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# GeneXpert in stool: Diagnostic yield in Intestinal Tuberculosis

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ABSTRACT

*Background:* Diagnosing intestinal (Luminal) tuberculosis is challenging due to limited yield of diagnostic modalities like CT scan, colonoscopy with blind ileal biopsies. GeneXpert MTB/RIF (Xpert) assays for diagnosing tuberculosis have been performed in the body secretions with excellent results. Its yield in stool is tested in this study.

*Objective:* The study aims to evaluate the yield of GeneXpert assay in stool of suspected cases of intestinal tuberculosis.

*Methods:* Hundred patients with suspected intestinal tuberculosis underwent routine biochemical tests, radiological investigations, colonoscopy with caecal and blind ileal biopsies for histopathology. Fresh stool samples were collected, processed for DNA extraction, tested using 2:1 ratio of GeneXpert reagent to sample to give positive or negative results for Mycobacterium tuberculosis and Rifampicin resistance.

*Results:* Out of hundred participants, 52% were female. Mean age was 28.21 ± 12.13. CT scan and colonoscopy findings suggestive of TB were present in 47% and 43% participants respectively. GeneXpert in stool was positive in 20% cases. Considering mucosal biopsy with histopathology of intestinal specimens as diagnostic of abdominal Tuberculosis, sensitivity and specificity of GeneXpert was 39.1% and 85.7% respectively.

*Conclusion:* Stool GeneXpert assay offers an alternative approach to detect intestinal tuberculosis rapidly with good diagnostic accuracy. Although it cannot replace the AFB culture and histopathology but contribute for early diagnosis and management.

## 1. Introduction

In Pakistan, with an estimated of 510,000 new TB cases emerging each year and approximately 15,000 drug resistant TB cases are reported in every year, is ranked fifth among high-burden countries worldwide and it accounts for 61% of the TB burden in the WHO Eastern Mediterranean Region. The country is also estimated to have the fourth highest prevalence of multidrug-resistant TB (MDR-TB) globally. Key reasons for emergence of drug resistance form of TB include delays in diagnosis, unsupervised, inappropriate and inadequate drug regimens, poor quality of medications, poor follow-up and lack of a social support program for high-risk populations.[1]

Intestinal tuberculosis (luminal) is an uncommon sequelae, occurring in less than 1% of local population.[2] It can affect any part from mouth to the anus but ileocecal tuberculosis accounts for more than 75% cases of abdominal TB.[3] Abdominal Tb is the 4th most common site of extra pulmonary Tuberculosis after lymphatic, genitourinary, bone and joints,miliary and meningeal TB.[4] Abdominal tuberculosis (TB) although less common in western countries, constitutes a major public health problem in developing countries and associated with significant morbidity and mortality.[5] Studies from Pakistan, West Africa and Turkey found abdominal TB to be a disease of young adults especially among females.[6]

Abdominal Tuberculosis may be primary infection or secondary following reactivation, usually from a primary pulmonary focus.[7] Difficulties arise in confirming diagnosis of intestinal tuberculosis (luminal), where it is a primary presentation, because of its limited diagnostic modalities and their yield.[8] Ultrasound abdomen, Barium studies, CT Scan mainly suggest diagnosis because tuberculosis may mimic Crohn's disease or intestinal malignancies.[9] Many patients refuse invasive procedures like colonoscopy with blind Ileal biopsies, which may be involved in 84% of cases of intestinal tuberculosis (luminal).[10] Access to higher up involvement of jejunum & other part of ileum is also not possible because of limited availability of enteroscopy. Thus, there

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is a need for a diagnostic test, which is noninvasive, readily available and gives a satisfactory diagnostic yield.

There is always need for simple and rapid diagnostic tools for early initiation of treatment in high- burden countries.[11] GeneXpert MTB/RIF (Xpert) assay, an automated molecular test for an automated molecular test for *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF), uses heminested real-time polymerase chain- reaction (PCR) assay to amplify an MTB specific sequence of the *rpoB* gene, which is probed with molecular beacons for mutations within the rifampin-resistance determining region.[12,13]

GeneXpert Tuberculosis is a NAAT (Nucleic Acid Amplification Test), which is now performed in sputum and different body fluids with an increased sensitivity and specificity and also with the advantage of diagnosis of Rifampicin Resistance within two hours.[11] Stool specimens in children with pulmonary tuberculosis have been examined by this technique with a higher sensitivity and specificity.[12] As children are unable to expectorate sputum, which they usually ingest.

