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Role of Tissue GeneXpert MTB/RIF Assay for Diagnosis of Intestinal Tuberculosis

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Abstract

Tuberculosis (TB) is a significant disease caused by *Mycobacterium tuberculosis,* affecting the respiratory, gastrointestinal, lymphoreticular, central nervous, musculoskeletal, reproductive, and hepatic systems. Intestinal TB, primarily affecting the ileocecal region, can affect any part of the gastrointestinal tract. Common symptoms include abdominal pain, fever, diarrhea, constipation, blood in stool, and potential complications. Intestinal TB diagnosis is challenging due to limited diagnostic techniques. Typical approaches include radiological scans and histopathological examinations, but their sensitivity limits their accuracy. Therapeutic response is proposed as a significant factor. Microbiological analysis, including polymerase chain reaction and acid-fast bacilli, is another measure. The GeneXpert Tuberculosis Nucleic Acid Amplification Test has low sensitivity for intestinal TB diagnosis. The National TB Control Program in Bangladesh focuses on timely identification and prevention of TB cases, with the aim of establishing GeneXpert as a diagnostic tool for intestinal tuberculosis, despite limited studies in Bangladesh.

A cross-sectional study was conducted on 55 suspected intestinal TB patients aged 18 years and above in Dhaka, Bangladesh. Data was collected through face-to-face interviews and a questionnaire. Participants were given anti-TB drugs for a two-month trial and underwent colonoscopy with biopsy. Histologically confirmed and non-caseating colitis patients were diagnosed with intestinal TB. Patients not responding to the trial were diagnosed as without TB. Additional mucosal biopsies were sent for the GeneXpert MTB/RIF assay. Obtained data was analyzed by using Statistical Package for Social Sciences version 23.

A study involving 45 individuals diagnosed with intestinal TB found that common symptoms included abdominal pain, loss of appetite, weight loss, and fever. The study also found a significant relationship between ESR and intestinal TB, with a mean of 56.6 mm. Colonoscopy revealed that 60% of patients had mucosal ulceration, while histopathological findings showed granuloma in 84.4% of cases. Ultrasonography revealed abnormal findings in 33 participants, with 54.5% having ascites and 30.3% having bowel wall thickening. The study found that GeneXpert was effective in detecting intestinal tuberculosis, with positive outcomes in 11 instances and 34 false negatives. All ten individuals without the disease were accurately classified as negative. The GeneXpert test had a sensitivity of 24.4% and a specificity of 100%, making it a valuable tool for detecting the condition.

The study, establishes the role of GeneXpert in diagnosing intestinal TB, despite the absence of endoscopy, contrast radiology tests, Acid-Fast Bacilli culture, and a small sample size of suspected cases in a tertiary health facility.

Keywords: Intestinal TB • GeneXpert • Anti-TB drugs • Colonoscopy • Acid-fast bacilli

Introduction

Tuberculosis (TB) is a disease of historical significance; having afflicted humanity for over four millennia caused by the bacteria *Mycobacterium tuberculosis* and is transmitted through airborne means between individuals [1]. The primary organ system impacted by TB is the respiratory system which is called pulmonary TB, with additional typically affected systems including the gastrointestinal system, the lymphoreticular system, the central nervous system, the musculoskeletal system, the reproductive system, and the hepatic system all of these are called extrapulmonary TB (EPTB) [2]. TB in the gastrointestinal system also known as Intestinal TB predominantly affects the ileocecal region, but can affect any part of the gastrointestinal tract, presenting as ulcerative, hypertrophic, or combined patterns [3]. Though most patients with intestinal tuberculosis are asymptomatic, the most common symptoms include abdominal pain, weight loss, fever, diarrhea or constipation, blood in the stool, abdominal mass, and in severe cases, complications such as gastrointestinal bleeding, obstruction, perforation, fistula formation, and malabsorption may occur [4].

According to the Global TB Report 2022, an estimated 10.6 million people were infected with tuberculosis in the year 2021 [5]. The prevalence of EPTB is estimated to range from 15% to 20% of the total tuberculosis patients [6]. Pakistan, which is the fifth largest TB burden country, showed that about 30% of all the notified TB was EPTB among which 21% were abdominal in origin [7]. In India, 20% were EPTB of total TB cases, of this, near about 13%

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were abdominal. In Bangladesh an estimated 360,000 people developed tuberculosis with 44,000 deaths, this has put TB as the number 3 cause of death among communicable, maternal, neonatal & nutritional disease in the year 2020 [8].

