RESEARCH ARTICLE

Comparison of Xpert MTB/RIF with AFB smear and AFB culture in suspected cases of paediatric tuberculosis in a tertiary care hospital, Karachi

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Abstract

Objective: To evaluate the sensitivity, specificity, positive predictive and negative predictive values of Xpert mycobacterium tuberculosis and resistance to rifampicin by comparing it with acid-fast bacilli smear and culture in suspected tuberculosis patients.

Methods: The retrospective study was conducted at the Aga Khan University Hospital, Karachi, and comprised patient data from January 2013 to December 2016. Data related to children with clinical suspicion of pulmonary and extra-pulmonary tuberculosis based on Modified Kenneth Jones criteria, aged 1 month to 18 years whose samples (respiratory or non-respiratory) were sent for Xpert mycobacterium tuberculosis and resistance to rifampicin and acid-fast bacilli smear and culture concurrently. Analysis was carried out by STATA 12 and MedCalc softwares.

Results: Of the 91 cases, 50(54.9%) related to females. The overall median age of the patients was 12.5 years (interquartile range: 8 years). Overall, 42(46.2%) cases had extra-pulmonary tuberculosis. The Xpert test had 66.7% sensitivity compared to smear microscopy 47.6%. Overall sensitivity, specificity, positive predictive value and negative predictive value were 95.7%, 72%, 51.2% and 98.3% respectively when the two tests were compared.

Conclusion: Xpert mycobacterium tuberculosis was found to be more sensitive than acid-fast bacilli smear and culture in both pulmonary and extra-pulmonary tuberculosis in children.

Keywords: Xpert MTB/RIF, AFB culture, Paediatric tuberculosis. (JPMA 69: 1273; 2019)

Introduction

In this era of vaccination, medical and technological advancement, tuberculosis (TB) remains the oldest and still a fatal communicable diseases.[1] It has emerged as a global epidemic and poses a serious threat to public health and is responsible for approximately 1.5 million deaths worldwide.[2] As per World Health Organisation (WHO) report 2016, globally out of 10.4 million new cases of TB, 10% i.e. 1.0 million, were children.[3] Among them 75% of childhood TB cases occurred in 22 high-TB burden countries and around 70% of them can be linked to a source TB case.[4] Majority of TB incidence belongs to India, Indonesia, China, Nigeria, Pakistan and South Africa, accounting for 60% of the new cases. Pakistan ranked fifth among the 22 high-burden countries.[5] The government of Pakistan in 2001 confirmed TB as a national emergency.[1] In Pakistan, an estimated TB prevalence was reported of 342 cases per 100,000 population, approximately 250,000 patients/year, according to the national population-based prevalence survey 2010-11.[4] Among them, 75 per cent were aged 15-54 years.[1] Although in Pakistan prevalence of TB in children is not exactly known, according to National TB Control Programme data (2001-04), 4% of the registered TB cases were of paediatric age group, responsible for 8-20% of overall mortality in children.[1] Despite the development and advances in medical technologies, the rapid and accurate diagnosis of TB, particularly in children, remains a challenge. This is because childhood TB presents with a wide spectrum of vague clinical features contrary to adult TB cases. Children also have non-specific radiological findings; they lack sputum production with low bacterial load; and low yield along with challenging nature of sputum induction and gastric lavage procedures. In case of extra-pulmonary TB (EPTB), diagnosis requires complex and invasive procedures for

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specimen collection like cerebrospinal and abdominal tap.[1] Furthermore, diagnostic errors of TB suspected cases like false-negative (FN) results, misdiagnosis or over-diagnosis are common in developing countries.[1] Early recognition and prompt treatment of TB cases is the key for successful management outcomes and also imperative for disease prevention and its transmission.

