture as the template with second PCR master mix. PCR conditions included 25 cycles of 45 s at 95°C, 45 s at 65°C, and 30 s at 72°C. PCR product size was 374 bp for the first PCR product and 158 bp for the second PCR product.

The in-house PCR method used in this study was designed to detect the *IS6110* region of *M. tuberculosis*. The first round of PCR was performed using 5 μL of genomic DNA in a total volume of 25 μL in the presence of 1 \times Ex *Taq* buffer (10 mmol/L Tris-HCl, 50 mmol/L KCl, 2 mmol/L MgCl₂), 2 U Ex *Taq* DNA polymerase (TAKARA, Japan), and 10 pmol of each primer (KBN-1: 5'-GGATGGTTCGCAGAGATCCBGC-3', KBN-2: 5'-CGATGCCCTCACGGTTCAGG-3'). PCR conditions were as follows: 35 cycles of 30 s at 95°C, 1 min at 60°C, and 1 min at 72°C, followed by a final extension step of 10 min at 72°C. For the second round of PCR, 1 μL of the first amplification mixture was used as the template in a total volume of 25 μL containing 10 pmol of each primer (KBN-3: 5'-GTCAGACGATTCGGAGTGG-3', KBN-4: 5'-TGATGATCGGCCGATGAACCG-3'). PCR conditions were as follows: 35 cycles of 30 s at 95°C, 1 min at 60°C, and 1 min at 72°C, followed by a final extension step of 10 min at 72°C. PCR product size was 297 bp and was analyzed by gel electrophoresis on 2% agarose gels stained with ethidium bromide.

Statistical analysis

We used the R version 2.9.2 (A free software environment for statistical computing and graphics, Copyright 2009 The R Foundation for Statistical Computing, ISBN 3-900051-07-0) for analyzing the histopathologic features and PCR results by χ^2 test and Fisher's exact test and for analyzing the increase of diagnostic sensitivity by Logistic regression analysis. *P* value less than 0.05 was considered statistically significant. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated.

RESULTS

Clinicopathologic findings of ITB and CD

Out of a total of 97 cases, 55 cases were ITB, and 42 cases were CD. The mean ages of the ITB and CD patients were 39 and 27 years, respectively. The most common site of involvement for ITB was the right colon