In intestinal tuberculosis (luminal), there are three types of lesion hypertrophic, ulcerative and ulcerohypertrophic lesions.[13] Ulcerative lesions having caseating granulomas can shed mycobacterium in stool as we hypothesize. Taking this hypothesis into consideration we undertook a prospective evaluation of the MTB/RIF test in stool to determine its yield in the intended targeted population & compared with the biopsy and histopathology specimens obtained on colonoscopy.

The aim of this study is to get benefit of this test in patients with suspected intestinal tuberculosis (luminal) by examining their stool samples for GeneXpert TB and evaluate its yield.

## 2. Objectives

To evaluate the yield of stool for GeneXpert in suspected cases of intestinal TB (luminal).

## 3. Methods

### 3.1. Patient population

All patients above 18 years of age, admitted with the presenting complains of fever (body Temp > 37′c), chronic unexplained diarrhea (duration lasting for more than 4 weeks), weight loss (10% or more loss of body weight over the last 3 months), abdominal pain were included in this study. Patients with either positive or negative chest radiographic findings for Pulmonary Tuberculosis and also positive or negative HIV serology were enrolled. While patients already taking Anti tuberculous therapy (ATT), Antiretroviral (HAART therapy), diagnosed cases of inflammatory bowel disease or malignancy and those who did not consent to be a part of the study were excluded.

### 3.2. Study design

This was a prospective Cross sectional study review of 100 consecutive admitted at Medical wards of Civil Hospital Karachi from January 2017 to January 2019 with suspected Intestinal Tuberculosis on clinical grounds with above mentioned complains were included in this study.

#### 3.2.1. Radiological tests

All patients had chest radiographs (PA view), Ultrasonography of the whole abdomen, Computed Topography Scan (CT) of the whole abdomen and pelvis. Single experienced radiologist was involved in reporting the findings to avoid person to person bias.

(a) Patients were considered as having positive chest X-ray findings of tuberculosis if they had either infiltration, apical fibrosis, cavitation or lymphadenopathy. Patients with normal X-ray Chest with clinical suspicion of Intestinal Tuberculosis were labelled Normal Chest X-ray.
(b) On ultrasonography, patients with either lymphadenopathy, ascites or thickened bowel loops were labelled as suspected intestinal tuberculosis.
(c) On Computed Topography scan of the abdomen and pelvis, patients were considered positive for abdominal Tuberculosis if they had one or more of the following findings which included enlarged lymph nodes, mesenteric or bowel wall thickness or ascites.
(d) On Colonoscopy patients were suspected to have intestinal tuberculosis if they had any one of the following findings like cecal ulcers, cecal scars, ileal ulcers or scars or inflammation.
(e) On mucosal biopsies (caecal and blind ileal) , the presence of inflammation with granulomas both caseating and non caseating were considered as diagnostic of intestinal Tuberculosis.

- *Stool for GeneXpert*:
  Stool samples were collected in containers and stored at −20oC if the testing took longer than two hours. Prior to GeneXpert testing, stool samples were processed for DNA extraction and then tested using 2:1 ratio of GeneXpert reagent in the sample. Testing is carried out on the MTB/RIF test platform (GeneXpert, Cepheid), which integrates sample processing and PCR in a disposable plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification and amplicon detection which precedes the addition of a bactericidal buffer to the sample before transferring a defined volume to the cartridge.[14] The MTB/RIF cartridge is then inserted into the GeneXpert device, which provides results within 2 h. All tests were done in Dow Diagnostic Research Laboratory to maintain uniform standard.
- *Sputum examination*:
  Patients of suspected Intestinal Tuberculosis having pulmonary symptoms such as prolonged cough (lasting for 4 weeks) or hemoptysis and positive findings on chest radiographs, three samples of sputum were collected early morning for AFB (smear, C/S and AFB GeneXpert) and was labelled as positive and negative in the presence and absence of AFB respectively.
- *Mucosal biopsy*:
  Showing presence of granulomas either caseating or noncaseating was labelled Biopsy Positive and was taken as diagnostic of Intestinal Tuberculosis to calculate the sensitivity and specificity of stool for GeneXpert.
- *HIV serology* was done by ELISA technique by taking 2cc of blood after aseptic technique.

## 4. Statistical analysis

Statistical analyses was performed using the Statistical Package for the Social Sciences (SPSS) Version 22.00. The data of categorical variables is presented as frequencies and percentages; the data of continuous variables is presented as the mean and standard deviation. Chi -square test was used for categorical variables. p-value of $< 0.05$ will be considered significant. Sensitivity and specificity of the test was calculated.

