Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Application value of tissue tuberculosis antigen combined with Xpert MTB/RIF detection in differential diagnosis of intestinal tuberculosis and Crohn's disease

Baoying Fei (3145681515@qq.com)
Tongde Hospital Of Zhejiang Province
Lin Zhou
Hangzhou Red Cross Hospital
Yu Zhang
Zhejiang Provincial People's Hospital
Linhe Luo
Tongde Hospital Of Zhejiang Province
Yuanyuan Chen
Hangzhou Red Cross Hospital

Research article

Keywords: Intestinal Tuberculosis, Crohn's disease, Xpert MTB/RIF, Antigen, Diagnosis

Posted Date: January 23rd, 2020

DOI: https://doi.org/10.21203/rs.2.12435/v4

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Version of Record: A version of this preprint was published at BMC Infectious Diseases on May 28th, 2021. See the published version at https://doi.org/10.1186/s12879-021-06210-8.

Abstract

Background: The purpose of this study was to evaluate the value of Xpert MTB/RIF detection and tuberculosis antigen detection of Mycobacterium tuberculosis cluster (MTBC) in intestinal tissues for differentiating intestinal tuberculosis (ITB) from Crohn's disease (CD).

Methods: A total of 110 patients who were clinically diagnosed with CD or ITB were monitored. Several specimens of intestinal tissue from endoscopic biopsy or surgical excision were used for culture and Xpert MTB/RIF for detection of MTBC, respectively. Four antigens (38KDa, ESAT-6, MPT64, Ag85 complex) of MTBC in intestinal tissue were detected by immunohistochemistry.

Results: A total of 42 cases of intestinal tuberculosis and 46 cases of CD were included in the experimental analysis. Perianal lesions and longitudinal ulcers were more common in CD patients ($p < 0.05$), while caseous granuloma and annular ulcers were more common in ITB patients ($P < 0.05$). The positive rate of MTBC detected by Xpert MTB/RIF in intestinal tissue samples of ITB patients was 33.33%, which was significantly higher compared to CD patients ($p < 0.05$) and compared to acid-fast staining smears (9.52%) ($p < 0.05$). The positive MPT64 expression rate in patients with intestinal tuberculosis was 40.48%, which was significantly higher than that observed in CD patients, which was 19.56% ($p<0.05$).

Conclusions: The detection of Xpert MTB/RIF in intestinal tissue is a rapid and useful method for establishing an early diagnosis of intestinal tuberculosis. The detection of Xpert MTB/RIF and MPT64 antigen in intestinal tissues have definitive value in the differential diagnosis of intestinal tuberculosis and Crohn's disease. The combination of these two methods could improve detection sensitivity.

Background

ITB and CD are chronic intestinal granulomatous diseases that share many similarities in clinical manifestations, imaging, endoscopy and intestinal histopathology. Over recent years, following the population migration, drug-resistant bacterial infection, and the spread of AIDS, the incidence of tuberculosis and ITB have significantly increased [1]. The incidence of CD has also been increasing in China [2]. Although numerous studies have addressed the clinical, pathological and molecular biological detections of the two diseases, the differential diagnosis of ITB and CD still represents a challenging clinical problem, especially in the developing countries with high incidence of tuberculosis where the misdiagnosis and missed diagnosis may lead to serious adverse consequences [3-5].

Xpert MTB/RIF is a new technology that has recently been approved by WHO [6]. It is an *in vitro* diagnostic technique based on semi-nested multiplex real-time fluorescent quantitative PCR using Gene Xpert full-automatic closed nucleic acid detection system. Primers and probes are designed to target the core sequence of rifampicin-resistant determinant regions which are characteristic of MTBC. It can be used to diagnose MTBC infection, but also to determine rifampicin resistance (mutation in rpoB sequence). The

whole detection process can be completed in 2 hours. Since its first applications, this technology has been gradually recognized and approved for its diagnostic value in tuberculosis detection, showing high accuracy, sensitivity of 89% and specificity of 98% in the diagnosis of adult pulmonary tuberculosis [7]. Previous studies have investigated the diagnostic value of Xpert MTB/RIF in extrapulmonary tuberculosis [8-10]; nevertheless, researches on the application of Xpert MTB/RIF in the diagnosis of ITB are still very rare.

MTBC secrete a variety of antigens with potential diagnostic significance [11-13]. 38KDa protein is a membrane protein, which is closely related to the transport of phosphate. It is an early secretory protein encoded by *Psts* gene that can stimulate T cells and B cells to develop corresponding immune response. ESAT-6 is an early secretory protein of MTBC that only exists in MTBC and a few pathogenic mycobacteria, but not in non-pathogenic mycobacteria and BCG. MPT64 exists in MTBC, and most BCG strains cannot secrete this protein. Ag85 complex is an acid transferase of Mycobacterium, where each strain of Mycobacterium can secrete Ag85, and thus has an important role in the late stage of cell wall synthesis of MTBC.

In this study, Xpert MTB/RIF detection system was used to detect MTBC. The immunohistochemical method was used to detect several tuberculosis antigens in the intestinal tissue samples of ITB and CD patients to clarify the role of Xpert MTB/RIF detection and tuberculosis antigen detection in the differential diagnosis of ITB and CD. Additionally, the clinical, endoscopic and pathological features of CD and ITB were also compared.