Due to the limited diagnostic techniques available and their yield, intestinal TB presents challenges in confirming the diagnosis when it is the major presentation [9]. Though clinical indicators, laboratory findings, endoscopic findings, radiographic signs, bacteriological findings, and histological discoveries are not considered definitive for diagnosing intestinal TB, the typical approach involves conducting thorough radiological such as computed tomography scan and histopathological examinations of tissue from biopsy to establish a diagnosis [10]. Nevertheless, the diagnostic accuracy of both methods is hindered by their limited sensitivity, thus the consideration of therapeutic response has been proposed as a significant factor in the diagnosis of intestinal TB [3]. Another measure of diagnosis is microbiological analysis which includes polymerase chain reaction, acid-fast bacilli, and mycobacterial culture [11]. The GeneXpert Tuberculosis Nucleic Acid Amplification Test is now used in sputum and other bodily fluids [12]. But it has a low sensitivity for the diagnosis of intestinal TB as evidenced by few studies [3].

The primary focus of the National TB Control Programme in Bangladesh is to prioritize the timely identification and prevention of TB cases [13]. The timely identification and commencement of antitubercular therapy and surgical intervention are crucial in mitigating adverse health outcomes and reducing the risk of death due to intestinal TB [10]. However, there are limited studies in Bangladesh that tried to establish GeneXpert as a diagnostic tool for intestinal TB. This study aimed to observe the role of tissue GeneXpert MTB/RIF assay for the diagnosis of intestinal tuberculosis in Bangladesh.

Methodology

This was a cross-sectional study among the 55 suspected intestinal TB patients based on clinical features, and aged ≥ 18 years attending the gastroenterology department of a tertiary hospital in Dhaka, Bangladesh from August 2019 to July 2020. The patients came from across the country, and if any of the participants were already on anti-TB drugs or if any female participants were pregnant they were excluded from the study. Suspected intestinal tuberculosis patients were those with strong clinical suspicion of intestinal tuberculosis, such as having abdominal pain, chronic diarrhea, per-rectal bleeding, features of intestinal obstruction (abdominal pain, vomiting, transient visible abdominal lump) or malabsorption, the feeling of abdominal lump and associated with evening rise of temperature/fever, weight loss, night sweat, and clinical examination findings include lymphadenopathy, doughy abdomen, and right iliac fossa mass [14]. Data was collected through a face-to-face interview using a pre-tested, semi-structured, interviewer-administered questionnaire with prior informed consent about the aim and procedure of the study. All the suspected intestinal TB participants were given anti-TB drugs for therapeutic trial for 2 months and underwent colonoscopy with biopsy which was sent for histopathology with AFB stain. A colonoscopy was done after adequate bowel preparation with a colonic lavage solution of polyethylene glycol or mannitol 20%. Multiple mucosal biopsies, including six to eight fragments from lesion areas, are fixed in formalin and sent for histopathology. All histologically confirmed (granuloma with caseation) and those histologically non-caseating and non-specific colitis patients who were responding to a therapeutic trial of two months of anti-TB drugs were diagnosed as having intestinal TB. And those with histologically non-caseating and non-specific colitis patients who were not responding to two months therapeutic trial of anti-TB drugs were diagnosed as without TB. Another six to eight fragments of mucosal biopsies, taken during colonoscopy in normal saline were sent for GeneXpert MTB/RIF assay.

Obtained data was analyzed by using Statistical Package for Social Sciences version 23. For the variable of age in years, the closest integer value was used. Continuous variables are expressed as mean ± standard deviation (SD). Categorical variables expressed as percentages. In the case of a normal distribution, the continuous variables compared by t-test. Categorical variables were compared by chi square test, and $p < 0.05$ was considered to be significant. Calculation of sensitivity, specificity, positive predictive value, and negative predictive value of the GeneXpert MTB for diagnosis of intestinal tuberculosis was done.

The study was conducted after approval from the ethical review committee. Written informed consent was taken. The participants had the freedom to refuse to participate or withdraw at any point from the study. The tests were conducted free of cost, other than that any other compensation was not given, and this was informed. There was invasive procedure involved for blood collection, colonoscopy with biopsy, and was done ensuring aseptic measures. In the event of any evident infections due to the study procedure, we assured the participants that adequate facilities would be provided. Anonymity and confidentiality were maintained given the highest priority.