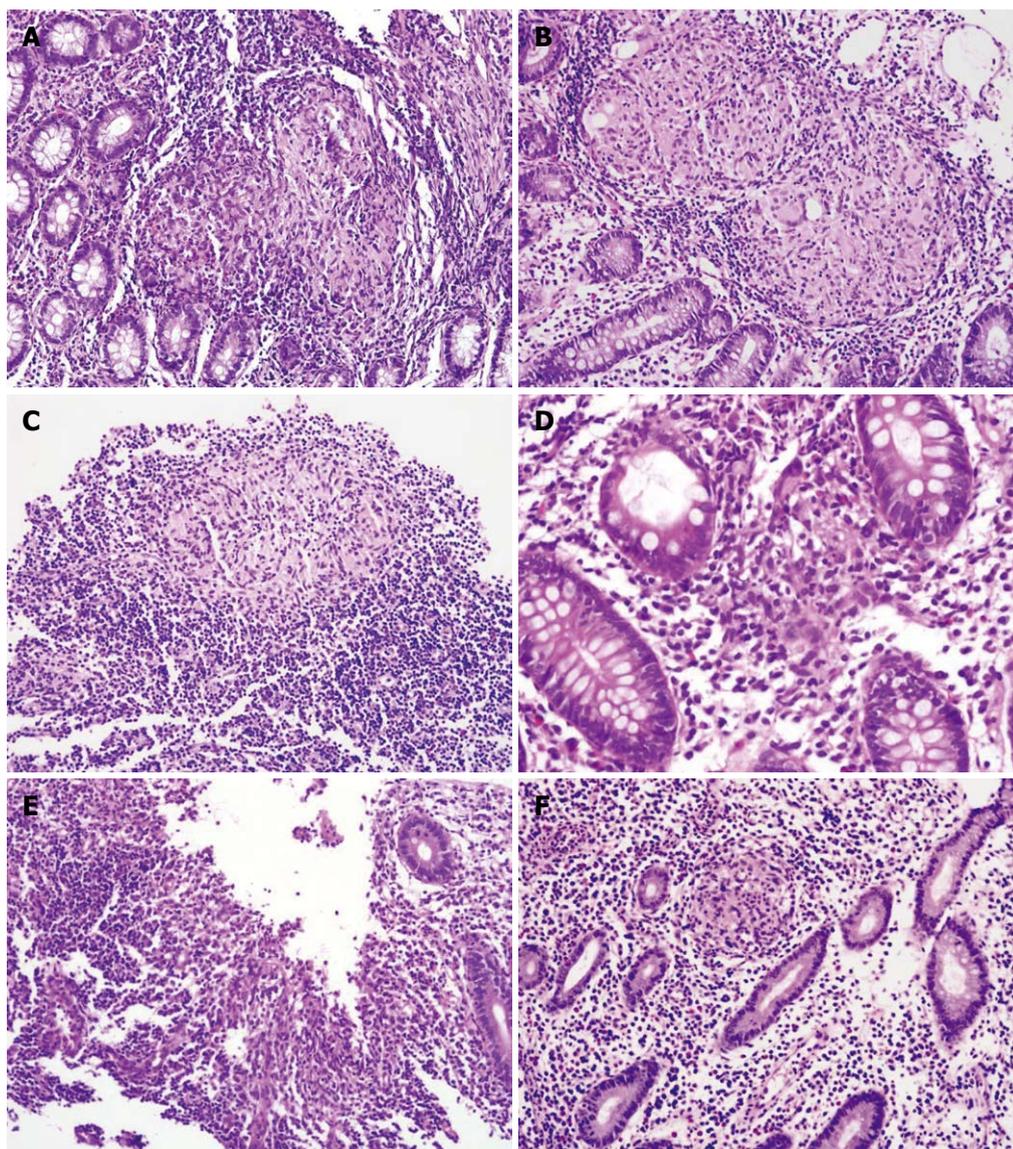


Figure 1 Histopathological parameters of intestinal tuberculosis (ITB) and Crohn's disease (CD). A: Confluent granuloma in ITB; B: Confluent granuloma with caseation necrosis and Langhans giant cells in ITB; C: Granuloma with lymphoid cuff in ITB; D: Vague granuloma in CD; E: Band of epithelioid histiocytes in ulcer base in ITB; F: Small granuloma in CD.

Table 1 Disease location of ITB and CD *n* (%)

Involving site	ITB	CD
Right colon	32 (58.2)	14 (33.3)
Left colon and rectum	4 (7.3)	4 (9.5)
Skip lesion or pancolitis	19 (34.6)	24 (57.1)

ITB: Intestinal tuberculosis; CD: Crohn's disease.

(58.2%), but many of the CD patients showed skip lesion or pancolitis (50.0%) (Table 1).

A summary of the histopathologic features is given in Figure 1 and Table 2. Granulomatous inflammation was seen in 52 cases (94.5%) of ITB and 25 cases (59.5%) of CD. The size and number of granulomas were larger in ITB than in CD ($P = 0.000$). Giant cells ($P = 0.015$), caseation necrosis ($P = 0.003$), confluent granulomas ($P =$

0.001), discrete granulomas ($P = 0.000$), and granuloma with lymphoid cuff ($P = 0.037$) were more frequently associated with ITB. Therefore, these histopathologic features were helpful in differentiating ITB and CD.