Methods

Patients

Patients who were clinically suspected of having CD and ITB between January 2016 and March 2018 were initially included in this study. Patients who received antituberculosis treatment within three months before the collection of specimens were excluded. The clinical, endoscopic, radiological and biopathological data of the subjects were collected, and the patients were followed up after treatment. Several specimens of intestinal tissue from endoscopic biopsy or surgical excision were collected. Four endoscopic biopsy samples were made into paraffin specimen, and another four to six endoscopic biopsy samples were used for culture and Xpert MTB/RIF for detection of MTBC.

The written informed consent was obtained from all the patients before the clinical trials started and the study was approved by the ethics committee of tongde hospital of zhejiang province.

Diagnostic criteria

The ITB diagnosis was established if at least one of the following criteria was met [14]: 1) histological evidence of a caseating granuloma, 2) histological demonstration of acid-fast bacilli(AFB), 3) intestinal granulomatous inflammation accompanied by histologically or microbiologically confirmed

extraintestinal TB, and 4) a positive MTBC culture. The CD diagnosis was based on well-established clinical,endoscopic, radiological, and histological parameters [15].

In patients for whom the differentiation between ITB and CD was uncertain, anti-TB therapy (ATT) was attempted for 2-3 months, and the final diagnosis was made based on the clinical and endoscopic response to the anti-TB therapy [16-17].The clinical response was determined by the loss of subjective symptoms. The endoscopic response was determined by the disappearance of ulcerations.

Xpert MTB/RIF detection of intestinal tissue specimens

Intestinal tissue specimens were decontaminated with 1% N-acetyl-L-cysteine *NaOH* (NALC-NaOH) and then were centrifuged. The tissue was mechanically homogenized and suspended with 1 ml of sterile saline solution. About 1 ml of specimen was taken, and 2 times volume of sample treatment solution was added. The solution was mixed by vortex oscillation for 10 seconds, kept at room temperature for 10 minutes, mixed again by vortex oscillation for 10 seconds, and then kept at room temperature for 5 minutes. The mixture of 2 ml was added to Xpert reagent kit, and the kit was put into Gene Xpert instrument for detection. After the reaction, the test results were directly observed under the window of the detection system.

Acid-fast staining and MTBC culture of intestinal tissues

Fresh intestinal tissue specimens were decontaminated with 1% NALC-NaOH and then were centrifuged. The tissue was mechanically homogenized and suspended in sterile saline solution. The treated samples were examined by acid-fast staining smear, MTBC culture with Lowenstein Jensen medium (LJ) and results determination. The specific procedures were carried out in accordance with “The Laboratory Science Procedure of Diagnostic Bacteriology in Tuberculosis”.

Immunohistochemical detection of several tuberculosis antigens in intestinal tissues

Four antigens of MTBC (38KDa protein, ESAT-6 protein, MPT64, Ag85 complex) were detected. Four micron thick tissue paraffin embedded slices were taken. Dewaxing, thermal repair and blocking were sequentially performed. Primary antibody was incubated overnight, and secondary antibody was incubated for 1 hour. The slices were washed with PBS, colored with DAB for 5 minutes, and sealed. The procedure strictly followed the instructions of the kit.

Statistical analysis

SPSS20.0 software was used for statistical analyses. Measurement data were expressed as mean ± standard deviation and T-test was used for comparison between groups. Countable data were expressed in rates, and X^2 test or Fisher’s exact probability method were used for comparison between groups. $P < 0.05$ was considered to be statistically significant.

Results

General information

Histological specimens were initially collected from 110 patients, where 22 patients were excluded for diagnosis of non-intestinal tuberculosis or CD. Finally, 42 cases of intestinal tuberculosis (14 cases were surgical specimens) and 46 cases of CD (3 cases were surgical specimens) were included in the experimental analyses. Of the 30 patients started on ATT for probable ITB, symptomatic improvement and endoscopic healing were noted in 22 patients.

There were 20 male (47.6%) and 22 female (52.4%) patients with intestinal tuberculosis.The age of onset was 18-59 years and the mean age was 30.5±11.8 years. There were 27 male (58.7%) and 19 female (41.3%) patients with CD. The age of onset was 17-61 years and the mean age was 31.5±12.2 years. There was no significant difference in gender and age between the two groups.

