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Original Article

Utility of Xpert MTB/RIF assay for diagnosis of childhood tuberculosis- Extrapulmonary and Pulmonary

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Abstract
Background: *Xpert MTB/RIF assay is now recommended by WHO for diagnosis of tuberculosis in children but evaluation data is limited.*
Methods: *The study was conducted on 186 consecutive specimens (both respiratory and non respiratory) collected between October 2013 to July 2016 from 171 children having MDR TB or/and clinically suspected tuberculosis. Of the 186 specimens, maximum were sputum 83 (44.6%) followed by gastric aspirate 46 (24.7%), CSF 34 (18.3%), tissue biopsies 8 (4.3%), pleural fluid 6 (3.2%), ascitic fluid 5 (2.6%) and lymph node aspirates 4 (2.2%).*
Each specimen was subjected to smear examination (direct and after decontamination), culture on Lowenstein Jensen (LJ) medium and Xpert MTB/RIF assay.
Results: *Analyses on per-sample basis showed that direct smear was positive in 54 (29.2%), culture in 78 (42.2%) and Xpert MTB/RIF assay in 77 (41.6%) specimens. Overall sensitivity and specificity of Xpert MTB/RIF was 93.6% and 96.2% while that of smear was 69.7% and 98.9% respectively against culture as the gold standard. Of the total 77 specimens positive by Xpert MTB/RIF, rifampicin resistance was observed in 7 (9.1%).*
Conclusion: *Xpert MTB/RIF assay is a promising tool for diagnosis as well as detection of rifampicin resistance because of its high sensitivity, specificity and rapidity. However, as more than 50% of clinically suspected children still remain unconfirmed, further refinement of the test before it can replace the current reference standards for diagnosis is to be looked into.*
Keywords: *Tuberculosis, Children, Xpert MTB/RIF, Multidrug resistant.*

Introduction

Despite being an important public health problem, childhood tuberculosis has remained a neglected disease. According to a WHO estimate, there are over half a million cases & 74000 deaths of children (without HIV infection) worldwide each

year[1]. In India 6-8% of all new cases of tuberculosis (TB) are in pediatric age group[2]. This is probably because unlike the adult TB, pediatric TB faces several diagnostic challenges like paucibacillary nature of infection, non specific clinical presentation with almost an equal affection of both pulmonary & extrapulmonary systems and difficulty in obtaining sufficient quantity of good clinical specimens[3]. All these problems make microbiological diagnosis of the pediatric tuberculosis an uphill task. The best available diagnostic tests are costly and the traditional ones (direct microscopy and culture) are insensitive and slow. Even in facilities with an access to full range of diagnostic tools, *Mycobacterium tuberculosis* (*M. tuberculosis*) is isolated from fewer than half of the children ultimately treated for TB[4]. Recently introduced, Gene Xpert MTB/RIF (Cepheid, USA) assay is a nucleic acid amplification (NAAT) test that can simultaneously identify *M. tuberculosis* complex bacteria and their resistance to rifampicin (RIF). The test was endorsed by WHO for the diagnosis of TB in 2011 but due to limited evaluation data there was no specific recommendation for its use in pediatric cases[5]. In October 2013, an updated systematic review recommended that Xpert MTB/RIF should be used rather than conventional microscopy as the initial diagnostic test in children suspected of having multidrug resistant TB (MDR TB) or HIV associated TB (strong recommendation) and that Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial test in all children suspected of having TB (conditional recommendation acknowledging resource limitations, very low quality of evidence)[6]. However, most of the data on Xpert MTB/RIF for diagnosis of TB in children has come from South Africa and there remains a need for further evaluations in other (diverse) settings. The available data from Indian children is scarce. Therefore, the present study was undertaken to estimate the burden of childhood tuberculosis and to detect rifampicin resistance in pediatric tuberculosis (a surrogate marker for multidrug resistance) in a tertiary care hospital of Punjab (North India).

