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Diagnostic Accuracy of Xpert Mtb/Rif Assay in Stool Samples in Intrathoracic Childhood Tuberculosis

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Abstract

Background: The study aims at finding out usefulness of Xpert in stool samples in children, as they usually swallow their sputum. It also simultaneously compares the results of stool Xpert with Xpert, smear and culture in gastric lavage and sputum samples.

Materials and methods: A diagnostic accuracy study included children (<15 years) with probable tuberculosis. Induced sputum, gastric aspirate and stool samples each were subjected to Xpert, AFB stain and culture. Diagnostic utility of stool Xpert was calculated with reference to liquid culture in sputum or gastric aspirate as gold standard.

Results: The study included 100 children. Stool Xpert was positive in 4 cases (4%). Overall cultures positivity was 26%. The total yield including culture and Xpert (sputum or gastric aspirate) was 45%. The sensitivity of stool Xpert was 11.54% and specificity 98.65% as compared to culture. There was association of stool Xpert positivity with sputum AFB (p 0.024), sputum Xpert (p 0.004) and gastric aspirate Xpert (p 0.039), while there was no significant association with X-ray pattern or malnutrition.

Conclusion: Stool sample for Xpert cannot replace gastric aspirate and induced sputum for diagnosis, and hence should not be used as a first line test.

Keywords: Childhood tuberculosis; Xpert Mtb/Rif assay; Stool samples

Introduction

Childhood tuberculosis presents unique challenges in diagnosis. The paucibacillary nature of the disease makes bacterial isolation difficult; hence diagnosis often relies on clinical and epidemiological characteristics, positive tuberculin skin test and radiological findings [1]. However, these are rather imperfect tools as radiology is subject to inter-observer variability and tuberculin test serves as a marker of infection rather than disease [2]. For microbiological tests to be effective, the sample must be representative of lower respiratory tract. These are also difficult in paediatric population as they are unable to produce deep cough for adequate sputum [3]. Gastric aspirate as a sample has the drawbacks that it is minimally invasive and necessitates fasting state [4]. Stool as a sample for intrathoracic tuberculosis has been explored on the premise that children usually swallow their sputum [5]. It is a convenient to obtain, non-invasive sample compared to sputum or gastric aspirate.

Smear microscopy gives quick results, but has a low sensitivity [6] whereas culture has a good sensitivity albeit requires long time [7]. Xpert Mtb/Rif (Xpert) test is a molecular method which has revolutionised the diagnosis of tuberculosis. In children with suspected drug resistant tuberculosis or cases with HIV and tuberculosis, Xpert has been recommended as the first line test by WHO [6]. However there are only a handful of studies regarding the same in stool samples in intrathoracic childhood tuberculosis with each of them giving discrepant results [3,7-9].

The detection of paediatric tuberculosis in a timely, efficient and effective manner through improvement in existing diagnostic Methods are a priority for global research. Stool molecular studies aim to find a rapid method of diagnosis, in a population where the paucibacillary nature of conventional samples does not allow immediate detection of the bacillus. This study thus explores the utility of stool Xpert in Indian patients with childhood intrathoracic tuberculosis. It further adds to the literature by providing a simultaneous comparison of stool Xpert with sputum and gastric aspirate Xpert, smear and culture as well. Our study includes ambulatory patients, rather than hospitalized ones; thus making it more relevant to community based setting.

Materials and Methods

The study was conducted at a tertiary care centre (All India Institute of Medical Sciences, New Delhi, India) on consecutive children (6 months to 15 years age) attending the paediatric tuberculosis clinic (outpatient department) between December 2014 and July 2016. A sample size of 100 was derived assuming the likely yield of stool Xpert as 70% with precision of 15%, confidence interval of 95% and possibility of culture confirmed tuberculosis as 40% among probable tuberculosis. The consensus definition by Graham et al. [10] was used in defining probable tuberculosis. A case was excluded if consent was not given, patient had received anti-tubercular therapy or Isoniazid prophylaxis for more than 4 weeks, had signs of upper airway

obstruction or saturation of <92% on room air. The study was approved by institutional ethics committee (AIIMS, Delhi).