Clinical features

Clinical features are shown in **Table 1**. The most common symptoms of both CD and ITB were abdominal pain and diarrhea. Abdominal pain was found among 41 patients (89.1%) with CD and 33 patients (78.6%) with ITB; the diarrhea was found in 37 patients (80.4%) with CD patients and 29 (69.0%) patients with ITB. There was no significant difference in abdominal pain and diarrhea among the groups. There were 10 cases (21.7%) among CD patients with perianal lesions, including 6 cases of anal fistula, 3 cases of perianal abscess and 1 case of anal fissure; and there was 1 case among ITB patients with perianal lesion (anal fissure). The observed difference was significant ($P < 0.01$). Longitudinal ulcer was found in 17 patients with CD (36.9%), which was significantly higher than that in ITB patients (6 cases, 14.3%). Annular ulcer was found in 25 patients with ITB (59.5%) which was significantly higher compared to CD patients (7 cases, 15.2%). Among CD patients, 11 (23.9%) had aphthous ulcer, 10 (21.7%) had paving-stone like changes in ulcers and 11 patients (23.9%) had pseudopolyps, which were significantly different from those found in patients with ITB (4 cases, 9.5%; 5 cases, 11.9%; 14 cases, 33.3%); the observed differences were not statistically significant. Caseous granuloma was found in three cases of ITB endoscopic biopsy specimens and 7 cases of ITB surgical specimens, which had definitive diagnostic significance. Non-caseous granulomas were found in 17 patients with ITB (40.5%) and in 12 patients with CD (26.1%); but there was no significant difference between the groups. The difference in lymphocyte aggregation at the bottom of lamina propria between the two groups was also non significant.

Detection and comparison of MTBC in intestinal tissues of ITB and CD patients

The positive rate of MTBC detected by Xpert MTB/RIF in intestinal tissues of ITB patients was 33.33%, which was significantly higher than that of CD patients ($p < 0.01$). The positive rate detected by Xpert MTB/RIF in 28 biopsy specimens of ITB patients was 14.28% (4/28), and that in 14 surgical specimens was 71.43% (10/14). None of them showed resistance to RIF. The positive rate of MTBC detected by Xpert MTB/RIF in intestinal tissue specimens of ITB patients (33.33%) was higher than that detected by tissue culture (21.43%), but the difference was not statistically significant. The positive rate detected by tissue culture in 28 biopsy specimens of ITB patients was 7.14% (2/28), and that in 14 surgical

specimens was 50% (7/14).The positive rate of MTBC detected by Xpert MTB/RIF in intestinal tissue specimens of ITB patients (33.33%) was significantly higher than that detected by acid-fast staining smear (11.9%). The results are shown in **Table 2**.

Clinical evaluation of detection of *M.tuberculosis* in intestinal tissues by Xpert MTB/RIF

The specificity of all three methods for detection of MTBC in intestinal tissues was very high. The specificity and positive predictive value of Xpert MTB/RIF for detection of MTBC in intestinal tissues were both 100%. The negative predictive value of Xpert MTB/RIF for detection of MTBC in intestinal tissues (62.2%) was higher than that of tissue culture (58.2%) and acid-fast staining (54.9%), but there was no significant difference. The results are shown in **Table 3.**

Expression of intestinal tuberculosis proteins in patients with intestinal tuberculosis and Crohn's disease

Psts1, MPT64, Ag85B and EAST-6 were expressed in the granular cytoplasm of intestinal granuloma cells in intestinal tuberculosis and Crohn's disease (**Figure 1**). The positive rate of MPT64 expression was 40.48% in intestinal tuberculosis patients and 19.56% in Crohn's disease patients, and the difference was statistically significant ($X^2 = 4.61$, $P < 0.05$). However, there was no statistically significant difference in the expression of the other three tuberculosis proteins between the two diseases (**Table 4**).

Detection of MTBC by intestinal tuberculosis protein combined with Xpert MTB/RIF

The sensitivity and specificity of combined detection of tuberculosis protein MPT64 and Xpert MTB/RIF in the diagnosis of pulmonary tuberculosis were 50.0% and 80.44%, respectively(**Table 5**). The sensitivity of combined detection was improved compared to the single detection sensitivity, but the specificity declined.

Discussion

CD and ITB share many similarities in clinical manifestations and signs, which make them difficult to differentiate [18]. Some studies have proposed certain criteria for identifying CD and ITB, but these criteria have been shown to have great limitations [19-21]. In this study, common symptoms of CD and ITB, such as abdominal pain, diarrhea and ascites, had no significant differences between two groups. Perianal lesions in CD patients were significantly higher than in ITB patients, suggesting that perianal lesions had certain value for the differentiation of the two diseases. Perianal lesions are often the first symptom of CD and are closely related to disease activity, which is why clinicians should pay close attention to them. The data in our study showed that the longitudinal ulcer and stenosis in CD patients were significantly higher than that in ITB patients, and the annular ulcer in ITB patients was significantly higher than that in CD patients. These data were consistent with previous reports [22, 23], and they suggest that the longitudinal ulcer and stenosis are the characteristic manifestations of CD, and that the annular ulcer is the characteristic manifestation of ITB. The pathological changes of CD and intestinal tuberculosis usually occur in the submucosa of the intestinal wall. Because of mucosal swelling, the endoscopic

biopsy tissues tend to be too small and the sampling too superficial, resulting in low positive rates. In the current study, there were only three cases of caseous granuloma in ITB endoscopic biopsy, while the pathological positive rate was much lower than that of surgical specimens, suggesting that the differential diagnostic value of endoscopic biopsy specimens in the two diseases was much lower than that of surgical specimens. Non-caseous granuloma was more common in ITB patients than in CD patients, but there was no statistically significant difference. Non-caseous granuloma alone are not enough to completely exclude ITB and should not be considered as a CD-specific manifestation.