Although, there are various diagnostic modalities and clinical algorithms developed for the diagnosis of TB in children, all of them have limitations. Bacteriological confirmation is generally recognised as the gold standard, but culture is a slow process and takes 2-6 weeks to yield a final result and requires infrastructure and technical expertise.[2] Furthermore, sensitivity of culture varies in children, ranging from 1.5% to 65%.[6] Smear microscopic examination for acid-fast bacilli (AFB) is a rapid method but does not provide any information about viability or resistance, and is not able to differentiate between TB and non-TB mycobacterium. Moreover, high number of bacilli (~5,000 to 10,000 bacilli per ml) is required to yield a positive result.[4,7] Several other serological and polymerase chain reaction (PCR) assays are also available, but with variable specificities and sensitivities.[8] Delays in the diagnosis not only increases morbidity and mortality, but also expand the risk for disease transmission and are also responsible for drug resistance TB.[2] Thus, TB control would greatly benefit from the advent of newer diagnostic tests for rapid identification. These tests rely mainly on rapid molecular diagnostic tests, including nucleic acid amplification techniques (NAAT) such as line probe assay and mycobacterium tuberculosis (MTB) deoxyribonucleic acid (DNA) and resistance to rifampicin (RIF) (Xpert MTB/RIF).[9] There has been a major positive shift in the dilemma previously faced while diagnosing this disease after this new molecular modality was introduced. X-pert MTB/RIF is an efficient test that not only identifies the microorganisms, but also documents associated RIF resistance in just two hours from clinical samples.[9] It requires minimal biosafety services and requires simple technical support. Initially this test was recommended for the practice in adult patients till 2010, but considering its efficacy it has been endorsed for children since 2013 as a means of helping in diagnosis for specific forms of EPTB, especially in countries facing high TB burden.[10,11] Xpert MTB/RIF's sensitivity and specificity is high in children for both diagnosing the disease and in the detection of RIF resistance. The sensitivity of the modality is 65% (95% confidence interval [CI]: 61-69%) and specificity is 99% (95% CI: 98-99%).[12]

The current study was planned to compare the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Xpert MTB/RIF with AFB smear and culture, together with RIF resistance frequency in children suspected with TB or EPTB. To the best of our knowledge, this is the first such study from Pakistan.

Materials and Methods

The retrospective study was conducted at Aga Khan University Hospital (AKUH), Karachi, and comprised data from January 2013 to December 2016. Karachi is Pakistan's largest city with an estimated population of over 16.2 million.[13] The data collected related to children with clinical suspicion of TB and EPTB based on Modified Kenneth Jones scoring criteria/Pakistan Paediatric Association Scoring chart,[14] aged from 1 month to 18 years of either sex who were either admitted or had visited the hospital's emergency department and whose samples (respiratory or non-respiratory) were sent for Xpert MTB/RIF, AFB smear and AFB culture concurrently for TB diagnosis. Any one of the 3 consecutive AFB smear and culture specimens tested positive was considered and compared with Xpert MTB/RIF. The pulmonary samples included broncho-alveolar lavage (BAL) specimen, tracheal aspirate and sputum, while EP samples included cerebrospinal fluid (CSF), gastric aspirate (GA), pleural fluid, pericardial fluid, synovial fluid, peritoneal fluid, urine, lymph node tissue and tissue of any other site or organ. Samples were received and processed at the AKUH clinical laboratory as per standard protocol. Data was collected by reviewing the medical charts on a standard proforma specifically designed for this study after the study was exempted from permission by the institutional ethics review committee.

The sterile samples for AFB smear and AFB culture were processed directly without decontamination. However, non-sterile samples were decontaminated using N-acetyl-L-cysteine-sodium hydroxide with a final concentration of 1.25%, and centrifugation was done for 20min at 3000g. This sediment was then re-suspended in 1-2 ml of phosphate buffer saline (PBS) and a final volume of 3ml was obtained. One drop of decontaminated sample was used to prepare smear, and Ziehl-Neelsen (ZN) staining was performed to see the

number of AFB per high power field (HPF; ×1000). Standard laboratory protocol was used and the Lowenstein-Jensen (LJ) and mycobacteria growth indicator tube (MGIT) were both inoculated. Identification of MTB was on the basis of ZN staining colony morphology and inhibition to para-nitrobenzoic acid (PNB). Further, 7H10 agar was used to perform indirect sensitivity testing using final critical concentrations of 0.2 µg/ml isoniazid (INH), rifampicin (RIF) 1µg/ml, ofloxacin (OFX) 2µg/ml, amikacin (AK) 4µg/ml, kanamycin[15] 5µg/ml, ethionamide (ETH) 5µg/ml, pyrazinamide (PZA) and capreomycin (CAP) 5µg/ml were tested by the MGIT 960 system. MTB H37Rv was the control strain used in every batch for quality control of susceptibility testing in accordance with the WHO recommendations.[16] The definition of resistance was taken as growth on drug-containing quadrants >1% in comparison to the number of colonies on drug-free quadrant. Susceptibility was defined as growth on the control quadrant with no growth or <1% on the drug-containing quadrant.