Results

Among the 55 suspected intestinal TB patients attending the gastroenterology department of a tertiary hospital in Dhaka, Bangladesh, intestinal TB was confirmed by clinical signs and symptoms, biopsy histopathology, and trial therapeutic anti-TB drug in 81.8% (n=45) and the rest (18.2%) was considered as not having intestinal TB.

Table 1 compares individuals with Intestinal Tuberculosis (TB) to individuals without TB in terms of age and sex. The mean age of participants with intestinal TB was 34.6 years. For age, there is no statistically significant difference (p=0.539), and for sex, there is also no significant difference between males and females in both groups (p=0.949).

Table 2 shows that common intestinal TB-related symptoms like abdominal pain, loss of appetite, and weight loss were found among all the participants in both with and without intestinal TB groups. Fever was almost universal in both, with a slightly lower rate in the Intestinal TB group (95.6%) compared to the group without Intestinal TB (100%). Notably, per rectal bleeding was

Table 1. Age and Sex of the participants (n=55).

Variables		Intestinal Tuberculosis (n=45)	No intestinal Tuberculosis (n=10)	p-value
Age	Mean±SD*	34.6±13.2	37.5±15.7	0.539a
Sex	Male	23 (51.1)	5 (50.0)	0.949b
	Female	22 (48.9)	5 (50.0)	

*SD = Standard Deviation
[a]Obtained by unpaired t-test
[b]p-value obtained by chi-square test

Table 2. Clinical symptoms, signs, and history of tuberculosis (n=55)*

Variables	Intestinal tuberculosis n=45 n(%)	No intestinal tuberculosis n=10 n(%)
Fever	43(95.6)	10(100.0)
Per rectal bleeding	5(11.1)	4(40.0)
Abdominal pain	45(100.0)	10(100.0)
Loss of appetite	45(100.0)	10(100.0)
Weight loss	45(100.0)	10(100.0)
Nausea/vomiting	39(86.7)	6(60.0)
Cough	8(17.8)	0(0.0)
Diarrhoea	5(11.1)	1(10.0)
Constipation	11(24.4)	4(40.0)
Anaemia	44(97.8)	7(70.0)
Ascites	18(40.0)	0(0.0)
Right iliac fossa mass	3(6.7)	1(10.0)
Past h/o tuberculosis	5(11.1)	0(0.0)
Family h/o tuberculosis	4(8.9)	0(0.0)

*Multiple response

significantly more common in the No Intestinal TB group (40%) compared to the Intestinal TB group (11.1%).

Table 3 shows that patients with intestinal tuberculosis exhibited a significant difference in mean of erythrocyte sedimentation rate (ESR) (56.6±17.7 mm vs. 40.4±16.1 mm, p=0.010) and Mantoux Tuberculin Skin Test (MT-test) reaction (9.0±6.5 mm *vs.* 2.0±2.2 mm, p=0.001) compared to those without the condition. While chest X-rays and plain X-rays of abdomen didn't significantly differ between the two groups. But, Ultrasonography (USG) of abdomen results showed statistically significant difference (73.3% vs. 30.0%, p=0.009). The USG findings were exclusive to intestinal tuberculosis cases (p=0.001). Furthermore, the site of lesions observed by colonoscopy differed significantly (p=0.001) between those with intestinal TB and those without it. Histopathological investigation showed significant (p=0.001) differences between the two groups. The majority (84.4%) of the patients with intestinal TB were found to have granuloma in the biopsy derived from the lesion site through colonoscopy.

In Table 4, it is evident that GeneXpert gave true positive results in 11 cases with intestinal tuberculosis, while 34 cases were missed (False negative). Additionally, all 10 patients without intestinal tuberculosis were correctly identified as negative (True negative), with no false-positive results.

Table 5 reveals the sensitivity, specificity, positive predictive values, negative predictive values, and accuracy of GeneXpert. The GeneXpert test was shown to have a sensitivity of 24.4% and a specificity of 100%, making it useful for identifying intestinal tuberculosis. For example, when a positive result was achieved, it was highly trustworthy in verifying the existence of the condition. Nonetheless, the negative predictive value (NPV) amounted to 22.7%, indicating constraints in ruling out the illness in situations where a negative outcome was obtained. Notwithstanding these drawbacks, the test's overall accuracy was 38.2%, meaning that only a tiny percentage of people with and without the illness were accurately categorized.