Patients presenting with fever >38°C for more than one week, cough for more than 2 weeks, or constitutional symptoms which persisted after treatment with antibiotics were subjected to chest radiograph. If the radiograph showed features consistent with intra-thoracic tuberculosis like hilar or mediastinal lymphadenopathy, consolidation, cavity, miliary pattern or pleural effusion as judged by expert paediatrician and the patient had either positive tuberculin skin test (≥ 10 mm induration) or history of contact in last 2 years with a case of tuberculosis, the child was labelled as probable tuberculosis. Exclusion criteria were applied to these cases, and consent was taken. Their clinical profile was reviewed and details of physical examination and nutritional status were recorded. The children were then asked to report for collection of gastric lavage and induced sputum on two consecutive days and bring stool samples when they come. In case of delay in bringing stool samples, they were asked to refrigerate the stool sample. Gastric aspirate and induced sputum were collected by paediatricians as per standard protocol [11]. Sputum induction was done only if expectoration was not possible.

Laboratory processing

Paired samples were collected from all patients on consecutive days except for stool (only a single stool sample was collected). These were pooled before processing. All the samples were subjected to Xpert, sputum and gastric lavage were subjected to smear microscopy and liquid culture (MGIT), while stool was cultured on Lowenstein Jensen (LJ) medium. The sputum and gastric lavage samples were decontaminated with NALC-NaOH method prior to smear and culture. Standard protocols for smear, culture and Xpert were followed [12,13].

About 200 mg of stool was homogenised in 1 mL Phosphate Buffer saline (PBS). It was then left for some time to allow the undissolved particles to settle. The supernatant was then processed by decontamination using the NALC-NaOH method. Xpert test was carried out by mixing 2 mL of this sample with the buffer provided in 2:1 ratio as per the manufacturer's instruction [14]. It was then manually invert mixed twice within 15 minutes at room temperature. 2 mL of this was subsequently loaded in the cartridge containing the buffer, reagent and probes and was inserted in the Xpert module. The test was read at 90 minutes. The result of 'Indeterminate' was considered as negative for the purpose of analysis. The technicians performing culture were blinded from the results of Xpert test and vice-versa.

Statistical analysis

Statistical analysis was performed using Stata version 14. The variables for diagnostic accuracy were calculated using mathematical formulas for sensitivity, specificity, positive and negative predictive value. The associations between stool Xpert positivity with positivity in smear, sputum or lavage Xpert were calculated using Fisher's exact test.

Results

About 100 eligible cases of probable tuberculosis were included in the study (Table 1) with the median age of 11 years (41% males). HIV testing was done only in cases with a strong clinical suspicion (as required by the treating physician), and 3 out of the 6 cases tested were positive for HIV infection. Most of the cases had either no or milder grades of malnutrition, only 9 cases had moderate to severe malnutrition (IAP grades III and IV). All the included cases had abnormal chest radiograph consistent with tuberculosis. Among the 56 cases who had hilar widening on radiograph, 27 had no associated parenchymal lesion. Isolated parenchymal lesion were seen in 15, well defined consolidation in 11, cavity in 7, pleural effusion in 9, and miliary pattern in 6 cases.

	Number (Total 100)
Age (years)	Mean 9.98 ± 3.67 (median 11 years)
	0-5 years-16
	5-10 years-26
	10-15 years-58
Sex	Males-41
	Females-59
Nutrition (Indian Academy of Paediatricians Classification)	Not malnourished -34
	Grade 1-35
	Grade 2-22
	Grade 3-5
	Grade 4-4
Radiological features	Parenchymal only-15
	Only hilar widening-27
	Parenchymal and hilar-29

	Consolidation-11
	Cavity-7
	Effusion-9
	Miliary-6
	Others-2
HIV status	Positive-3
	Negative-3
	Not done-94

Table 1: Baseline characteristics of enrolled patients.

	Total	Positive	Negative	Indeterminate/Contaminated
Xpert				
Stool	100	4	94	2
Sputum	100	27	72	1
Gastric aspirate	100	37	62	1
Culture				
Stool	64	0	62	2
Sputum	99	18	77	4
Gastric aspirate	100	25	73	2
Smear microscopy				
Sputum	100	7	93	
Gastric aspirate	100	6	94	

Table 2: Results of microbiological tests in the study population.

	Sensitivity	Specificity	PPV	NPV
Stool Xpert	11.54%	98.65%	75.00%	76.04%
	(2.45-30.15%)	(92.70-99.97%)	(24.60-96.50%)	(73.37-78.52%)
Sputum Xpert	73.08%	89.19%	70.37%	90.41%
	(52.21-88.43%)	(79.80-95.22%)	(54.25-82.63%)	(83.28-94.69%)
Gastric aspirate Xpert	76.92%	77.03%	54.05%	90.48%
	(56.35-91.03%)	(65.79-86.01%)	(42.44-65.25%)	(82.33-95.09%)
PPV-positive predictive value, NPV-negative predictive value, MGIT-Mycobacterial Growth Indicator tube (Liquid culture)				

Table 3: Diagnostic accuracy of Xpert test with reference to MGIT positivity in sputum or gastric aspirate.