When all cases of ITB and CD were divided into four groups according to histopathologic parameters, almost all cases of group I and II were ITB patients except for one CD patient in group II. However, more than half of the ITB patients (52.7%) and nearly all of the CD patients (98%) were in group III or group IV (Table 3). Hence, groups I and II could be combined to form an "ITB group" although there was one case of CD. Groups III and IV could be combined to form a "confusing group" because differential diagnosis between ITB and CD was difficult based on histopathologic parameters. According to this two-tiered classification, diagnostic sensitivity, specificity, PPV, and NPV were 47.3%, 98%, 96.3%, and 58.6%, respectively.

Table 2 Comparison of histopathologic parameters between ITB and CD *n* (%)

Histologic parameters	Cases		<i>P</i> value
	ITB (<i>n</i> = 52)	CD (<i>n</i> = 26)	
Size of granuloma			0.000
Small (< 200 μm)	6 (11.5)	13 (50.0)	
Medium (200-400 μm)	7 (13.5)	7 (26.9)	
Large (> 400 μm)	39 (75.0)	5 (19.2)	
Granuloma/piece > 1 ¹	24 (46.2)	9 (34.6)	0.331
Giant cells	25 (45.5)	5 (19.2)	0.015
Caseation necrosis	14 (26.9)	0 (0.0)	0.003
Confluent granuloma	24 (46.2)	2 (7.8)	0.001
Discrete granuloma	30 (57.7)	3 (11.5)	0.000
Lymphoid cuff	31 (59.6)	9 (34.6)	0.037
Ulcer with histiocytes ²	17 (30.9)	3 (11.5)	0.056

¹More than one granuloma per biopsy piece; ²Ulcer with band of epithelioid histiocytes.

Table 3 Classification of ITB and CD according to histopathologic parameters *n* (%)

Group	ITB (<i>n</i> = 55)	CD (<i>n</i> = 42)	Re-classification of ITB (<i>n</i> = 55)
I	14 (25.5)	0 (0.0)	ITB group: 26 (47.3)
II	12 (21.8)	1 (2.3)	
III	23 (41.8)	15 (35.7)	Confusing group: 29 (52.7)
IV	6 (10.9)	26 (61.9)	

Group I showing caseation necrosis; Group II showing more than four kinds of histopathologic parameters without caseation necrosis; Group III showing one to four kinds of histopathologic parameters without caseation necrosis; Group IV without any histopathologic parameters.

TB-PCR in positive and negative control

Among the 9 AFB culture-positive samples, 8 cases were identically TB-PCR positive based on analyses done with kit <A> and kit . However, only 6 cases were TB-PCR positive by the in-house PCR method. Ten normal samples were all negative on three kinds of TB-PCR tests (Table 4).

TB-PCR in ITB and CD

When using kit <A>, 25 samples among 55 ITB cases were PCR positive (sensitivity 45.5%). In contrast, 37 samples among 42 CD cases were PCR negative (specificity 88.1%). When kit was used, 20 samples were PCR positive among the ITB cases (sensitivity 36.4%), and all samples were PCR negative among the CD cases (specificity 100%). Both PCR tests were significantly different between ITB and CD (*P* = 0.000). Figure 2 demonstrates positive bands and negative results of PCR tests using kits <A> and . The sensitivity of the in-house PCR method (5.5%) was much lower than that of either kit (Table 5).

Diagnostic value of TB-PCR in combination with histopathologic parameters

Among the ITB patients, group I showed the highest TB-PCR positivity, which gradually decreased in groups

Table 4 Results of TB-PCR in gold standard

	Positive control (<i>n</i> = 9)	Negative control (<i>n</i> = 10)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Kit <A>	8	10	88.9	100	100	90.9
Kit 	8	10	88.9	100	100	90.9
In-house	6	10	66.7	100	100	71.4

TB-PCR: Polymerase chain reaction for *Mycobacterium tuberculosis*; PPV: Positive predictive value; NPV: Negative predictive value.