A cartridge automated machine was used to perform Xpert MTB/RIF testing (Cepheid, USA). Samples were processed directly from Xpert TB/RIF test, according to the manufacturer's protocol. Sample reagent was put in the untreated specimen at a ratio of 2:1, which was then manually agitated and kept for 10min at room temperature. It was then shaken again and kept for 5min. Subsequently, 2ml of inactivated material was transferred to the test cartridge and this was then inserted into the test platform. Thus, data interpretation from these MTB/RIF tests was software-based and not user-dependent.[17]

STATA 12.0 was used to carry out descriptive analysis. Frequencies with percentages were reported for categorical variables, like gender, type of TB, type of clinical specimen etc. For quantitative variables, such as age mean/median and standard deviation (SD) / interquartile range (IQR) were reported depending upon data distribution. MedCalc was used to calculate sensitivity, specificity, PPV and NPV along with 95% confidence interval (CI) by analysing 2×2 tables.

Xpert MTB/RIF was compared with both AFB smear and AFB culture taken as reference; samples that were positive or negative using the reference method were taken as true positive (TP) and true negative (TN). If a sample was Xpert MTB/RIF positive but negative by the reference method, then it was considered false positive (FP). Similarly, Xpert MTB/RIF negative and reference method positive samples were considered FN samples.

Results

Of the 98 specimens initially tested, 43(43.8%) were GeneXpert-positive and all (100%) were RIF sensitive; 23(23.5%) were positive for AFB smear and 46 (46.9%) were culture positive for MTB. Of the 98 samples, 91(93%) met the eligibility criteria and comprised the study sample. Of them, 42(46.2%) had EPTB; and 50(54.9%) were females. The overall median age was 12.5 years (IQR: 8 years) (Table-1).

Sensitivity, specificity, PPV and NPV were noted for each type of specimen tested (Table-2). The overall sensitivity, specificity, PPV and NPV of Xpert MTB/RIF in all samples were compared to AFB culture (Tables-3A-B).

Table-1: Demographic statistics of suspected cases of paediatric tuberculosis (TB) in a tertiary care hospital, Karachi.

Variables		n/N (%)
Age Median (Range)		12.5 years (IQR: 8.0 years)
Gender	Female	50/91 (54.9%)
Type of TB	Pulmonary	22/91 (24.1%)
	Extra pulmonary	42/91 (46.2%)
	Disseminated	27/91 (29.7%)
Clinical features suggestive of TB		87/91(95.6%)
Radiological findings suggestive of TB		85/91(93.4%)
Mantoux test	Performed	28/91 (30.8%)
	Negative	6/28 (21.4%)
	Positive	1/28 (3.6%)
	Results Unknown	21/28 (75%)
Type of specimen	Respiratory	30/98 (30.6%)
	Non-respiratory	68/98 (69.4%)
Type of Respiratory samples	Sputum	16/39 (41%)
	BAL	8 /39 (20.5%)
	Tracheal	6 /39 (15.4%)
	Gastric Aspirates	9 /39 (23.1%)
Type of Non-respiratory samples	CSF	27/59 (45.7%)
	Pleural fluid	3/59 (5.1%)
	Peritoneal fluid	5/59 (8.5%)
	**Tissue specimens	24/59 (40.7%)
Investigation Methods	Xpert MTB/RIF Positive	43/98 (43.8%)
	Smear positive	23/98 (23.5%)
	Culture positive	46/98 (46.9%)

BAL: Broncho alveolar lavage; CSF: Cerebrospinal fluid; IQR: Interquartile range.
**Lymph node(6), Pleura(3), Abscess(7), Intestine(5), Cerebellum(1), Spine(1), Skin(1)

Table-2: Comparison of Xpert MTB/RIF with AFB smear as gold standard.

Xpert MTB/RIF	AFB Smear (Gold Standard)															
	BAL		Sputum		Gastric Aspirate		Tracheal Aspirate		CSF		Pleural Fluid		Peritoneal Fluid		Tissue	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
+	3	3	6	1	2	4	0	2	5	4	0	1	1	0	5	6
-	0	2	0	9	0	3	1	3	0	18	0	2	0	4	0	13

Specimen	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
All samples	95.7 (78.1, 99.9)	72 (60.4, 81.8)	51.2 (41.9, 60.3)	98.3 (88.8, 99.7)
BAL	100 (29.2, 100)	40 (5.3, 85.3)	50	100
Sputum	100 (54.1, 100)	90 (55.5, 99.8)	85.7 (48.3, 97.5)	100
Gastric Aspirate	100 (15.8, 100)	42.9 (9.9, 81.6)	33.3 (20.8 ,48.7)	100
Tracheal Aspirate	0	60 (14.7,94.7)	0	75 (59.5,86)
CSF	100 (47.8,100)	81.8 (59.7, 94.8)	55.6 (34, 75.2)	100
Pleural Fluid	0	66.7 (9.4,99.2)	0	100
Peritoneal Fluid	100 (2.5, 100)	100 (39.8,100)	100	100
Tissue	100 (47.8, 100)	68.4 (43.5,87.4)	45.5 (30.1, 61.7)	100

MTB/RIF: Mycobacterium tuberculosis and resistance to rifampicin; AFB: Acid-fast bacilli; BAL: Broncho aleveolar lavage: CSF: Cerebrospinal fluid; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value.