## 5. Results

Out of 100 participants enrolled, 52% were female. The mean age was 28.21 + 12.13. Seventy four 74 (74%) of patients presented with fever and 86 (86%) presented with diarrhea lasting for more than 4 weeks. (Table 1). On clinical examination lymphadenopathy was present in 27 (27%) and ascites was present in 30 (30%) participants. HIV serology was positive in 11% participants and none of them were taking Antiretroviral therapy. Stool GeneXpert was positive in 20% participants.

**Table 1**
Association of clinical features of patients suggestive of abdominal TB with confirmed diagnosis of abdominal TB on Stool GeneXpert.

| Symptoms (n%) | Stool For Gene Expert Positive n(%) | p Value |
|---|---|---|
| Fever (74) | 18 (24.3) | **0.05** |
| Abdominal Pain (71) | 18 (25) | **0.02** |
| Diarrhea (86) | 19 (22) | 0.17 |
| Weight Loss (84) | 18 (21.4) | 0.33 |
| Cough (30) | 9 (30) | 0.08 |

chi square test was applied between Clinical Features and stool GeneXpert.
$P \leq 0.05$ is considered as significant.

### 5.1. X-ray chest

34 patients had x-ray findings favoring tuberculosis. Amongst these 34 patients only 6 had stool Xpert positive, while the remaining 14 patients who had stool GeneXpert positive had normal chest x-ray.

### 5.2. Ultrasound abdomen

On abdominal Ultrasound, thickened bowel loops were present in[22] (22%) patients while ascites was present in 47% patients.

### 5.3. CT Scan abdomen and pelvis

CT scan findings suggestive of abdominal TB were present in 47% participants. (Table 2). Specific findings included bowel thickening which was present in 46%, mesenteric thickening in 24% and enlarged lymph nodes in 64% patients. (Table 3)

### 5.4. Coloncoscopy

Colonoscopy findings suggestive of intestinal Tuberculosis were present in 43% of the patients (Table 2). caecal scars, caecal ulcers and ileal inflammation present in 9%, 24% and 25% of patients respectively. (Table 3)

### 5.5. HIV serology

11 out of 100 patients were found to be HIV reactive and none of them were taking anti retroviral therapy. Out of those 11 patients only one patient had GeneXpert positivity i.e. 9%.

### 5.6. Sputum for AFB + VE/GeneXpert positive

Four patients were found to have sputum AFB and GeneXpert positive. Among these four patients only one had Stool GeneXpert positivity. Two out of the 20 stool GeneXpert positive patients did not produce any sputum, while 17 stool GeneXpert positive patients had negative sputum microbiological results.

Amongst the 20 patients positive for stool Xpert, 4 patients had CT scan findings suggestive of Abdominal Tuberculosis ($p = 0.006$) while colonoscopy findings were positive in 9 patients ($p = 0.517$). Histopathology was also positive in 9 patients, who were stool Xpert positive. ($p = 0.013$).

Amongst the 23 patients with biopsy-positive intestinal Tuberculosis i.e. histopathology showing granulomatous inflammation with or without necrosis 9 had positive stool for GeneXpert, the overall sensitivity of GeneXpert in stool samples was 39.1% while the specificity was 85.7% ($p = 0.013$) with a positive and negative predictive value of 45% and 82.5% respectively.

**Table 3**
Findings of patients with abdominal TB compared with stool for gene Xpert.

| | Positive findings n(%) = =100 | Stool Xpert Positive n(%) = =20 | p value |
|---|---|---|---|
| RADIOGRAPHIC FINDINGS ON CT SCAN | | | |
| Enlarged lymph nodes | 64(64) | 11(55) | 0.247 |
| Mesenteric Thickening | 24(24) | 01(5) | 0.019 |
| Bowel thickening | 46(46) | 04(20) | 0.008 |
| TYPES OF LESIONS ON COLONOSCOPY | | | |
| Cecal scars | 09(9) | 01(5) | 0.427 |
| Cecal Ulcers | 24(24) | 03(15) | 0.228 |
| Ileal inflammation | 25(25) | 07(35) | 0.191 |
| BIOPSY AND HISTOPATHOLOGY | | | |
| Granulomatous Inflammation with necrosis | 16(16) | 08(40) | 0.003 |
| Granulomatous Inflammation without necrosis | 07(7) | 01(5) | 0.574 |

Chi square test was applied between Diagnostic findings and positive stool GeneXpert testing.
$P < 0.05$ is considered significant.