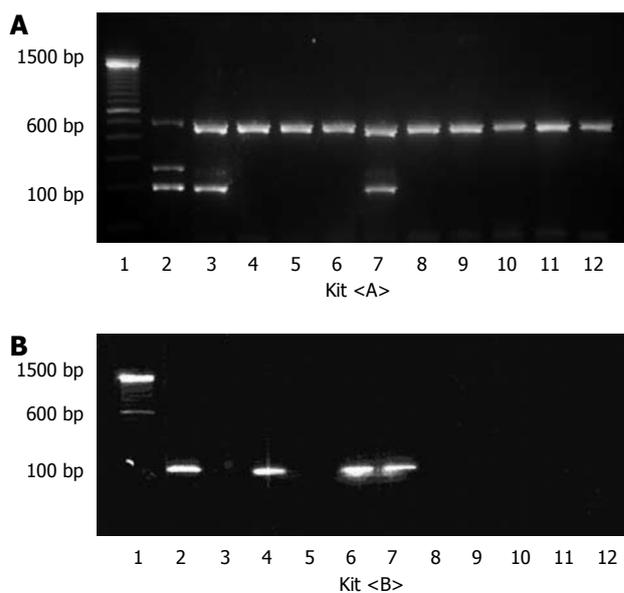


Figure 2 Polymerase chain reaction (PCR) product by kit <A> and kit . A: Number 3 and 7 showing positive result by kit <A>; **B:** Number 2, 4, 6, 7 showing positive result by kit .

II, III, and IV (Table 6). On two-tiered classification, the ITB cases belonging to the “ITB group” showed 57.7% (kit <A>) and 53.8% (kit) TB-PCR positivity. In the “confusing group” there were 10 additional PCR-positive cases among the ITB cases based on kit <A> analysis, and 6 additional PCR-positive cases based on ITB analysis by kit . The PCR positive cases in the “confusing group” of ITB could be reclassified as the “ITB group”. The one CD case classified as group II by histopathologic evaluation revealed negative TB-PCR. After a combination of histopathologic and PCR analyses with kit , the diagnostic sensitivity of ITB was significantly increased (*P* = 0.010) to 65.5% (Table 7). Although the sensitivity of kit <A> was better than that of kit , the diagnostic usefulness was not validated due to low specificity. The diagnostic sensitivity and specificity of histopathologic findings and adjuvant effect of TB-PCR is summarized in Table 7.

DISCUSSION

A number of reports have documented the characteristics of ITB^[15-17] and CD^[18-20], and some have also compared the histopathological features. Pulimood *et al.*^[8,9]

Table 5 Results of TB-PCR in ITB and CD

TB-PCR test	ITB (n = 55)		CD (n = 42)		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	P value
	+	-	+	-					
Kit <A>	25	30	5	37	45.5	88.1	83.3	55.2	0.000
Kit 	20	35	0	42	36.4	100	100	54.5	0.000
In-house	3	52	0	42	5.5	100	100	44.7	0.256

Table 6 Histopathologic parameters and TB-PCR n (%)

Confirmed diagnosis	Group of HPP	n	TB-PCR positive cases		
			Kit <A>	Kit 	In-house
ITB	I	14 (25.5)	9 (64.3)	10 (71.4)	2 (14.3)
	II	12 (21.8)	6 (50.0)	4 (33.3)	0 (0.0)
	III	23 (41.8)	9 (34.6)	6 (23.1)	1 (3.8)
	IV	6 (10.9)	1 (33.3)	0 (0.0)	0 (0.0)
CD	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	II	1 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)
	III	15 (35.7)	4 (15.4)	0 (0.0)	0 (0.0)
	IV	26 (61.9)	1 (6.3)	0 (0.0)	0 (0.0)

Table 7 Comparison of diagnostic accuracy of ITB before and after combination of histopathology and TB-PCR