Table-3 A: Sensitivity, Specificity, PPV, and NPV of Xpert MTB/RIF with AFB culture as Gold standard.

Specimen Type	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
		Overall		
All samples	66.7 (50.5,80.4)	73.2 (59.7,84.2)	65.1 (54,75.2)	74.6 (65,82.2)
Pulmonary	76.2 (52.8, 91.8)	72.2 (46.5,90.3)	76.2 (59.4, 87.5)	72.2 (53.5,85.5)
Extra-pulmonary	57.1 (34, 78.2)	73.7 (56.9, 86.6)	54.6 (38.6, 69.7)	75.7 (64.7, 84.1)
Sputum	85.7 (42,99.6)	88.9 (51.8,99.7)	85.7 (48,97.5)	88.9 (56.2,98.03)
BAL	83.3 (35.9,99.6)	50 (1.3, 98.7)	83.3 (54.4, 95.4)	50 (9.4,90.6)
Gastric Aspirate	100 (47.8,100)	60 (14.7,94.7)	71.4 (46.1, 88)	100
CSF	54.5 (23.4, 83.3)	81.3 (54.4, 96)	66.7 (38.7, 86.4)	72.2 (56.6, 83.8)
Tracheal	25 (0.6, 80.6)	50 (1.3, 98.7)	50 (10.1, 90)	25 (6.9, 60)
Pleural	0	66.6 (9.4, 99.2)	0	100
Peritoneal	0	75 (19.4, 99.4)	0	75 (63, 84.1)
Tissue	66.7 (29.9, 92.5)	66.7 (38.4,88.2)	54.6 (33.9, 73.8)	76.9 (55.3, 90)
Lymph node	100 (39.8,100)	50 (1.3, 98.7)	80 (50, 94.1)	100

MTB/RIF: Mycobacterium tuberculosis and resistance to rifampicin; AFB: Acid-fast bacilli;, BAL: Broncho aleveolar lavage: CSF: Cerebrospinal fluid; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value.

Discussion

The study highlighted that Xpert MTB/RIF had higher sensitivity in children compared to the conventional methods. With culture as the gold standard, Xpert MTB/RIF has shown better diagnostic sensitivity and specificity in pulmonary specimens compared to extra-pulmonary specimens when it was used to diagnose suspected childhood TB cases. This observation was further improved for smear-positive cases showing 100% sensitivity even for extra-pulmonary samples precisely in CSF and tissue specimens. Matthew Bates et al. also observed that the sensitivity of Xpert MTB/RIF assay in sputum was significantly more at 90% compared to smear microscopy 30% in detecting childhood TB cases.[18] In pulmonary specimens, the sensitivity and specificity were more in sputum samples compared to GA, BAL and tracheal aspirates. In a cross-sectional study from Uganda, Moorine P.S. et al. evaluated diagnostic strength of Xpert MTB/RIF in childhood pulmonary TB and revealed high sensitivity and specificity ratings at 79.4% and 96.5% correspondingly in sputum specimen which were also comparable with our findings.[19] Detjen A. et al. in one comprehensive systematic review stated pooled sensitivity of 62% and a pooled specificity of 99% for Xpert MTB/RIF in contrast to sputum culture in children.[6] Likewise, Xpert MTB/RIF showing higher sensitivity in BAL specimen in the present study which was also consistent with a study conducted in South Africa in which Xpert MTB/RIF was evaluated on BAL specimens in suspected intra-thoracic childhood TB. Comparatively, out of 14 patients, 9(64%) cases were

Table-3 B: Sensitivity, Specificity, PPV, and NPV of Xpert MTB/RIF with AFB culture as Gold standard in smear positive and smear negative cases.