Material & Methods

The study was conducted on 186 consecutive specimens (both respiratory and non respiratory) collected between October 2013 to July 2016 from 171 children (presenting in paediatrics department) clinically suspected of having tuberculosis or/and MDR TB. Table 1 shows various signs and symptoms that were taken into consideration for inclusion of children in this study. MDR TB was suspected in children having history of contact with MDR TB patient.

The study was approved by the Institutional Ethics Committee. Written and informed consent was obtained from a parent or legal guardian before collecting the specimens.

Of the 186 specimens, maximum were sputum 83 (44.6%) followed by gastric aspirate 46 (24.7%), CSF 34 (18.3%), tissue biopsies 8 (4.3%), pleural fluid 6 (3.2%), ascitic fluid 5 (2.6%) and lymph node aspirates 4 (2.2%).

Specimen processing

The specimens collected according to WHO guidelines for Xpert MTB/RIF were processed in the Microbiology Department. In case of unavoidable delay, the specimens were kept at 2-8^{0}C up to a maximum of 7 days.

A pre-treatment step was adopted depending upon the sample type; non-sterile specimens were decontaminated with standard NALC NaOH (1% final concentration) procedure and concentrated by centrifugation[7]. For sterile specimens, only mechanical homogenisation was performed before resuspension in saline.

Each specimen was subjected to smear examination (direct and after decontamination), culture on Lowenstein Jensen (LJ) medium and Xpert MTB/RIF assay.

a) ZN staining- Two drops of processed sample were placed on a slide and were subjected to ZN staining. It was then microscopically examined for the presence of AFB taking sufficient time[8].

b) Culture- The decontaminated specimen was cultured onto two slopes of LJ medium and incubated at 37^0C for 8 weeks before declaring them sterile. In case of positive culture, growth was confirmed by ZN staining and biochemical reactions[8].

c) Xpert MTB/RIF assay- The protocol was strictly followed according to manufacturer's instructions.

Statistical Analysis

The analyses were presented on a per-sample basis and not on per-patient basis. The difference between the positivity of Xpert MTB/ RIF assay and smear examination was calculated using McNemar test. The sensitivities and specificities of Xpert MTB/RIF assay and smear examination were calculated taking culture on LJ media as gold standard.

Results

Of the 171 children, 15 provided two different types of specimens thus making the total number of specimens processed as 186.

Out of 186 specimens, one showed error in Xpert MTB/RIF and was excluded from the study. Of the remaining 185, 83 (44.9%) were respiratory and 102 (55.1%) were non respiratory. In all, direct smear was positive in 54 (29.2%), culture in 78 (42.2%) and Xpert MTB/RIF assay in 77 (41.6%) specimens. The ratio of smear positivity to Xpert MTB/RIF positivity was 1:1.4 and the difference in positivity of Xpert MTB/RIF and smear examination was statistically significant (p value= <0.001 using McNemar test) (Table 2). Specimen wise positivity showed that Xpert MTB/RIF was positive in 74.7% of respiratory and 14.7% of non respiratory specimens. All the respiratory specimens which were positive on Xpert MTB/RIF also showed growth on LJ medium. However, in non respiratory specimens, culture detected 1% more (15.7%) specimens than those detected by Xpert MTB/RIF (14.7%) (Table 2).

Taking culture as reference standard, over all sensitivity and specificity of Xpert MTB/RIF was found to be 93.6% and 96.2% while that of smear was 69.7% and 98.9% respectively (Table 3). As Xpert MTB/RIF was positive in 93.7% (15/16) and 100% (62/62) of culture confirmed non-respiratory and respiratory specimens respectively, the sensitivity and specificity of Xpert MTB/RIF was found to be 100% each among respiratory specimens and 68.8% and 96.3% among non respiratory specimens respectively (Table 4).

Of the total 77 specimens positive by Xpert MTB/RIF, rifampicin resistance was observed in 7 (9.1%) that included 3 respiratory and 4 non-respiratory specimens.