## 6. Discussion

Abdominal TB is the 4th most common site of extra pulmonary Tuberculosis after lymphatic, genitourinary, bone and joints, miliary and meningeal TB[4] while the incidence of abdominal TB is steadily on the rise especially in developing countries, symptoms can easily be overlooked due to similarity with other infectious causes . Difficulty in having a confirmed diagnosis arise when patients do not have both clinical and laboratory evidence of pulmonary disease. Due to limited yield of imaging and endoscopic techniques to confirm intestinal tuberculosis, there is a need for developing a test to identify this treatable disease timely. With the availability of GeneXpert assay, giving an early diagnosis of TB with detecting Rifampicin resistance in sputum and different extrapulmonary tissues with increased sensitivity and specificity, this study was designed. This study is of its first kind to test stool sample for GeneXpert assay in patients with suspected intestinal TB (luminal) with and without pulmonary disease.

Awasthi et al.[15] reported abdominal pain, anorexia and fever as the most common presenting symptoms in a retrospective analysis of patients who underwent laparotomy for intestinal obstruction, while in our study diarrhea and weight loss were the most common symptoms present in 86% and 84% patients respectively, followed by abdominal pain and fever as it was the main selection criteria amongst our patients. Sarkar et al. also described diarrhea as the main clinical presentation along with pain. [16]Coexistence of HIV with pulmonary and extrapulmonary TB is extremely common and a possible explanation of this coexistence is the decreased immune response in patients suffering from HIV. The clinical features of patients suffering from both HIV and

**Table 2**
Diagnostic performance of diagnostic modalities in diagnosis of abdominal TB.

| Diagnostic modality | Findings suggestive of TB n (%) = =100 | Biopsy positive $n = 23$ | Sensitivity* | Specificity * |
|---|---|---|---|---|
| CT scan | 47(47) | 10 | 43.5% | 51.9% |
| Colonoscopy | 43(43) | 13 | 56.5% | 61% |
| Stool for GeneXpert | 20(20) | 09 | 39.1% | 85.7% |

*Biopsy and histopathology is used as the reference standard.

Abdominal TB presented with worse clinical presentations and grave clinical outcomes.

Patient with HIV and low CD4 count presents with chronic diarrhea and weight loss. In such situation diagnosing intestinal TB is very important before starting HAART therapy. Eleven out of 100 patient had HIV serology positive and among them one patient tested positive for GeneXpert on stool examination.

Although abdominal Tuberculosis can affect any age group with no gender predisposition, it is usually found in young adults. In a study conducted by Kapoor et al.[17], most affected patients were between 21 and 40 years of age with a slight female predominance, which was similar to our study, where mean age of the patients was 28.21 ± 12.13 with 52% female patients.

It was found in our study that routine laboratory and radiological tests have limited diagnostic value in the diagnosis of abdominal Tuberculosis with no statistical significance in biopsy proven patients with ESR levels and chest radiographs.

When comparing positive GeneXpert in stool sample with sputum analysis it was found in our study that out of 4 sputum AFB positive patients only 1 had positivity in stool sample. Although stool samples have readily been used in the determination of positive TB cultures & GeneXpert in children[12]who could not produce sputum, no studies have been conducted to assess stool for GeneXpert in sputum positive patients.

Historically radiology has been the best early investigation for diagnosis of abdominal TB by either ultrasound and CT scan. Most common findings in CT abdomen in suspected TB patients is mural thickening affecting the ileocecal region, which may be involving the ileum only or caecum but more frequently involving both regions simultaneously along with peritoneal fat stranding. In more severe cases there may be grossly thickened & adherent loops with lymphadenopathy (regional) mesenteric thickening giving a mass affect around ileocecal junction.[18,19]

Abdominal lymphadenopathy on imaging is the most common manifestation of abdominal TB, seen in 55–66% of patients, and may or may not be associated with other abdominal organ involvement.[20] Although only on imaging, etiology cannot be proven unless we proceed towards lymph node biopsy. Our study also showed abdominal lymph nodes in 46(46%) patients, however majority of the patients showed a combination of two or more findings on CT scan.