At present, WHO recommends Xpert MTB/RIF for rapid diagnosis of pulmonary tuberculosis and some forms of extrapulmonary tuberculosis [24, 25]. Currently, there are few reports on Xpert MTB/RIF detection in intestinal mucosa in China and abroad [26, 27]. In the present study, the intestinal tissue specimens from 42 cases of intestinal tuberculosis and 46 cases from Crohn's disease were examined by Xpert MTB/RIF. The results showed that the positive rate of MTBC detected by Xpert MTB/RIF in intestinal tissue samples of ITB patients was 33.33%, which was higher than that of CD patients (0%), and the specificity was 100%. This suggested that the detection of Xpert MTB/RIF in intestinal mucosa might have an important role in the differential diagnosis of intestinal tuberculosis and Crohn's disease. In a retrospective study from India ,of 37 intestinal TB patients, colonic biopsy samples underwent Xpert MTB/RIF analysis and the Xpert MTB/RIF assay has low sensitivity (8.1%) [27]. In this study, the positive rate of MTBC detected by Xpert MTB/RIF in endoscopic biopsy specimens was much lower compared to surgical specimens, which might be because the endoscopic biopsy specimens were superficial and small. MTBC was not evenly distributed in intestinal tissues, and low levels of MTBC extracted from mucosal biopsy specimens may lead to false negative results of Xpert MTB/RIF in tissues.

The gold standard for the etiological diagnosis of tuberculosis is *M.* tuberculosis culture. However, the growth of M. tuberculosis usually takes 3-8 weeks, and the positive rate of culture is low, which often causes difficulties in diagnosis. Although the routine acid-fast staining method takes only 2-3 hours, this approach has very low sensitivity and specificity of strain identification. Even though it can detect the acid-fast *Mycobacterium*, it cannot distinguish *M. tuberculosis* from other *mycobacterium*. The sensitivity and accuracy of PCR for pathogen detection provides a useful and new method for the diagnosis of *M. tuberculosis* [28-30].In this study, the detection of MTBC by Xpert MTB/RIF in the intestinal mucosa was compared with traditional methods, and the results showed that the sensitivity of Xpert MTB/RIF was 33.3%, which was significantly higher than that of acid-fast staining (11.9%) and higher than that of bacillus culture (21.4%). The above results indicated that Xpert MTB/RIF had superior sensitivity compared to acid-fast staining in detection of MTBC, it also had superior detection time compared with MTBC culture, and it had higher specificity. Therefore, the Xpert MTB/RIF was better approach for the early diagnosis and treatment of intestinal tuberculosis.

MTBC culture with Mycobacterium Growth Indicator Tube (MGIT) system was reported to be positive in 20% to 42% in colonoscopic biopsy specimens[31-32]. In our study,the positive rate of MTBC detected by tissue culture in ITB patients was 21.43%(7.14% in biopsy specimens and 50% in 14 surgical

specimens,LJ medium). Lower yield of MTBC culture in biopsy specimens may be associated with culture medium and the number of colonoscopic biopsies.The yield of MTBC culture on LJ medium is lower than that on MGIT system. However it should be noted that false-positive rates for MGIT system was higher than LJ methods[33].In a recently report, additional four (total eight) colonoscopic biopsies improved the yield of TB culture positivity over four biopsies by 11.4% to 14.3%[32]. If for improving AFB culture positivity, Increasing the number of colonoscopic biopsy specimens to eight should be considered,although it cost more time.

Over recent years, following the development of immune technology, an increasing attention has been paid to the study of MTBC antigen [13, 34]. At present, the main samples used for MTBC antigen diagnosis are body fluid and bacterial culture medium [35, 36]. Reports on the application of tuberculosis antigen in ITB tissues are very rare. There are only two reports on 38KDa antigen with limited number of ITB cases (≤ 10 cases). Ihama *et al.* have performed immunohistochemical detections of 38KDa antigen in intestinal paraffin specimens of 10 ITB patients, revealing that 40% of the patients were positive, which in turn suggested that immunohistochemical detection with MTBC monoclonal antibody might be an effective and simple diagnostic method for ITB [37]. Ince and colleagues have performed immunohistochemical detection of 38KDa antigen in 45 tissue specimens (including 8 colon tissue specimens, 8 skin specimens, 5 lung tissue specimens and 24 lymph node specimens), and found that 73% of tuberculosis patients were positive, while only 2 out of 28 patients with CD were positive, suggesting that immunohistochemical detection of 38KDa antigen could be used to distinguish the early diagnosis of ITB from CD [38]. Currently, there are no reports on the expression of pstS1 (38KDa) and East-6 antigens in intestinal tissues. The results of this study showed that the expression of Ag85B, pstS1 (38KDa) and East-6 antigen proteins in intestinal tuberculosis and Crohn's disease had no significant difference. The positive expression rates of Ag85B, pstS1 (38KDa) and East-6 antigen in intestinal tissues were 9.52%, 23.8% and 26.2%, respectively. The sensitivity of these three proteins immunostaining detection in intestinal tissues was low, and could not be improved by combined detection of these three proteins. Future studies should investigate whether other detection methods such as PCR could improve the sensitivity.

