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Original Research Article

Evaluating the diagnostic accuracy of gene xpert analysis in pediatric pulmonary tuberculosis: a prospective clinical study in North-Western India

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ABSTRACT

Background: The diagnosis of tuberculosis (TB) by microbiological tests is a major challenge particularly in children. The use of Xpert analysis, a rapid genetic testing modality is not widely reported in our locality. The aim of the study to evaluate the diagnostic accuracy of Gene xpert analysis in diagnosis of pediatric Pulmonary TB.
Methods: A prospective hospital-based study was conducted among 140 participants with symptomatology pertaining to pulmonary TB as per Revised national tuberculosis control program (RNTCP, India) criteria. The Xpert testing (GXT) was performed as per standards and was compared with erythrocyte sedimentation rate (ESR), tuberculin test (TT) and chest X-rays (CXR). The obtained results were reported in terms of Sensitivity %, Specificity %, Positive Predictive Value % (PPV) and Negative Predictive Value % (NPV) for comparisons. The receiver operating curve (ROC) analysis was employed to evaluate the accuracy of diagnosis.
Results: The GXT was positive (10.71 %) in suspected TB patients. TT has significantly (10 %) with a73.33% sensitivity, 93.60% specificity and a PPV of 57.89 % when compared with xpert. The ESR showed a sensitivity of 53.33% and a specificity of 56%. The CXR showed sensitivity of 93.33%. The ROC analysis showed that TT had a higher confidence interval (0.699-0.970) t5`han other methods. The Rifampicin resistance was found 7.5% (n=2) of 15 GXT positive cases.
Conclusions: The xpert based diagnosis of gastric lavage samples after a tuberculin test (TT) had high sensitivity and specificity, followed by chest X ray while the ESR had lower clinical accuracy. The 'gene xpert analysis' is highly useful rapid tool for diagnosis of children with TB.

Keywords: Children, Pulmonary tuberculosis, Tuberculosis, Tuberculin test, Xpert analysis

INTRODUCTION

Tuberculosis (TB) is infectious microbial disease (caused by Mycobacterium tuberculosis) which is one of the top ten causes of death in the world as per the global Tuberculosis Report, published by the World Health Organization (WHO). The children with TB account for about 1.2 million and associated mortality of 170,000 per year.[1,2] The TB is a global burden with 33.3 % of the world's population infected. The 11% of 9 million TB cases are reported to occur in children.[2] In India, as many as 500 children die of TB each year, and more than three-quarters of every 1 million children suffer from TB each year.[3] The clinical presentation of TB, particularly in young children, is subtle and often unrecognizable. Discrete changes such as weight loss or lack of weight

gain, fatigue, or a decreased interest in play may occur frequently which are unnoticed by the family.[3] Due to these factors and difficulty of diagnosis, the extent of TB in children in India is unclear.4 TB in children is a neglected disease because it manifests differently from adults.

The diagnostic delay is reported to occur in TB children owing to various reasons. The Mycobacterium culture is the gold standard method but takes time (2-6 weeks) and needs good laboratory settings. The children cannot expectorate or produce a small amount of sputum. The respiratory tract secretes very few bacteria, so sputum smear microscopy can only detect a limited number of bacteria.[4,5] The gastric juice aspirate (GA), induced sputum (IS), nasopharyngeal aspirate, bronchoalveolar lavage fluid (BAL) are ideal in such instances where the sputum microbiology is not sensitive.[5,6] Likewise in malnourished, the Mantux test may be negative because the cavity caused by tuberculosis is rarely seen in children. The chest X-rays (CXR) are not always helpful except for teenagers.[7] About 95% of children with tuberculosis under the age of 12 have a negative smear.[8] The undiagnosed /underdiagnosed childhood tuberculosis is also a sentinel marker for the active transmission of tuberculosis in the community.[9] Thus, there is a need to use accurate, feasible, rapid, affordable and possible near-case tuberculosis diagnostic tests in resource-limited settings.

The Xpert MTB/RIF analysis is a computerized molecular test based on semi-nested real-time polymerase chain reaction (RT-PCR) and molecular beacon technology for rpoB gene.[10] The Xpert MTB/RIF analysis can identify the resistance of MTB and rifampicin resistance (RIF) within 2 and is recommended test for pediatric TB as per the WHO.[10,11] The existing research on Xpert MTB / RIF has only focused on the application of Xpert MTB / RIF in children in various specimens.12-15 In spite of high recent quality meta-analysis endorsing the use of gene-Xpert in diagnosis of TB, 16 the diagnostic utility of Xpert was not compared it with conventional screening tools in our locality. Given the incidence of TB in India and issues of underrated diagnosis in children, a rapid test must be evaluated for its diagnostic accuracy as opposed to available routine or gold standard tests. The current study aimed to validity of gene-Xpert over ESR, CXR and TT in diagnosis of TB in children.