Table 1 Symptoms and signs taken into consideration based on site of infection*

Site of infection	Symptoms
CNS	Irritability, restlessness, neck stiffness, vomiting, seizures
Gastro-intestinal tract	Abdominal pain, diarrhea alternating with constipation
Cardiorespiratory	Shortness of breath, chest pain, pleural effusion

*Weight loss/ no gain in weight, fever, cough, reduced playfulness, refusal to feed were considered for all kinds of specimens

Table 2 Positivity of specimens by various tests

Specimen	Smear	Culture	Xpert
Respiratory (n=83)	53 (63.8%)	62 (74.7%)	62 (74.7%)
Non respiratory (n=102)	1 (0.9%)	16 (15.7%)	15 (14.7%)
Total	54[a]	78	77[b]

[a,b]- Significant difference with p-value= <0.005

Table 3 Overall Sensitivity and Specificity of Smear and Xpert MTB/RIF taking culture as the gold standard

	No. of specimens	Sensitivity		No. of specimens	Specificity
Smear					
Smear + ; Culture + (TP)	53	69.7%	Smear - ; Culture - (TN)	87	98.9%
Smear - ; Culture+ (FN)	23		Smear + ; Culture- (FP)	88	
Xpert					
Xpert + ; Culture + (TP)	73	93.6%	Xpert - ; Culture - (TN)	100	96.2%
Xpert - ; Culture + (FN)	5		Xpert + ; Culture - (FP)	4	

Table 4 Specimen wise Sensitivity and Specificity of Smear and Xpert MTB/RIF

Type of specimens	Sensitivity		Specificity	
	Xpert MTB/RIF	Smear	Xpert MTB/RIF	Smear
Respiratory	100%	83.8%	100%	95%
Non respiratory	68.8%	7.1%	96.3%	100%

Discussion

Xpert MTB/RIF detected 41.6% (77/185) specimens from clinically suspected children (171) which was significantly more (p-value = <0.001) than those detected by smear examination. Similar findings have also been reported by other authors too[9,10] and indicates that Xpert MTB/RIF is more sensitive for diagnosis of childhood TB.

Culture was positive in 42.2% (78/185) of specimens. Specimen wise distribution showed that while equal number of respiratory specimens was positive by both culture and Xpert MTB/RIF, there was slight discrepancy among the positivity of non-respiratory specimens. Xpert MTB/RIF failed to identify 33.3% (5/15) smear negative, culture positive non-respiratory specimens which collaborates the findings of Pang *et al*[11]. In their study 35% of the smear negative, culture positive gastric lavage specimens were missed by Xpert MTB/RIF. Therefore reliance on Xpert MTB/RIF as initial diagnostic test alone, especially in case of non-respiratory specimens, may result in non/delayed treatment of the tubercular children. On the other hand, Xpert MTB/RIF detected 4.8% (4/83) smear and culture negative non respiratory specimens. As these specimens were from clinically suspected children and included two children having history of contact with tubercular patient, the addition of Xpert MTB/RIF to the pediatric diagnostic algorithm not only increased the overall number of microbiologically detected cases, but also shortened the time for diagnosis.

Specimen wise sensitivity and specificity of Xpert MTB/RIF (Table 4) showed that this test was more helpful in diagnosis of non-respiratory than respiratory specimens (in comparison to smear). Not many studies are available on the accuracy of this test in children. Giang *et al*[4] found it to be more sensitive than smear but less sensitive than culture (MGIT) in a study on HIV negative pediatric TB (both respiratory and non respiratory) cases. Nicol *et al*[10] observed that this test detected twice as many cases as did smear examination in children of pulmonary tuberculosis.

Rifampicin resistance was detected in 7/77(9.1%) specimens positive by Xpert MTB/RIF. An earlier study from our institution has reported an overall resistance of 9.9%[12] showing thereby that resistance in pediatric age group is no less than the overall resistance in our area. However, Giang *et al*[4] from Vietnam have reported comparatively lower resistance (4.3%) in pediatric specimens. All the 7 resistant cases (4 non-respiratory and 3 respiratory) were new except one respiratory specimen which highlights the importance of simultaneous detection and drug susceptibility testing in pediatric population.