Jørstad *et al.* used immunohistochemistry to detect MPT64 antigen in 132 cases of extrapulmonary tuberculosis and non-tuberculosis specimens. MPT64 detection had the best performance in TB lymphadenitis patients and children with TB, suggesting that MPT64 detection could be used for routine diagnosis under low resource allocation to improve the diagnosis of extrapulmonary tuberculosis, especially for tuberculous lymphadenitis and children with TB [39]. Purohit *et al.* studied 51 cases of pulmonary tuberculosis and 38 control specimens of non-pulmonary tuberculosis.The results suggested that anti-MPT64 immunostaining detection was a rapid and sensitive method for early specific diagnosis of Mycobacterium tuberculosis infection [40]. This technique can easily be incorporated into routine pathological laboratories. Currently, there are no reports on the expression of MPT64 antigen in intestinal tissues. In this study, the positive rates of MPT64 antigen in intestinal tuberculosis and Crohn's disease were 40.48% and 19.58%, respectively. The observed difference was statistically significant, which

suggested that MPT64 antigen has definitive value in the differential diagnosis of intestinal tuberculosis and Crohn's disease. Sharma *et al.* used real-time immuno-PCR (RT-I-PCR) assay for the quantitative detection of a cocktail of mycobacterial MPT64 (Rv1980c) and PstS1 (Rv0934) in TB patients. The RT-I-PCR assay revealed high sensitivity 83.3% especially for the rapid diagnosis of smear-negative pulmonary TB and paucibacillary extrapulmonary TB samples[41]. More researches needs to be done to investigate whether other methods such as PCR may improve the sensitivity of MPT64 antigen detection in intestinal tissues.

Conclusions

In a word, the detection of Xpert MTB/RIF in intestinal tissue is a rapid and useful method for establishing an early diagnosis of intestinal tuberculosis. The detection of Xpert MTB/RIF and MPT64 antigen in intestinal tissues have definitive value in the differential diagnosis of intestinal tuberculosis and Crohn's disease. The combination of these two methods could improve detection sensitivity.

List Of Abbreviations

MTBC *Mycobacterium tuberculosis* cluster

ITB intestinal tuberculosis

CD Crohn's disease

Declarations

Acknowledgements

This study was supported by public projects of science technology department of zhejiang province (grant number: 2016C33112)

Conflict of interest

The authors declared that they have no conflicts of interest to this work.We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted

Declarations

Ethics approval and consent to participate

The written informed consent was obtained from all the patients before the clinical trials started and the study was approved by the ethics committee of tongde hospital of zhejiang province.

A clear justification in the manuscript has been given to explain that the new procedure was deemed more appropriate than usual clinical practice to meet the patient's clinical need.

Consent for publication

Consent for publication has been obtained from related individual persons whose data were included in the manuscript .

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors have gained informed consent for publication of the dataset from participants at the point of recruitment to the trial.

Competing interests

The authors declare that they have no competing interests

Funding

This study was supported by public projects of science technology department of zhejiang province (grant number: 2016C33112)

Authors' contributions

Bao-Ying Fei designed research and wrote the paper. Lin Zhou developed the idea for the study. Yu Zhang performed four antigens of MTBC detection in intestinal tissue by immunohistochemistry.Yuan-yuan Chen performed Xpert MTB/RIF examination in intestinal tissue . Lin-He Luo collected and analysed the data. . All authors read and approved the final manuscript.

Acknowledgements

This study was supported by public projects of science technology department of zhejiang province (grant number: 2016C33112)

References

1. Glaziou P, Floyd K, Raviglione MC. Global Epidemiology of Tuberculosis.Semin Respir Crit Care Med. 2018;39(3):271-85.
2. Cui G, Yuan A. A Systematic Review of Epidemiology and Risk Factors Associated With Chinese Inflammatory Bowel Disease. Front Med (Lausanne). 2018;5:183.
3. Kedia S, Das P, Madhusudhan KS, et al. Differentiating Crohn's disease from intestinal tuberculosis. World J Gastroenterol.2019;25(4):418-32.