METHODS

Study settings

A prospective clinical study was conducted from March 2017 to September 2017 (a period of 6 months) in Government Medical College, Surat, India. A sample of 140 participants was recruited by XXX sampling. The participants with in the age group 0-14 in OPD and 0-12 age group in IPD with symptoms of TB according to RNTCP criteria were enrolled in the study.[17] The participants with comorbidities, who were previously or already diagnosed with TB and those who were unwilling to participate in the study were excluded. The study was approved by the Ethics Committee. The patients and parents/guardians on behalf of the children and adolescents included in this study had given written informed consent to participate in the study.

Data collection

The sample recruited after subjecting to set criteria, was clinically examined by single examiner (principal investigator). The TB diagnosis was made based on the 2016 RNTCP guidelines on pediatric diagnosis and management.[17] The gastric lavage samples were collected as per standards.[17] The outcomes measured for the participants included investigative tests namely: (i) Tuberculin test (TT) (RT-23; Guindy Laboratory, Chennai, India). (ii) Complete Blood test/ ESR and (iii) standard CXR. These 3 tests were compared with Xpert (on gastric lavage specimens) using the automated real-time DNA amplification test for rapid and simultaneous detection of TB and Rifampicin resistance (XPERT® MTB/RIF assay; Cephid Invitro Diagnostics). Also, the rifampicin resistance (RIF) has been evaluated in this study for those patients in whom the xpert test is positive or reactive.

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of AFB microscopy, MTB culture, and Xpert MTB/RIF assay were calculated. The data analysis was done using the Statistical package for social sciences (IBM SPSS; version 25.0, USA) software.

RESULTS

The study was conducted on a sample of 140 participants. The sample consisted of 57.2% (n=80) male subjects and 42.8% (n=60) of females (Figure 1).

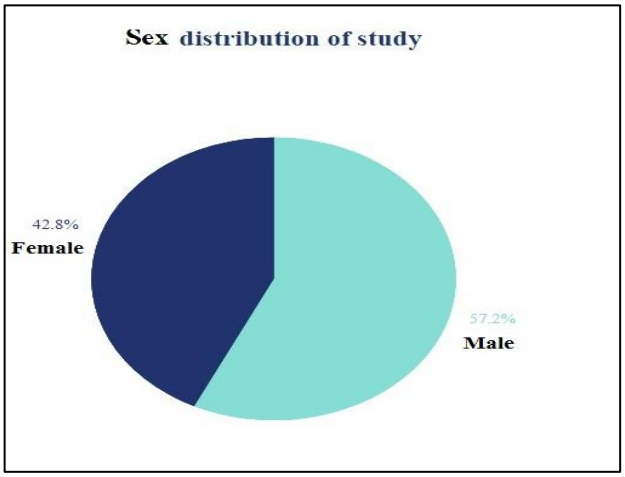

Figure 1: Sex distribution.

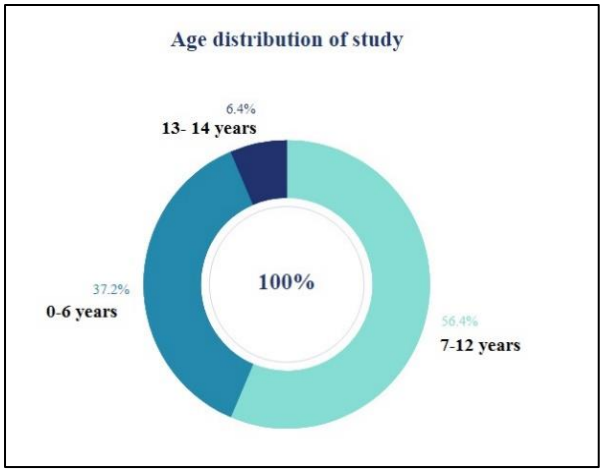

Figure 2: Age distribution.

Around 56.4% (n=79) belonged to age group of 0-6 years, 52 (37.2%) belonged to age group 7-12 years and 6.4% (n=9) belonged to 13-14 years (Figure 2).