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	P value
Histopathology	47.3	100	100	59.2	
Combination with kit <A>	65.5	88.1	87.8	66.1	0.390
Combination with kit 	58.2	100	100	64.6	0.010
Combination with in-house PCR	49.1	100	100	60.0	0.320

HPP: Histopathologic parameters.

evaluated several histological parameters in colonoscopic biopsies to distinguish between ITB and CD. They examined characteristics of granuloma such as size, number, confluence, caseation necrosis, location, and presence of microgranulomas, as well as ulceration pattern, focally enhanced colitis, and disruption of submucosa. They reported that all of these characteristics were significantly different between ITB and CD, but other factors, such as architectural alteration, chronic inflammation, and discontinuous inflammation, were not significantly different. In our study, the histopathological features that had been reported as favorable factors for ITB in the literature were also statistically significant in differentiating these two diseases. More than a half of ITB cases (52.7%) showed less than five kinds of these histopathologic parameters confused with CD microscopically. Therefore, differential diagnosis of ITB and CD based on histopathologic features may be limited.

PCR detection of mycobacteria in histopathological specimens has been introduced as a rapid and useful technique for the diagnosis of pulmonary and extrapulmonary tuberculosis^[13,21,22]. *IS6110* was originally described in 1990 as a 1.36 kb insertion sequence with extensive numerical and positional polymorphism, found only in the *M. tuberculosis* complex (MTBC). Many members of the MTBC contain multiple *IS6110* copies^[23]. Because there are multiple copies of the *IS6110* insertion sequence in the MTBC, *IS6110* is an attractive target for PCR amplification. However, some MTBC strains do not contain the *IS6110* sequence^[24], so false negative results may occur. Past studies have shown that detection of the *IS6110* gene in *M. tuberculosis* has variable sensitivity (30%-90%)^[11,25-28] and specificity (60%-100%)^[12,13,21,29].

MPB64 is a mycobacterial antigen specific to the *M. tuberculosis* complex. This antigen is secreted from

M. tuberculosis, *Mycobacterium bovis* (*M. bovis*), and some strains of *M. bovis* BCG during bacterial growth^[30,31]. In a study of pleural and meningeal tuberculosis, the sensitivity and specificity of nested-PCR using *MPB64* were 70% and 88%^[32]. The study of Singh *et al*^[33] used two PCRs targeting *IS6110* and *MPB64* and found that sensitivity is 77.8% better than PCR targeting *IS6110* only (48.2%).

The current study used conventional in-house PCR, which detects the *IS6110* gene sequence, along with two newly developed kits manufactured by <A> and . Kit <A> detects the *MPB64* and *IS6110* genes simultaneously using the duplex nested PCR method, and kit detects the *IS6110* gene sequence using only the nested PCR method. Among 9 positive control cases, 8 cases (88.9%) were PCR positive for both kits. The sensitivity of the in-house PCR test (66.7%) was lower than that of both kits. Ten negative control cases were all negative for three kinds of PCR test. The kits we used showed higher sensitivity and specificity than in-house PCR. The false negative rates of the kits were lower than in-house PCR test.

The PCR test performed on the endoscopic biopsy specimens showed a dramatic drop in sensitivity in both kits, as well as in the in-house method compared to the control samples. False negative results were probably due to paucibacilli. All samples were obtained from endoscopic biopsies, so the tissue samples were small in amount. *M. tuberculosis* would not be distributed evenly in the tissue, furthermore, each case tested for PCR was only a 50 μm thick sample from each paraffin block of endoscopic biopsy. Hence, some sections among the ITB samples might not have contained *M. tuberculosis*. The absence or only a few copies of *IS6110* gene sequence in a proportion of *M. tuberculosis*^[34,35] may also bring about a false negative result.