The Xpert MTB/RIF (G4 version 5 and software version 4.4a) error rate was acceptably low (0.5%, n=1/186) in this study and is comparable to other studies [4,13].

There were certain limitations of the study. 1) As Xpert MTB/RIF does not make distinction between infection and disease, follow-up of the children should have been done. 2) Because of logistic constrains, we could not perform MGIT and culture was put up only on the solid medium (LJ). 3) Resistance detected by Xpert MTB/RIF was not compared with phenotypic drug susceptibility testing.

Since, for the diagnosis of pediatric tuberculosis, rapid confirmation of the result is of utmost importance, Xpert MTB/RIF assay is a promising tool for diagnosis as well as detection of rifampicin resistance because of high sensitivity, specificity and rapidity. It also serves as a user friendly diagnostic tool. However, as more than 50% of clinically suspected children still remain unconfirmed, further refinement of the test before it can replace the current reference standards for diagnosis is to be looked into.

What is already known	WHO recommended that Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial test in all children suspected of having TB
What this study adds	It further adds to the fact that Xpert MTB/RIF is a sensitive, specific and rapid test. Moreover data from this region of North India was lacking which is provided by this study.

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Conflict of interest: None

References

1. World Health Organisation. Guidance for national tuberculosis programmes on the management of tuberculosis in children second edition. Geneva, Switzerland;2014. http://apps.who.int/iris/bitstream/10665/112360/1/9789241548748_eng.pdf. Accessed 10th August 2017
2. TB India 2007 RNTCP status report. Central TB Division, Directorate General of Health Services, New Delhi 2008; p.32
3. Mathew JL, Vijayasekharan D, Singh S. Is Xpert MTB/RIF assay in gastric lavage aspirate useful for diagnosis of smear-negative childhood pulmonary tuberculosis? Indian Pediatr. 2014;51:1007-11.
4. Giang do C, Duong TN, Ha DT, Nhan HT, Wolbers M, Nhu NT *et al*. Prospective evaluation of GeneXpert for the diagnosis of HIV- negative pediatric TB cases. BMC Infect Dis. 2015;15:70.
5. World Health Organization. Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System - Policy Statement. Geneva, Switzerland: World Health Organization; 2011. WHO/HTM/TB/2011.4.
6. World Health Organization. Automated Real-Time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF Assay for the Diagnosis of Pulmonary and Extrapulmonary TB in Adults and Children. World Health Organization, Geneva, Switzerland: Policy update; 2013.
7. Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, *et al*. Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. Eur Respir J. 2012;40:442-7.
8. Watt B, Rayner A, Harris G. Mycobacterium. In: Collee JG, Fraser AG, Marmion BP, Simmons A (eds), Mackie and McCartney Practical Medical Microbiology. 14th ed., Vol. 2, Churchill Livingstone, London 1996;329-41.
9. Rachow A, Clowes P, Saathoff E, Mtafya B, Michael E, Ntinginya EN, *et al*. Increased and expedited case detection by Xpert MTB/RIF assay in childhood tuberculosis: a prospective cohort study. Clin Infect Dis. 2012;54:1388-96.
10. Nicol MP, Workman L, Isaacs W, Munro J, Black F, Eley B, *et al*. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children

admitted to hospital in Cape Town, South Africa: a descriptive study. Lancet Infect Dis. 2011;11:819–24.

11. Pang Y, Wang Y, Zhao S, Liu J, Zhao Y, Li H. Evaluation of the Xpert MTB/RIF assay in gastric lavage aspirates for diagnosis of smear-negative childhood pulmonary tuberculosis. Pediatr Infect Dis J. 2014;33:1047-51.
12. Kaur R, Jindal N, Arora S, Kataria S. Epidemiology of rifampicin resistant tuberculosis and common mutations in rpoB gene of Mycobacterium tuberculosis - a retrospective study from six districts of Punjab (India) using Xpert MTB/RIF assay. Journal of Laboratory Physicians. 2016;8:96-100.
13. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? J Clin Microbiol. 2011;49:2540-5.