POLYMERASE CHAIN REACTION TARGETING MULTIPLE SITES IN EARLY DETECTION OF ABDOMINAL TUBERCULOSIS

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ABSTRACT

Diagnosis of abdominal tuberculosis is challenging as the specimens are paucibacillary in nature. Polymerase chain reaction (PCR) is a diagnostic tool increasingly being used for the detection of Mycobacterium tuberculosis in such specimens. The efficiency of PCR as a molecular method in the detection of Mycobacterium tuberculosis depends on the targeted gene. Use of multiple targets in detection of Mycobacterium tuberculosis by PCR increases the sensitivity of the assay. We report a 57 year old male patient who presented to the tertiary care centre with increased frequency of defecation associated with mucous and pain. The clinical findings were found to be suggestive of tuberculosis. Tissue biopsy following colonoscopy was sent for histopathological investigations and for PCR targeting two multi-copy genes, IS6110 and TRC4. The histopathological findings were consistent with tuberculosis. PCR was performed with the biopsy sample by targeting IS6110, TRC4 and MPB64. PCR targeting IS6110 and MPB64 were negative whereas PCR targeting TRC4 was found to be positive. This case report suggests that PCR for tuberculosis must be performed by targeting multiple genes to increase the case finding among patients with tuberculosis.

Keywords: *Abdominal tuberculosis, Colonoscopy, Colonoscopy, IS6110, MBP64, MTB, PCR, TRC4.*

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INTRODUCTION

Tuberculosis (TB) affects one third of the global population[1] and is of major global health concern because of its diverse clinical presentations and the diagnostic dilemma it presents. Extra-pulmonary tuberculosis is difficult to diagnose as it deals with paucibacillary specimens. The delay in diagnosis affects disease management and increases the risk for mortality.[2] Therefore, there is an urgent need to reduce the time to laboratory diagnosis of TB and initiate early treatment. In this report, we present the laboratory diagnosis of an immunocompetent patient with abdominal tuberculosis using polymerase chain reaction (PCR) targeting multiple genes.

CASE REPORT

A 57 years old male patient presented to the tertiary care centre with increased frequency of defecation associated with mucous and pain. Frequency of defection was reported to be 5 to 7 episodes per day, was not associated with blood or foul smell. He also reported history of fever with evening rise of temperature for 1 week, cough for 1 month, weight loss for 3 months and loss of appetite for 3 months. There was no history of nausea, vomiting or diarrhoea. Though the patient reported having undergone colonoscopy elsewhere, he did not know the reason and did not give any history of prolonged medication. He had surgery for haemorrhoids, 3 years back. The patient was an alcoholic and smoker. He is not a known case of diabetes mellitus, hypertension or pulmonary tuberculosis. He is not allergic to any food or drugs. There was no family history of tuberculosis.

Tissue biopsy specimen was received in the laboratory following colonoscopy for histopathological investigations and for polymerase chain reaction (PCR) targeting two multi-copy genes, *IS6110* and *TRC4* which are specific for *Mycobacterium tuberculosis*. The impression from colonoscopy revealed a high suspicion of gastro-intestinal tuberculosis (Fig. 1).

The histopathology examination showed extensive ulceration with ileal mucosa and necrotising granulomatous inflammation which was constant with tuberculosis (Fig. 2).

Prior to PCR assay, the DNA from the specimen was extracted using a standard kit based method (MTB amplification kit, Bangalore Genei) and a nested PCR was performed targeting *IS6110* gene. The same DNA was used for performing conventional PCR targeting *TRC4* gene. An internal control was included in the assay to confirm that the result was not false negative due to amplification inhibition. The PCR targeting *IS6110* gene was found to be negative (Fig. 3a), while the PCR targeting *TRC4* gene was found to be positive (Fig. 3b). Another PCR targeting *MPB64* gene was performed with same DNA, but was found to be negative (Fig. 3c).