The sensitivity of kit <A> was better than that of kit . Twenty-five cases of ITB (45.5%) were posi-

tive by kit <A>, but 20 cases (36.4%) were positive by kit . This suggests that the combined method could reduce false negativity in the samples without the *IS6110* sequence, as Singh *et al*³³¹ reported. However, five cases of CD were TB-PCR positive by kit <A>. Therefore, the specificity of kit <A> was not as good as that of kit (100%). The false-positive results on the duplex method might be due to contamination or the influence of gene sequences on each other during the experiments.

The histopathologic findings were very informative in differentiating ITB and CD. However, more than half of all ITB cases (52.7%) and nearly all CD cases fell in the "confusing group". So, in order to increase diagnostic accuracy, it is better to interpret histopathologic features in conjunction with PCR results. Because of the high specificity of TB-PCR, we could ascertain ITB if the TB-PCR test was positive. The TB-PCR test could detect *M. tuberculosis* in 20.7%-34.5% of these gray zone ITB cases, and the diagnostic sensitivity of ITB was significantly increased ($P = 0.010$) from 47.3% to 58.2% with kit , but the low sensitivity of the test in small biopsy pieces was problematic. Sufficient tissue sampling might improve results. Also, it is necessary to check the clinical features and endoscopic findings first.

In conclusion, kit <A> and kit TB-PCR were more sensitive in detecting *M. tuberculosis* in endoscopic biopsy specimens when compared to the in-house PCR method. The TB-PCR test with kit targeting *IS6100* combined with histopathologic examination appears to be a helpful technique in differentiating ITB and CD in endoscopic biopsy samples.

COMMENTS

Background

The incidence of intestinal tuberculosis (ITB) is increasing with resurgence of tuberculosis (TB). Because of similar clinical, endoscopic, radiological, and microscopic findings the differential diagnosis of ITB with Crohn's diseases (CD) is very difficult. Similar histopathological features of chronic colitis are seen in both ITB and CD but with some delicate differences. Recent studies have shown that polymerase chain reaction for *Mycobacterium tuberculosis* (TB-PCR) is useful for diagnosis of TB.

Research frontiers

Some characteristics of individual granulomas and some other histopathological features might be helpful for differential diagnosis between ITB and CD and TB-PCR targeting *IS6100* and/or *MPB64* is sensitive and specific for diagnosis of TB. But which and how many kinds of characteristic histopathological features may be helpful for differential diagnosis and whether combination with TB-PCR targeting *IS6100* and/or *MPB64* in endoscopic biopsy specimen may improve differential diagnosis are not well known.

Innovations and breakthroughs

In this study, the authors found that caseation necrosis and more than four kinds of histopathological features in an endoscopic biopsy specimen are very informative in differential diagnosis of ITB and CD and these histopathological features combined with TB-PCR can improve the differential diagnosis. Otherwise the commercial kits are more sensitive and specific in TB-PCR in endoscopic biopsy specimen than in-house TB-PCR.

Applications

By selecting and observing endoscopic biopsy specimens from patients of ITB and CD and comparing TB-PCR using commercial kits and in-house TB-PCR, this study is beneficial for the differential diagnosis between ITB and CD practically, and provides initial insight into a method of diagnosis of TB.

Peer review

This paper is very nicely written and makes a good contribution.