4. Seo H, Lee S, So H, et al. Temporal trends in the misdiagnosis rates between Crohn's disease and intestinal tuberculosis.World J Gastroenterol.2017;23(34):6306-14.
5. Wei JP, Wu XY, Gao SY, et al. Misdiagnosis and Mistherapy of Crohn's Disease as Intestinal Tuberculosis: Case Report and Literature Review. Medicine (Baltimore). 2016;95(1):e2436.
6. Geneva: World Health Organization .Xpert MTB/RIF implementation manual：Technical and operational 'How-to' practical considerations.
7. Steingart KR, Schiller I, Horne DJ, et al. Xpert(R) MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults.The Cochrane database of systematic reviews.2014;(1):CD009593.
8. Mazzola E, Arosio M, Nava A, et al.Performance of real-time PCR Xpert ®MTB/RIF in diagnosing extrapulmonary tuberculosis. Infez Med. 2016;24(4):304-9.
9. Kohli M, Schiller I, Dendukuri N, et al.Xpert® MTB/RIF assay for extrapulmonary tuberculosis and rifampicin resistance. Cochrane Database Syst Rev. 2018;8:CD012768.
10. Prakash AK, Datta B, Tripathy JP, et al.The clinical utility of cycle of threshold value of GeneXpert MTB/RIF (CBNAAT) and its diagnostic accuracy in pulmonary and extra-pulmonary samples at a tertiary care center in India. Indian J Tuberc. 2018;65(4):296-302.
11. Gutlapalli R, Sykam A, Tenali SP, et al.Detection of Tuberculosis in HIV Co-infected Individuals: Use of Multiple ELISA Responses to 38kDa, Lipoarabinomannan and ESAT- 6 of M. tuberculosis. J Clin Diagn Res. 2016;10(2):KC01-4.
12. Bai L, Chen Y, Bai Y, et al. Fullerene-doped polyaniline as new redox nanoprobe and catalyst in electrochemical aptasensor for ultrasensitive detection of Mycobacterium tuberculosis MPT64 antigen in human serum. Biomaterials.2017;133:11-9.
13. Karbalaei Zadeh Babaki M, Soleimanpour S, Rezaee SA.Antigen 85 complex as a powerful Mycobacterium tuberculosis immunogene: Biology, immune-pathogenicity, applications in diagnosis, and vaccine design.Microb Pathog.2017;112:20-9.
14. Lee YJ, Yang SK, Byeon JS, Myung SJ, Chang HS, Hong SS, et al.Analysis of colonoscopic findings in the differential diagnosis between intestinal tuberculosis and Crohn's disease. Endoscopy. 2006;38(6):592-7.
15. Travis SP, Stange EF, Lémann M, Oresland T, Chowers Y, Forbes A, et al.European evidence based consensus on the diagnosis and management of Crohn's disease: current management. Gut. 2006 Mar;55 Suppl 1:i16-35.
16. Pratap Mouli V, Munot K, Ananthakrishnan A, Kedia S, Addagalla S, Garg SK, et al.Endoscopic and clinical responses to anti-tubercular therapy can differentiate intestinal tuberculosis from Crohn's disease. Aliment Pharmacol Ther. 2017;45(1):27-36.
17. Sharma V, Mandavdhare HS, Dutta U.Letter: mucosal response in discriminating intestinal tuberculosis from Crohn's disease-when to look for it? Aliment Pharmacol Ther. 2018 ;47(6):859-60.
18. Ma JY, Tong JL, Ran ZH.J Intestinal tuberculosis and Crohn's disease: challenging differential diagnosis.Dig Dis. 2016;17(3):155-61.

19. Limsrivilai J, Shreiner AB, Pongpaibul A, et al.Am J Gastroenterol. Meta-Analytic Bayesian Model For Differentiating Intestinal Tuberculosis from Crohn's Disease. 2017;112(3):415-27.
20. Jin T, Fei B, Zhang Y, et al.The diagnostic value of polymerase chain reaction for Mycobacterium tuberculosis to distinguish intestinal tuberculosis from crohn's disease: A meta-analysis. Saudi J Gastroenterol. 2017;23(1):3-10.
21. Lee JM, Lee KM.Endoscopic Diagnosis and Differentiation of Inflammatory Bowel Disease. Clin Endosc. 2016;49(4):370-75.
22. Gan H, Mely M, Zhao J, et al. An Analysis of the Clinical, Endoscopic, and Pathologic Features of Intestinal Tuberculosis. J Clin Gastroenterol. 2016;50(6):470-75.
23. Jung Y,Hwangbo Y,Yoon SM,et al.Predictive Factors for Differentiating Between Crohn's Disease and Intestinal Tuberculosis in Koreans. Am J Gastroenterol. 2016;111(8):1156-64.
24. Zumla A, George A, Sharma V, et al. The WHO 2014 global tuberculosis report—further to go.The Lancet Global Health. 2015;3 (1):e10–e12
25. MacLean E, Sulis G, Denkinger CM, et al.Diagnostic accuracy of stool Xpert MTB/RIF for the detection of pulmonary tuberculosis in children: a systematic review and meta-analysis. J Clin Microbiol. 2019; pii: JCM.02057-18.
26. Bellam BL, Mandavdhare HS, Sharma K, Shukla S, Soni H, Kumar-MP, et al.Utility of tissue Xpert-Mtb/Rif for the diagnosis of intestinal tuberculosis in patients with ileocolonic ulcers. Ther Adv Infect Dis. 2019;6:2049936119863939.
27. Kumar S, Bopanna S, Kedia S, Mouli P, Dhingra R, Padhan R, et al.Evaluation of Xpert MTB/RIF assay performance in the diagnosis of abdominal tuberculosis. Intest Res. 2017 ;15(2):187-94.
28. Leung KS, Siu GK, Tam KK, et al.Diagnostic evaluation of an in-house developed single-tube, duplex, nested IS6110 real-time PCR assay for rapid pulmonary tuberculosis diagnosis. Tuberculosis (Edinb). 2018;112:120-25.
29. Lim JH, Kim CK, Bae MH.Evaluation of the performance of two real-time PCR assays for detecting Mycobacterium species.J Clin Lab Anal. 2019;33(1):e22645.
30. Rahman MM, Rahim MR, Khaled A, et al.Molecular Detection and Differentiation of Mycobacterium Tuberculosis Complex and Non-tuberculous Mycobacterium in the Clinical Specimens by Real Time PCR. Mymensingh Med J. 2017;26(3):614-620.
31. Patel B, Yagnik VD. Clinical and laboratory features of intestinal tuberculosis.Clin Exp Gastroenterol. 2018;11:97-103.
32. Mehta V, Desai D, Abraham P, Gupta T, Rodrigues C, Joshi A, et al.Do additional colonoscopic biopsies increase the yield of Mycobacterium tuberculosis culture in suspected ileo-colonic tuberculosis?Indian J Gastroenterol. 2018;37(3):226-30.
33. Diriba G, Kebede A, Yaregal Z, Getahun M, Tadesse M, Meaza A, et al.Performance of Mycobacterium Growth Indicator Tube BACTEC 960 with Lowenstein-Jensen method for diagnosis of Mycobacterium tuberculosis at Ethiopian National Tuberculosis Reference Laboratory, Addis Ababa, Ethiopia. BMC Res Notes. 2017;10(1):181.