Stool Xpert was positive in 4 cases, negative in 94 and indeterminate in 2 cases (Table 2). Of these, 1 was Rifampicin (Rif) indeterminate and other 3 Rif sensitive. Stool Xpert was positive in 3/26 (11.53%) of patients with culture positivity while in 1/74 (1.3%) among patients with negative culture. The sensitivity, specificity, positive and negative predictive value of stool Xpert when compared to MGIT positivity in either sputum or gastric aspirate was 11.54%, 98.65%, 75.00%, and 76.04% respectively (Table 3). As only 4 cases were stool Xpert positive, the significance of factors associated with it cannot be attributed much value. Stool Xpert was nonetheless significantly associated with positive smear microscopy (p 0.024), positive Xpert on sputum (p 0.004) or gastric lavage (p 0.039). No significant correlation was found

between stool Xpert positivity and X-ray pattern, BCG vaccination status, Mantoux positivity, HIV status and malnutrition.

Xpert detected Mtb in 27 cases and 37 cases in induced sputum and gastric aspirate respectively. The overall yield of sputum and gastric aspirate Xpert combined was 40. When this was compared with positive cultures, Xpert detected 21 on 26 culture positive cases (80.77%), and 19 of culture negative cases (25.67%). The sensitivity of Xpert with reference to culture was 76.92% and 73.08% (Table 3) in gastric aspirate and sputum samples respectively. All the four cases with positive stool Xpert had both sputum and gastric aspirate Xpert positivity.

MGIT done on sputum and gastric aspirate was positive in 18 and 25 cases respectively. When the report from either was considered, culture detected 26 cases out of 100. Both sputum and gastric aspirate culture were positive in 3 of the 4 cases with stool Xpert positivity. The fourth patient was followed up clinically and had shown response to anti-tubercular treatment, thus was a truly a case of tuberculosis clinically.

Smear microscopy for acid fast bacilli (Table 2) was positive in 7 cases in sputum samples while in only 6 cases in gastric aspirate. The combined result of positive smear microscopy from sputum or gastric aspirate detected 11 cases. When compared with Xpert, smear microscopy was positive in 8/40 (20%) with Xpert positivity while 3/60 (5%) with Xpert negative results. When smear positivity was compared to stool Xpert, two cases with positive sputum smear and one case with positive gastric aspirate smear were also positive by stool Xpert. Effectively smear microscopy detected 6 of 26 (23.07%) culture confirmed tuberculosis.

Discussion

This study was done to evaluate the utility of Xpert test in stool samples in childhood tuberculosis. In our study, stool Xpert was positive in 4 cases only. The sensitivity and specificity were 11.54% and 98.65% respectively as compared to culture. Thus stool Xpert detected 3 on 26 (11.53%) of culture positive cases. This does not concur with previous studies (Table 4). In one study [8], stool Xpert was positive in 3 of 4 cases (75%) with positive cultures. While in another study [9], Xpert identified 8 out of 17 (47%) culture positive cases. In this study, among HIV positive, stool Xpert was positive in 4 of 5 (80%) cases, while in HIV negative, the positivity was found in only 4 out of 12 cases (33%). A study in Kenya [3] had stool Xpert sensitivity 100% and specificity 89.36%. However this study compared stool Xpert with smear positivity, which is not the gold standard for comparison. Another study conducted in Egypt [7] among HIV negative children had the sensitivity and specificity of stool Xpert 83.33% and 98.73% compared to sputum culture. The good yield in this study could have been due to high sputum smear positivity (16%) and sputum culture positivity (31%).

Study population	Total cases, Median age	HIV positive cases	Sputum smear positive	Reference standard	Yield of reference standard	Stool Xpert Sensitivity Specificity
Cape Town+ [8]	17	2 (11%)	?	GA culture	23.50%	75% of culture positive
Africa* [9]	115, 30 months	14.80%	3.47%	Sputum culture	14.78%	47% of culture positive
Kenya [10]	91, 3 years	?	11.30%	Sputum smear	11.30%	100%, 89.36%
Egypt [11]	115	0%	16%	Sputum LJ	31%	83.33%, 98.73%
Our study	100, 11 years	3%	7%	Sputum or gastric aspirate MGIT	26%	11.54%,
+ Also evaluated gastric aspirate Xpert- could detect 75% of culture positive cases, * also evaluated sputum Xpert-could detect 65% of culture positive cases.						