Table 3. Laboratory test findings of the participants (n=55)

Variables	Intestinal tuberculosis	No intestinal tuberculosis	p-value
	n=45	n=10	
	Mean±SD	Mean±SD	
Erythrocyte sedimentation rate (mm)	56.6±17.7	40.4±16.1	0.010[a]
Mantoux tuberculin skin test (mm)	9.0±6.5	2.0±2.2	0.001[a]
	n (%)	n (%)	
Chest X-ray			
Normal	38(84.4)	10(100.0)	0.182[b]
Abnormal	7 (15.6)	0(0.0)	
Plain X-ray Abdomen			
Normal	40(88.9)	10(100.0)	0.269[b]
Abnormal	5(11.1)	0(0.0)	
Colonoscopy Finding			
Mucosal ulcerations	27(60.0)	7(70.0)	0.403[b]
Nodules	8(17.8)	0(0.0)	
Ulcero-nodular lesions	5(11.1)	3(30.0)	
Multiple lesion	5(11.1)	0(0.0)	
Site of lesion			
Terminal ileum	4(8.9)	0(0.0)	0.001[b]
Caecum	5(11.1)	7(70.0)	
Ileo-cecal region	25(55.6)	0(0.0)	
Ascending colon	1(2.2)	1(10.0)	
Rectum	1(2.2)	0(0.0)	
Affecting multiple sites	9(20.0)	2(20.0)	
Histopathology			
Granuloma	38(84.4)	0(0.0)	0.001[b]
No granuloma	7(15.6)	10(10.0)	
Ultrasonography of abdomen			
Normal	12(26.7)	7(70.0)	0.009[b]
Abnormal	33(73.3)	3(30.0)	
Ultrasonography findings (n=36)			
Dilated bowel loops	3(9.1)	0(0.0)	0.001[b]
Bowel wall thickening	10(30.3)	0(0.0)	
Ascites	18(54.5)	0(0.0)	
Enlarged abdominal lymph nodes	2(6.1)	3(100.0)	

[a]p-value obtained by unpaired t-test
[b]p-value obtained by fisher exact test

Discussion

This was a cross-sectional observational study conducted in a tertiary hospital in Dhaka, Bangladesh during the period of August 2019 to July 2020 among 55 patients suspected of intestinal tuberculosis. Based on clinical symptoms, signs, histopathological findings, and response to therapeutic anti-TB drugs 45 were diagnosed as having intestinal TB.

This study found that the mean age of the intestinal TB participants was 34.6 years. A study in Thailand from 2009-2020 reported the mean age of intestinal TB was 49 years [15]. A retrospective study among intestinal TB patients in China from 2008 to 2021 found the mean age was 43.2 years [16]. Another study in China reported a mean age of 37.3 years [17]. In India a prospective study conducted from 2011-2013 observed 33.7 years mean age among the participants [18]. In Pakistan, the mean age was found to be 33 years [19]. All these studies and the present study showed almost similar mean age of above 30 years. The current study revealed that the common symptoms were abdominal pain(100.0%), loss of appetite (100.0%), weight loss (100.0%), and fever (95.6%) among those diagnosed with intestinal TB. The study in Thailand observed lower percentages than the current study findings of Bangladesh but yet the most prominent symptoms were abdominal pain (52.5%), weight loss (52.5%), and fever (44.9%) [15]. Similarly, in China the study by Zeng et al. observed that among patients with intestinal TB common clinical symptoms were weight loss (67.4%), followed by abdominal pain (65.2%), and fever (39.1%) [16], although another study in china showed a slightly higher rates of abdominal pain (88.2%), weight loss (75.3%), asnd fever (57.6%) which were the most common reported symptoms [17]. In India among intestinal TB patients, the most common symptoms also were abdominal pain (76.0%), weight loss (60.9%), and fever (72.5%) [18]. So was in Pakistan where abdominal pain was found among 93% and fever was prevalent among 64% [19]. In a study in Turkey, the most complaints of the patients with intestinal TB were abdominal pain (51.2%), and weight loss (51.2%) [20]. The rate of clinical presentation varies between this current study and other studies, but all of them show that the most common symptoms were the same among the patients with intestinal TB despite geographical variations.

Table 4. Performance of GeneXpert with reference to clinical findings, histopathology, and therapeutic trial of anti-tubercular drug (n=55).