REFERENCES

- 1 Glaziou P, Floyd K, Raviglione M. Global burden and epidemiology of tuberculosis. *Clin Chest Med* 2009; **30**: 621-636, vii
- 2 Annual Report on the Notified Tuberculosis Patients in Korea. Seoul: Korean center for Disease Control and Prevention, Korean Institute for Tuberculosis, Reported in 2008. Available from: URL: <http://dis.cdc.gov.kr>
- 3 Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, Dye C. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* 2003; **163**: 1009-1021
- 4 Horvath KD, Whelan RL. Intestinal tuberculosis: return of an old disease. *Am J Gastroenterol* 1998; **93**: 692-696
- 5 Arnold C, Moradpour D, Blum HE. Tuberculous colitis mimicking Crohn's disease. *Am J Gastroenterol* 1998; **93**: 2294-2296
- 6 Epstein D, Watermeyer G, Kirsch R. Review article: the diagnosis and management of Crohn's disease in populations with high-risk rates for tuberculosis. *Aliment Pharmacol Ther* 2007; **25**: 1373-1388
- 7 Ouyang Q, Tandon R, Goh KL, Ooi CJ, Ogata H, Fiocchi C. The emergence of inflammatory bowel disease in the Asian Pacific region. *Curr Opin Gastroenterol* 2005; **21**: 408-413
- 8 Pulimood AB, Peter S, Ramakrishna B, Chacko A, Jeyamani R, Jeyaseelan L, Kurian G. Segmental colonoscopic biopsies in the differentiation of ileocolic tuberculosis from Crohn's disease. *J Gastroenterol Hepatol* 2005; **20**: 688-696
- 9 Pulimood AB, Ramakrishna BS, Kurian G, Peter S, Patra S, Mathan VI, Mathan MM. Endoscopic mucosal biopsies are useful in distinguishing granulomatous colitis due to Crohn's disease from tuberculosis. *Gut* 1999; **45**: 537-541
- 10 Bascuñana CR, Belák K. Detection and identification of mycobacteria in formalin-fixed, paraffin-embedded tissues by nested PCR and restriction enzyme analysis. *J Clin Microbiol* 1996; **34**: 2351-2355
- 11 Amarapurkar DN, Patel ND, Amarapurkar AD, Agal S, Baigal R, Gupte P. Tissue polymerase chain reaction in diagnosis of intestinal tuberculosis and Crohn's disease. *J Assoc Physicians India* 2004; **52**: 863-867
- 12 Peneau A, Moinard D, Berard I, Pascal O, Moisan JP. Detection of mycobacteria using the polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 1992; **11**: 270-271
- 13 Negi SS, Anand R, Pasha ST, Basir SF, Gupta S, Khare S, Lal S. Detection of *M. tuberculosis* in clinical samples of diversified nature by IS6110 based PCR. *J Commun Dis* 2006; **38**: 325-332
- 14 Gan H, Ouyang Q, Bu H. [The value of polymerase chain reaction assay in the diagnosis of intestinal tuberculosis and differentiation from Crohn's disease] *Zhonghua Neike Zazhi* 1995; **34**: 30-33
- 15 Franklin GO, Mohapatra M, Perrillo RP. Colonic tuberculosis diagnosed by colonoscopic biopsy. *Gastroenterology* 1979; **76**: 362-364
- 16 Howell JS, Knapton PJ. Ileo-caecal tuberculosis. *Gut* 1964; **5**: 524-529
- 17 Tandon HD, Prakash A. Pathology of intestinal tuberculosis and its distinction from Crohn's disease. *Gut* 1972; **13**: 260-269
- 18 Morson BC. Pathology of inflammatory bowel disease. *Gastroenterol Jpn* 1980; **15**: 184-187
- 19 Hofstaedter F. Granuloma formation in the different phenotypes of Crohn's disease. *Gut* 2005; **54**: 180-181
- 20 Cook MG, Dixon MF. An analysis of the reliability of detection and diagnostic value of various pathological features in Crohn's disease and ulcerative colitis. *Gut* 1973; **14**: 255-262