Table 4: Summary of studies on Xpert in stool.

The recovery of tubercle bacilli in stool could be influenced by many factors like additional intestinal source in disseminated tuberculosis, higher sputum bacillary load in cavitary or primary progressive tuberculosis, etc. In patients with HIV positivity and malnutrition, poor immunity may lead to ineffective suppression of the bacillary load and thus an improved yield from stool.

The possible reasons for low positivity rate in our study could be very small proportion of HIV positive cases (3%). None of these HIV positive cases were positive by stool Xpert. In previous studies, it was noticed that all sputum smear positive cases were also positive by stool Xpert [3,7,10]. As our study had low rate of sputum smear positivity (7%), this is also a plausible explanation for low yield of stool Xpert. Another possible explanation could be lack of strict adherence to the stool sample storage guidelines by the patients' relatives.

MGIT positivity for sputum (100 samples) were 18% and for gastric aspirate was 25%. Similar results are seen in other studies. In the study by Mukherjee quoted above, culture positivity from sputum and gastric lavage were 17.9% and 32.5% respectively. In yet another study, cultures for gastric aspirate are positive in 30 to 40% cases [15].

Sputum smear staining was positive in 7 cases in induced sputum, while in 6 of gastric aspirate samples. The relatively low yield is also seen in other studies on paediatric tuberculosis [16,17] where the sputum smear was positive in less than 15% and in less than 10% cases of gastric aspirate[18]. Also our study was conducted in ambulatory patients rather than hospitalised patients. Hence they had a significant proportion of children with milder disease and isolated hilar adenopathy, in whom bacterial yield is less.

Sputum smear could only identify 11% cases while Xpert detected 40% when both induced sputum and gastric aspirate were combined. Thus Xpert detected 4 times as many cases as smear microscopy. This is due to requirement of very little number of bacilli for detection by Xpert as it is a molecular test. This also suggests that Xpert can replace smear microscopy as a first line test. The three cases which were smear

positive but Xpert negative and negative or contaminated culture could represent either contaminated sample or non-tubercular mycobacteria.

The bacteriological yield in other studies [11] usually ranges between 25 to 30%. The inclusion of Xpert lead to this increased yield. Overall Xpert detected 21 on 26 culture positive cases (80.77%), and 19 of culture negative cases, thus making proving it to be very useful in diagnosis of childhood tuberculosis. The possible reasons why gastric aspirate Xpert results superseded culture could be as it can detect even fewer bacilli being a molecular test and that even dead bacilli can be picked up by Xpert testing. Another possible explanation could be that prior to culture, the samples were subjected to decontamination, while Xpert samples (except stool were not). This could have led to washing away of bacilli in the supernatant fluid.

The strengths of the study include prospective recruitment of the cases, uniformity in collecting all samples (gastric aspirate, induced sputum and stool) from each patient, subjecting all of these samples to Xpert and culture, correlation of Xpert with smear positivity and a good diagnostic yield overall 45 cases (45%) with culture yielding 26 cases and Xpert 40 cases. The study was done in ambulatory patients rather than hospitalised cases and is thus more likely to represent the actual population of childhood tuberculosis.

The limitations include the no component of follow up to determine response to treatment, HIV testing not done in all cases, single stool testing, lack of strict adherence by caregivers in storage of stool samples before depositing in lab and pooling samples of two days prior to analysis (storage of the first sample may have decreased the yield). Another limitation could be wider age range and that even older patients were subjected to gastric lavage. However this can be justified as gastric lavage did contribute a significant proportion to diagnosis.

Conclusion

Thus, our study results implicate that stool samples are not very good specimen for detection of intrathoracic tuberculosis in children due to pauci-bacillary nature of childhood tuberculosis, presence of blood or inhibitors in faeces [15], intermittent shedding of bacilli, limited utility in culture negative cases and detection of dead bacilli. Stool Xpert cannot by itself be used as a first line test and should be used only as complimentary test. Our study was an exploratory pilot study and further studies are needed to validate it. The stool as a sample needs to be enhanced by improved methods of collection and storage, concentration techniques, processing two or three serial samples, and combining the test with other modalities like sputum and aspirate to improve diagnostic performance. Further studies should focus on these areas.

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