Variables	Intestinal tuberculosis	No intestinal tuberculosis
	n=45	n=10
GeneXpert		
Positive	11 (True positive)	0(False positive)
Negative	34 (False negative)	10 (True negative)

Table 5. Sensitivity, specificity, positive predictive values, negative predictive values, and accuracy of GeneXpert.

Performance of GeneXpert	Percentage
Sensitivity	24.4
Specificity	100
Positive predictive value	100
Negative predictive value	22.7
Accuracy	38.2

The current study provided evidence that there was a significant (p=0.010) relation between ESR and intestinal TB and the mean was 56.6 mm. A study in China also observed elevated ESR levels among the patients with intestinal TB where the mean was 38.4 mm [16]. Another study in China also observed elevated ESR among 62.5% of their participants [17]. While in Turkey elevated ESR was found among 64% of patients with intestinal TB [20]. There was also significant (p=0.001) relation with MT-test induration in mm and among patients with intestinal TB and the mean was 9 mm. A study in England found that only 22% of intestinal TB patients tested positive by MT-test [21].

The present study performed colonoscopy among all the participants and found that 60% of the patients with intestinal TB had mucosal ulceration though there was no significant relation found. Similar finding was observed in India where about 58% were diagnosed as having mucosal ulcers [18]. A slightly higher rate was observed in the study in Pakistan where mucosal ulceration by colonoscopy was found among 80% [19]. Similar finding was observed in China reporting 79.6% as having mucosal ulceration [17] and the same rate was also reported in Thailand (79.6%) [15].

Significant (p=0.001) relation was identified between the site of lesion and intestinal TB in the present study with the most observed site for lesion among patients with intestinal TB being the ileo-cecal region (55.6%). In China, a study reported most common site of involvement was the ileocecal area (76.1%) [16], and another study reported that 65.6% were in the same region [17]. In India a study found the ileocecal valve was involved in 84.05% of the intestinal TB participants [18].

Histopathological findings were also significant (P=0.001) among participants and 84.4% of the intestinal TB patients were found to have granuloma. Similar finding was observed in Thailand where granuloma was present in 78.4% of patients [15]. A lower rate was observed in a study in Pakistan which found common histological features on biopsy specimens was the presence of granuloma in 68% of patients [19]. In China, the proportion of granuloma was 70.6% by histopathology in a study [17], and another study found it was among 44.4% of participants [16], in India it was seen among about 71% [18].

In the ultrasonography statistical significance was seen among the participants. On USG 33 participants with intestinal TB had abnormal findings among which 54.5% were found to have ascites followed by bowel wall thickening (30.3%). The most common USG finding was also ascites In Pakistan (79%) [19], and in Turkey (36.3%) [20].

The present study found that GeneXpert is effective in detecting intestinal tuberculosis, with positive outcomes in 11 instances and 34 false negatives. All ten individuals without the disease were accurately classified as negative. The GeneXpert test showed a sensitivity of 24.4% and a specificity of 100%, making it a valuable tool for detecting the condition. However, the NPV is 22.7%, suggesting limitations in excluding the condition when a negative result is achieved. Despite these limitations, the test has an overall accuracy rate of 38.2%. Same specificity (100.0%) was also found in a study in India, although the sensitivity (32%), NPV (46.9%), and accuracy (57.5%) were a bit higher [22]. A previous study showed that the GeneXpert test has true diagnostic potential with moderate sensitivity of 63 to 73% for tissue [23]. Another study also showed the sensitivity of the GeneXpert test to detect TB in extra-pulmonary sites to be 53 to 95% [24]. This current study found a lower sensitivity of the GeneXpert test as a diagnostic tool to detect intestinal TB.

This study has some limitations. The study did not perform an endoscopy to observe and collect samples from the upper gastrointestinal tract or small intestine. Contrast radiology tests were not performed. The study was unable to perform a culture of Acid-Fast Bacilli or by Polymerase Chain Reaction. The study was conducted among a small number of suspected intestinal TB who sought treatment in the selected tertiary health facility, so the results are not generalizable. Despite the limitations, this study establishes the role of GeneXpert in cases of intestinal TB diagnosis.

Conclusion

As GeneXpert is an inexpensive and rapid test for intestinal TB diagnosis, this should be routinely performed alongside MT-test, colonoscopy, histopathology, and USG. All the tested positive GeneXpert from biopsy tissue individuals can be considered as intestinal TB. Although the negative result may not exclude intestinal TB and further evaluation is required.

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Conflict of Interest

There is no Conflict of interest.

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