- 21 **Negi SS**, Khan SF, Gupta S, Pasha ST, Khare S, Lal S. Comparison of the conventional diagnostic modalities, bactec culture and polymerase chain reaction test for diagnosis of tuberculosis. *Indian J Med Microbiol* 2005; **23**: 29-33
- 22 **Dar L**, Sharma SK, Bhanu NV, Broor S, Chakraborty M, Pande JN, Seth P. Diagnosis of pulmonary tuberculosis by polymerase chain reaction for MPB64 gene: an evaluation in a blind study. *Indian J Chest Dis Allied Sci* 1998; **40**: 5-16
- 23 **McEvoy CR**, Falmer AA, Gey van Pittius NC, Victor TC, van Helden PD, Warren RM. The role of IS6110 in the evolution of Mycobacterium tuberculosis. *Tuberculosis (Edinb)* 2007; **87**: 393-404
- 24 **van Soolingen D**, de Haas PE, Hermans PW, Groenen PM, van Embden JD. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of Mycobacterium tuberculosis. *J Clin Microbiol* 1993; **31**: 1987-1995
- 25 **Gunisha P**, Madhavan HN, Jayanthi U, Therese KL. Polymerase chain reaction using IS6110 primer to detect Mycobacterium tuberculosis in clinical samples. *Indian J Pathol Microbiol* 2000; **43**: 395-402
- 26 **Kulkarni S**, Vyas S, Supe A, Kadival G. Use of polymerase chain reaction in the diagnosis of abdominal tuberculosis. *J Gastroenterol Hepatol* 2006; **21**: 819-823
- 27 **Balamurugan R**, Venkataraman S, John KR, Ramakrishna BS. PCR amplification of the IS6110 insertion element of Mycobacterium tuberculosis in fecal samples from patients with intestinal tuberculosis. *J Clin Microbiol* 2006; **44**: 1884-1886
- 28 **Negi SS**, Anand R, Pasha ST, Gupta S, Basir SF, Khare S, Lal S. Diagnostic potential of IS6110, 38kDa, 65kDa and 85B sequence-based polymerase chain reaction in the diagnosis of Mycobacterium tuberculosis in clinical samples. *Indian J Med Microbiol* 2007; **25**: 43-49
- 29 **Noordhoek GT**, Kolk AH, Bjune G, Catty D, Dale JW, Fine PE, Godfrey-Faussett P, Cho SN, Shinnick T, Svenson SB. Sensitivity and specificity of PCR for detection of Mycobacterium tuberculosis: a blind comparison study among seven laboratories. *J Clin Microbiol* 1994; **32**: 277-284
- 30 **Wiker HG**, Nagai S, Harboe M, Ljungqvist L. A family of cross-reacting proteins secreted by Mycobacterium tuberculosis. *Scand J Immunol* 1992; **36**: 307-319
- 31 **Nakamura RM**, Velmonte MA, Kawajiri K, Ang CF, Frias RA, Mendoza MT, Montoya JC, Honda I, Haga S, Toida I. MPB64 mycobacterial antigen: a new skin-test reagent through patch method for rapid diagnosis of active tuberculosis. *Int J Tuberc Lung Dis* 1998; **2**: 541-546
- 32 **Martins LC**, Paschoal IA, Von Nowakowski A, Silva SA, Costa FF, Ward LS. Nested-PCR using MPB64 fragment improves the diagnosis of pleural and meningeal tuberculosis. *Rev Soc Bras Med Trop* 2000; **33**: 253-257
- 33 **Singh HB**, Singh P, Jadaun GP, Srivastava K, Sharma VD, Chauhan DS, Sharma SK, Katoch VM. Simultaneous use of two PCR systems targeting IS6110 and MPB64 for confirmation of diagnosis of tuberculous lymphadenitis. *J Commun Dis* 2006; **38**: 274-279
- 34 **Diaz R**, Kremer K, de Haas PE, Gomez RI, Marrero A, Valdivia JA, van Embden JD, van Soolingen D. Molecular epidemiology of tuberculosis in Cuba outside of Havana, July 1994-June 1995: utility of spoligotyping versus IS6110 restriction fragment length polymorphism. *Int J Tuberc Lung Dis* 1998; **2**: 743-750
- 35 **Yuen LK**, Ross BC, Jackson KM, Dwyer B. Characterization of Mycobacterium tuberculosis strains from Vietnamese patients by Southern blot hybridization. *J Clin Microbiol* 1993; **31**: 1615-1618

S- Editor Wang JL L- Editor O'Neill M E- Editor Zheng XM