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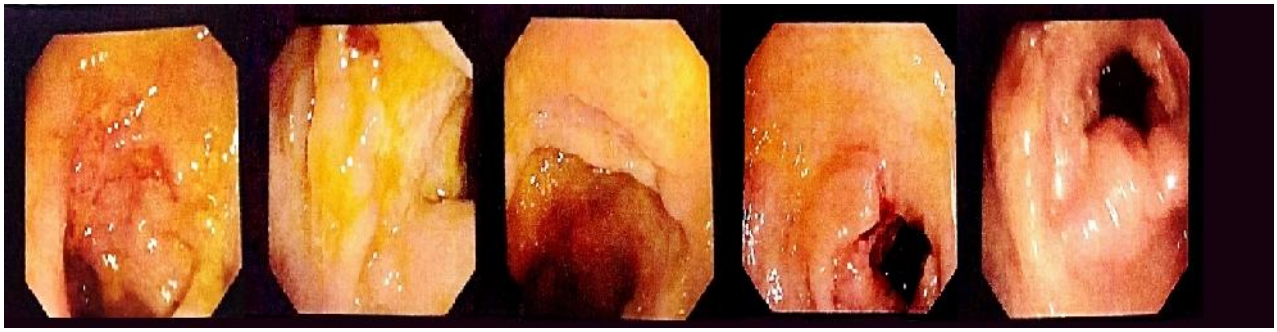

Fig. 1: The colonoscopy findings:The colonoscopy picture shows presence of multiple ulcers in the terminal ileum and colon region.

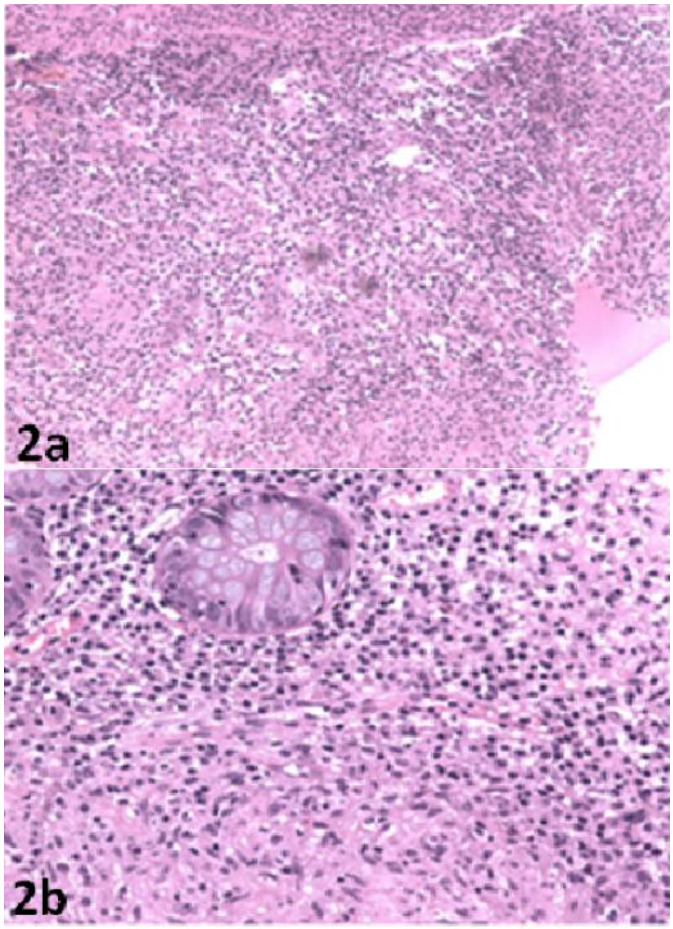

Figure 2a & 2b: The histopathological findings of the biopsy specimen under low and high power objectives: (H & E staining, 2a.100x 2b.400x). The tissue segment shows presence of granulomatous inflammation with epitheloid cells and lymphocytes, granulomas seen along with gastric gland under 2a. Low power objective and 2b. High power objective.