34. Coppola M,Ottenhoff TH.Genome wide approaches discover novel Mycobacterium tuberculosis antigens as correlates of infection, disease, immunity and targets for vaccination. Semin Immunol. 2018;39:88-101.
35. Da Silva RJ, da Silva Corrêa R, Sardella IG, de Paulo Mulinari AC,Mafort TT, Santos AP, et al.IgA and IgG antibody detection of mycobacterial antigens in pleural fluid and serum from pleural tuberculous patients. BMC Immunol. 2019;20(1):36.
36. Meier NR, Jacobsen M, Ottenhoff THM, Ritz N.A Systematic Review on Novel Mycobacterium tuberculosis Antigens and Their Discriminatory Potential for the Diagnosis of Latent and Active Tuberculosis. Front Immunol. 2018;9:2476.
37. Ihama Y,Hokama A, Hibiya K. Diagnosis of intestinal tuberculosis using a monoclonal antibody to Mycobacterium tuberculosis. World J Gastroenterol. 2012;18(47):6974-80.
38. Ince AT, Güneş P, Senateş E, et al.Can an immunohistochemistry method differentiate intestinal tuberculosis from Crohn's disease in biopsy specimens?Dig Dis Sci. 2011;56(4):1165-70.
39. Jørstad MD, Marijani M, Dyrhol-Riise AM, et al.MPT64 antigen detection test improves routine diagnosis of extrapulmonary tuberculosis in a low-resource setting: A study from the tertiary care hospital in Zanzibar. PLoS One. 2018;13(5):e0196723.
40. Purohit MR,Sviland L,Wiker H, et al. Rapid and Specific Diagnosis of Extrapulmonary Tuberculosis by Immunostaining of Tissues and Aspirates With Anti-MPT64. Appl Immunohistochem Mol Morphol. 2017;25(4):282-88.
41. Sharma S, Sheoran A, Gupta KB, Yadav A, Varma-Basil M, et al.Quantitative detection of a cocktail of mycobacterial MPT64 and PstS1 in tuberculosis patients by real-time immuno-PCR. Future Microbiol. 2019;14:223-233.

Tables

Table 1 Clinical, endoscopic, and histological features in the differentiation of CD and ITB

	ITB (n=42)	CD (n=46)	P
Clinical presentation			
Age, years (mean±sd)	30.5±11.8	31.5±12.2	0.83
Sex(male:female)	20:22	27:19	0.10
Abdominal pain	33(78.6%)	41(89.1%)	0.10
Diarrhea	29(69.0%)	37(80.4%)	0.09
Perianal disease	1 (0.03%)	10(21.7%)	0.01
Endoscopic features			
Longitudinal ulcer	6(14.3%)	17(36.9%)	0.01
Annular ulcer	25(59.5%)	7(15.2%)	0.00
Aphthous ulcers	4(9.5%)	11(23.9%)	0.05
Cobblestone appearance	5(11.9%)	10(21.7%)	0.11
Pseudopolyps	14(33.3%)	11(23.9%)	0.12
Histological features			
Caseous granuloma	10(23.8%)	0(0.00%)	0.00
Non-caseous granulomas	17(40.5%)	12(26.1%)	0.07
Lymphocyte aggregation	19(45.2%)	17(37.0%)	0.13

Table 2 Detection and comparison of MTBC in intestinal tissues of ITB and CD patients

	ITB (n=42)	CD (n=46)	
Xpert MTB/RIF□□□	14□33.3%□	0	
Tissue Culture□□□	9□21.4%□	0	
Acid-fast Staining Smear□□□	5□11.9%□	1□2.17%□	