Specimen Type	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
AFB Smear Positive				
All samples	95 (75.1, 99.9)	0	86.4 (85.1,87.5)	0
Sputum	100 (54.1, 100)	0	100 (54.1, 100)	0
BAL	100 (29.2, 100)	0	100 (29.2,100)	0
Gastric Aspirate	100 (15.8, 100)	0	100 (15.8, 100)	0
CSF	100 (47.8, 100)	0	100 (47.8, 100)	0
Tracheal	0	0	0	0
Pleural	0	0	0	0
Peritoneal	0	0	0	0
Tissue	100 (29.2, 100)	0	60 (60, 60)	0
Lymph node	100 (15.8,100)	0	100	0
AFB Smear Negative				
All samples	40.9 (20.7, 63.7)	77.4 (63.8, 87.7)	42.9 (27, 60.3)	75.9 (68.4, 82.1)
Sputum	0	88.9 (51.8, 99.7)	0	88.9 (86.4,91)
BAL	66.7 (9.4, 99.2)	50 (1.3, 98.7)	66.7 (28.8, 90.8)	50 (10.8, 89.3)
Gastric Aspirate	100 (15.8, 100)	60 (14.7, 94.7)	50 (25.5, 74.5)	100
CSF	16.7 (0.4, 64.1)	81.3 (54.4, 96)	25 (4.1, 72.3)	72.2 (62.9, 80)
Tracheal	33.3 (0.8, 90.6)	50 (1.3, 98.7)	50 (10.8, 89.3)	33.3 (9.2, 71.2)
Pleural	0	66.7 (9.4, 99.2)	0	100
Peritoneal	0	100 (29.2, 100)	25 (0.6, 80.6)	75 (75, 75)
Tissue	50 (11.8, 88.2)	76.9 (46.2, 95)	50 (21.8, 78.2)	76.9 (58.7, 88.7)
Lymph node	100 (15.8,100)	50 (1.3, 98.7)	66.7 (33.3,88.9)	100

MTB/RIF: Mycobacterium tuberculosis and resistance to rifampicin; AFB: Acid-fast bacilli; BAL: Broncho aleveolar lavage: CSF: Cerebrospinal fluid; CI: Confidence interval; PPV: Positive predictive value; NPV: Negative predictive value.

confirmed by culture whereas BAL Xpert MTB/RIF was positive in 7 (78%).[20] In facilities where resources for collection of induced sputum are not available, GAs can be valuable in diagnosing pulmonary TB in children. In this study, sensitivity of GA specimen was better than sputum though it was less specific. Matthew Bates et al. from sub-Saharan Africa revealed 33/48 (68·8%) sensitivity and 735/740 (99·3%) specificity in gastric lavage.[18] However, collection of a GA needs hospitalisation and may not be logistically feasible in many settings. Conversely in an analysis that was retrospective described by Petrovic S. et al. documented better diagnostic yield of BAL specimen compared to sputum and gastric lavage samples in children with smear negative pulmonary TB.[21] BAL is invasive and resource intensive compared to either induced sputum and GA or may be possible only in secondary/tertiary care settings.

In our study, the sensitivity in serous fluids like pleural and peritoneal specimens was low compared to tissue biopsy specimen. Subordinate sensitivity for pleural fluid samples (44.4%) also reported by Lawn SD et al. and other body fluids including pericardial, peritoneal and synovial fluids (50%) whereas sensitivity exceeded 75% for tissue specimens and fine-needle aspirates (88.3%).[22] Vadwai, V. et al. and Tortoli, E. et al. both observed higher sensitivity and specificity for tissue specimens compared to serous fluid samples.[23,24] Diagnostic yield of tissue specimen was comparatively higher compared to fluid samples. A South African hospital-based study in 72 children by Coetzee et al. reported X-pert MTB/RIF sensitivity in lymph node tissue 80% and a specificity of 93.8% when compared against a standard composite reference of cytology and/or culture.[25] WHO also recommend the use of X-pert MTB/RIF as diagnostic tool for TB lymph nodes in adults and children.[26] Our study findings also showed high sensitivity for both smear-positive and smear-negative TB lymphadenitis. Similarly, in childhood TB meningitis (TBM) cases, CSF has shown around 50% overall sensitivity which was reached to 100% in smear-positive TBM cases. This finding in the index study was comparable to a recent study conducted by Rakesh Bhatia et al. from India which observed 38.2% overall sensitivity of Xpert MTB/RIF in CSF compared to culture.[27]

Major limitation of this study includes the retrospective nature and small sample size. Additionally, diagnostic utility of Xpert MTB/RIF in malnourished and children positive for human immunodeficiency virus (HIV) was not

evaluated. Despite the limitations, we recommend the utilisation of Xpert MTB/RIF in diagnosing paediatric TB.

Conclusion

Xpert MTB/RIF showed better sensitivity than smear microscopy when compared with gold standard culture method and can be considered a reliable tool for early diagnosis of pulmonary and EPTB in paediatric smear-negative cases as well. It is also considered a good tool in the detection of RIF resistance. We propose that larger diagnostic prospective studies in children from different settings are needed to validate this diagnostic impression.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: None.

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