On colonoscopy caecal ulcers and inflammation were found in 49% of patients. Predominant findings of transverse ulceration and patulous ileocecal valve on colonoscopy have also been reported by Patel B and Yagnit VD.[10] Similarly, Prabhu PR et al.[21] reported ileal or ileocecal involvement with nodularity and ileocecal valve destruction, presence of circumferential ulcers, short-segment strictures and mucosal nodularity in colon with or without pseudopolyps but these may be overlapping features of Crohn's disease.

A retrospective study in diagnosed patients of intestinal TB, colonoscopy helped to confirm the diagnoses in 77% of patient. The most common findings were ileocecal inflammation followed by ulcer & ileal stricture.[22]

Histopathology of intestine has been the gold standard investigation for the diagnosis of intestinal TB with findings such as epithelioid cell granulomas with caseation are characteristic histological features of Tuberculosis. Thickening of the wall in the hypertrophic and ulcerohypertrophic types was mostly due to extensive granulomatous inflammation and, in a few instances, due to submucosal fibrosis, edema, and serosal fibrosis[13] but these were the findings of biopsy specimens on laparotomy of intestinal perforation patients. In our study 23% patients had granulomas on colonoscopy biopsy with 16% showing caseation and 7% showing noncaseating granulomas & among these 16 patients [8](40%) has stool for GeneXpert positive. Lee YJ et al.[23] on histology of colonoscopic biopsy specimen found caseous granulomas or AFB smear in 23% of cases.

So, it can be postulated that there is more chance of having GeneXpert positivity where there is caseation necrosis, which probably may be more liable to shed bacteria in stool. The possibility that positive GeneXpert in stool could be due to swallowed sputum with AFB could be a possibility but results from our study suggest otherwise; as out of 4 patients who had positive sputum cultures and GeneXpert only one had stool GeneXpert positive while 17 of the stool GeneXpert positive patients had negative GeneXpert in sputum.

On the other hand, 7 patients had granulomas without necrosis, which may mimic Crohn's disease; among these only 1 patient had stool for GeneXpert positive. A larger group of patients having nonspecific colitis 31(33.3%) on biopsy, 7(35%) had stool for GeneXpert positive. According to our study taking colonoscopy biopsy as reference standard the sensitivity of GeneXpert assay in stool was found to be 39% while specificity was found to be 85.7%. In the light of these findings stool for GeneXpert can be used as an inexpensive, readily available and a rapid initial diagnostic modality with a high specificity.

In different studies PCR assays[24] and GeneXpert[12] have been used in tissues (biopsy specimen).Although Xpert test have been performed on mucosal biopsy specimen taken on colonoscopy with decreased sensitivity but 100% specificity in two different studies.[25,26] No published data is available in which stool for GeneXpert is tested in patients with intestinal Tuberculosis. Our study estimated the sensitivity of GeneXpert in stool samples as 39.1% which is slightly higher,while specificity was lower than a study conducted by Bellam et al.[25] but they tested mucosal biopsy specimens for GeneXpert and the sensitivity, specificity, negative predictive value, positive predictive value, and accuracy of GeneXpert-Mtb/Rif was calculated as 32% (CI: 14.95–53.50%), 100% (78.2–100), 46.88% (40.27–53.59%), 100 & 57.50 (40.89–72.89%) respectively while Kumar et al.[26] also predicted the sensitivity, specificity, positive predictive value, and negative predictive value of the GeneXpert MTB/RIF assay was 8.1%, 100%, 100%, and, 64.2%, respectively, but this was again tested on mucosal biopsy specimens taken on colonoscopy.

Our study was a cross sectional study which aimed to assess the yield of Stool GeneXpert in comparison with the preexisting diagnostic modality of colonoscopy with mucosal biopsy and histopathology which has been used historically for diagnosing Intestinal Tuberculosis.

Limitation of this study is that biopsy specimen was not tested for GeneXpert or AFB culture, which may have given a better yield in diagnosing intestinal tuberculosis.

We can recommend that in patients having radiological suspicion of TB and in whom colonoscopy biopsy show nonspecific colitis or noncaseating granulomas, we can have this test stool for GeneXpert to have a confirmed diagnosis of intestinal TB.

## 7. Conclusion

GeneXpert testing in stool samples offers an alternative approach to diagnose Intestinal Tuberculosis that show rapid results with fairly good diagnostic accuracy. Although these tests cannot replace the conventional AFB smear, culture identification or histopathological observations but they contribute significantly for an early diagnosis of abdominal Tuberculosis and exert a positive impact on the early diagnosis and management of the disease.

## Declaration of Competing Interest

None.

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