Table 3 Clinical evaluation of detection of MTBC in intestinal tissues

	sensitivity (95%CI)	specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
Xpert TB/RIF	33.3% (20.0-49.6)	100% (90.4-100)	100% (73.2-100)	62.2% (50.1-73.0)
Tissue culture	21.4% (10.8-37.2)	100% (90.4-100)	100% (62.9-100)	58.2% (46.6-69.1)
Acid-fast Staining Smear	11.9% (4.5-26.4)	97.8% (87.0-99.9)	83.3% (36.5-99.1)	54.9% (43.5-65.8)

Table 4 Expression of intestinal tuberculosis proteins in patients with ITB and CD

	ITB (n=42)	CD (n=46)	P
Psts1 □□□	4□9.52%□	9□19.56%□	0.1
MPT64 □□□	17□40.48%□	9□19.56%□	0.02
Ag85B □□□	10□23.81%□	11□23.91%□	0.2
EAST-6 □□□	11□26.19%□	15 □32.61%□	0.15

Table 5 Detection of MTBC by intestinal tuberculosis protein combined with Xpert MTB/RIF

	ITB (n=42)	CD (n=46)
Xpert MTB/RIF□+□or MPT64□+□	21□50.0%□	9□19.56%□
Xpert MTB/RIF□-□and MPT64□-□	21□50.0%□	37□80.44%□

Figures

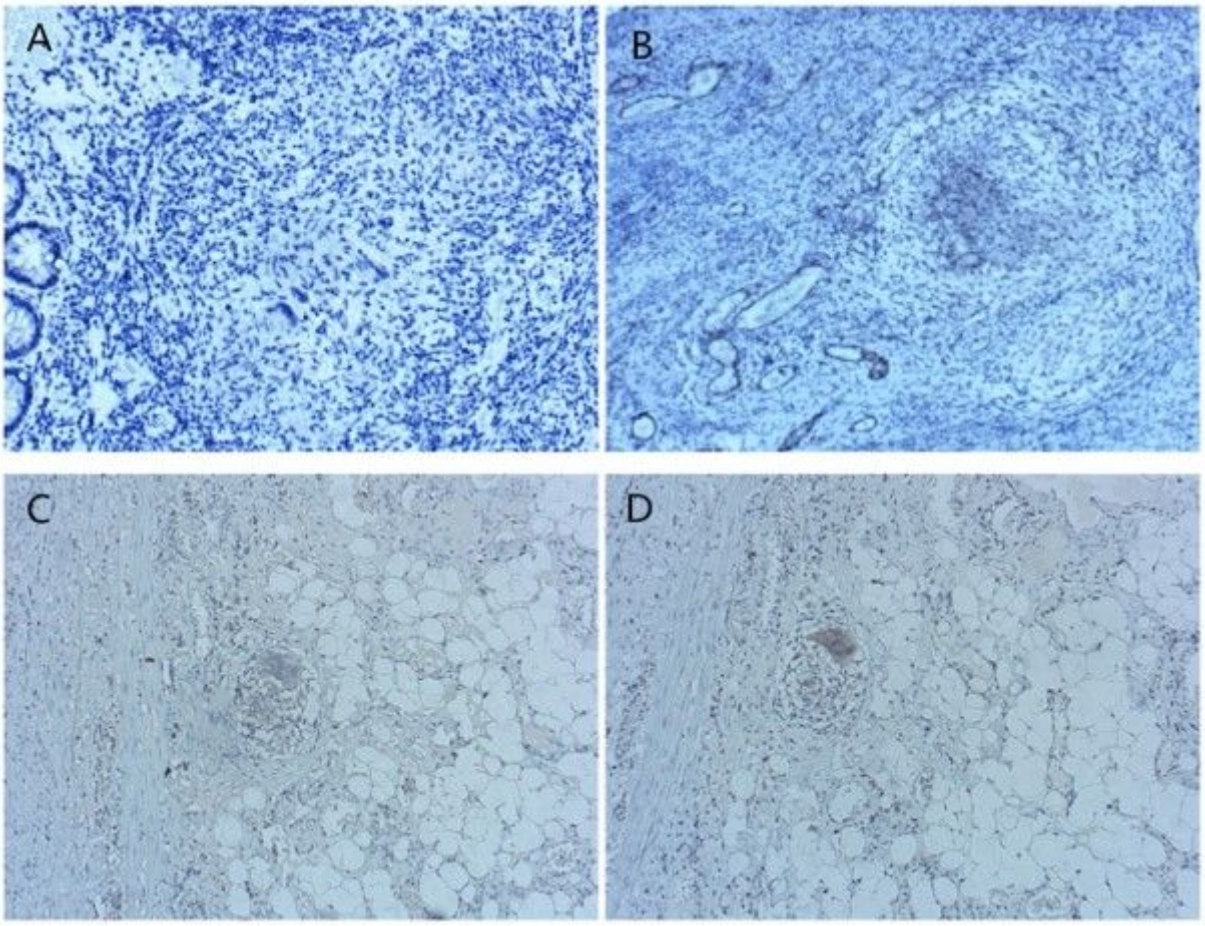

Figure 1

four tuberculosis proteins immunohistochemical view of the colonic specimen of ITB or CD patients (× 200). A: Psts1; B: MPT64;C: Ag85B; D: EAST6