DISCUSSION

Abdominal tuberculosis (TB) may involve any part of the gastrointestinal tract and with various clinical manifestations such as chronic abdominal pain, fever, weight loss, changes in bowel habits, abdominal mass, ascites, nausea, and vomiting.[3] It can also mimic other diseases and conditions, including inflammatory bowel disease, malignancy, and infectious diarrhoea and it is often difficult to diagnose.[3]

Therefore, clinicians often request for a combination of tests such as radiologic, endoscopic, histological findings, microbiological and molecular techniques. The microbiological culture method is considered the gold standard for the diagnosis of tuberculosis. Solid culture method takes 4-6 weeks, while the liquid culture method takes few days.[1,4] It has been reported that combination of histopathological and microbiological culture techniques can increase the diagnostic rate in over 60 % of patients.[5]

In abdominal tuberculosis, tissue from the affected area is the specimen of choice but these extra-pulmonary specimens are paucibacillary in nature.[1,4] Therefore, most laboratories have adapted to the rapid molecular techniques such as polymerase chain reaction. These assays are rapid and accurate even in paucibacillary conditions. It is a nucleic acid amplification based assay, which target specific genes from the genome of *Mycobacterium tuberculosis*. Several target sites such as *IS6110, TRC4, MPB64, 65kDa, 38kDa, 85B sequence, Pab, devR, GCRS, MTB40*, etc have been evaluated for detection of *Mycobacterium tuberculosis*.[1,2,6,7,8] Among these, *IS6110* the repetitive / multi-copy gene (present in multiple copies in the genome) is the most common gene targeted.[1] Similarly, *TRC4* is a multi-copy gene discovered and patented (235025) by scientists from National Institute for Research in Tuberculosis, Chennai, India.[6] A study reported that targeting the multi-copy gene *IS6110* performed better than targeting the other single copy genes.[7] But, some strains from South India lack insertion sequence *IS6110* in their genome.[1] Thus, targeting only *IS6110* may lead to false negatives and mis-diagnosis. In this report, the negative PCR for *IS6110* region in the absence of amplification inhibition suggests that it could be due to strains that lack even a single copy of *IS6110*.

Few studies have suggested using an additional target site such as *TRC4* to increase the diagnostic performance of PCR.[6,9] The negative PCR targeting *MPB64* explains the limitations of targeting a single copy gene; similarly, detection of *Mycobacterium tuberculosis* DNA by PCR for *TRC4* reveals the importance of targeting multiple sites, especially the multi-copy genes.

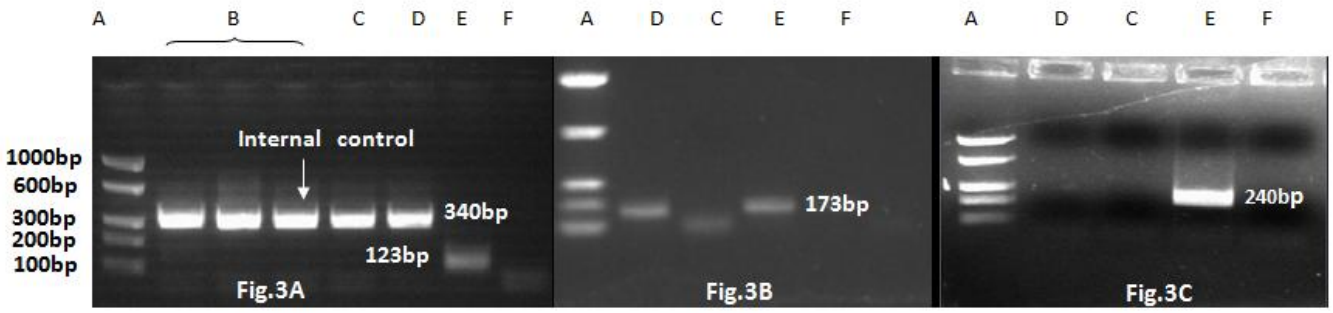

Fig.3A IS6110 gel image. **Fig.3B. TRC4 gel image.** **Fig.3C. MPB64 gel image**

A. M.wt marker B.Samples C.Negative Control D. Colonoscopy Biopsy E.Positive Control F. NTC (No template control)

Gel image shows Fig.3a.PCR targeting IS6110 gene was found to be negative. Fig.3b. PCR targeting TRC4 gene was found to be positive and Fig.3c. PCR targeting MPB64 gene was performed with same DNA, was found to be negative.

We conclude that the genome of *Mycobacterium tuberculosis* is wide and complex and requires detection of multiple PCR target sites. The accuracy of PCR assay mainly depends on these target sites. Moreover, PCR positivity can help to rule-in TB in an endemic region like India even when other laboratory tests are not conclusive. We suggest PCR for tuberculosis must be performed by targeting multiple genes to increase the case finding among patients with tuberculosis.

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