The results of Xpert analysis demonstrated 10.71% (n=15) positive. The ESR showed a sensitivity of 53.33% and a specificity of 56%. The CXR showed sensitivity of 93.33%. The TT had sensitivity of 73.3% and a specificity of 93.6%. The CXR and TT were positive in 10 % of cases each, while the ESR predicted only 5.7% of the patients (Table 1). A receiver operating curve (ROC) analysis (Figure 3) was performed, and TT had a higher confidence interval (0.699-0.970) than other methods (Table 2).

Table 1: Diagnostic accuracy of ESR, CXR, TT with genexpert.

Diagnostic accuracy	ESR	CXR	TT
Positive	08 (5.7%)	14 (10 %)	14 (10%)
Sensitivity (%)	53.33	93.33	73.33
Specificity (%)	56.00	12.80	93.60
Positive predictive value	12.70	11.38	57.89
Negative predictive value	90.91	94.12	96.69
Positive LR (%)	1.21	1.07	26.67
Negative LR (%)	0.83	0.52	11.46

In this study, 15 of 140 children were subjected to gastric lavage by a genexpert and they were positive. Among these 15 positive reports, 7.5% (n=2) had RIF, among which 1 child was found to have intermediate resistance to rifampicin.

Table 2: Area under the curve of the various methods.

Test result variable (s)	Area	Std. error	Asymptotic sig.	Asymptotic 95% Confidence Interval	
				Lower bound	Upper bound
ESR	0.547	0.079	0.556	0.392	0.701
CXR	0.531	0.076	0.698	0.382	0.679
Tuberculin	0.835	0.069	0.000	0.699	0.970

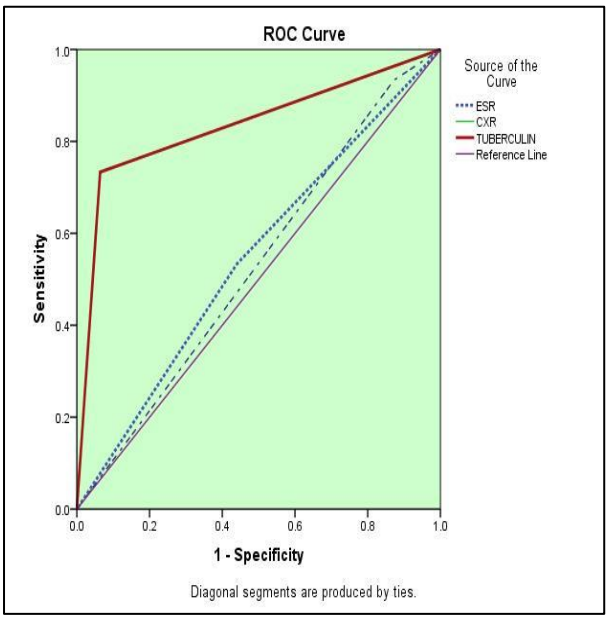

Figure 3: ROC curve showing diagnostic accuracy of TB.

DISCUSSION

Tuberculosis (TB) in children is associated high mortality of every year and is designated global burden. 2 The clinical presentation being unrecognizable the extent of TB in children in India is unclear.[4] Added to this is the lack of rapid and reliable tests, which causes the delay in diagnosis and timely treatment of tuberculosis in children. The Xpert genetic analysis can be used as a useful quick alternative test for the diagnosis of tuberculosis in children.[16,18] The gene Xpert assay is a tuberculosis detection method based on a single tube, cassette real-time PCR detection. Even in smear-negative specimens, it is very sensitive to the detection of Mycobacterium tuberculosis. Thus, the current study employed the comparisons of diagnostic accuracy between existing tests (TT, CXR, ESR) and xpert gene analysis for TB in children. The tuberculin test (TT) showed best accuracy, followed by the CXR and ESR. TT was positive in 10% of suspected TB patients in current study which is in line with to other studies like Chadha et al and Rao et al The TT is a delayed hypersensitivity reaction (type IV).[19,20] It is not 100% specific as its positivity doesn't differentiate between Tubercular Mycobacterium to Non- Tubercular

Mycobacterium or may show false positivity due to reactivation of previous BCG Vaccination.[21] Also, the false negative result may be deactivation of PPD by excessive heat or freezing or storage issues.[21] The CXR, the positive signs of TB was noted in 10% of patients. It was lower than the earlier report (21.3 %).[22] The positive predictive value of ESR is very low 12.70% suggesting no diagnostic significance as a single test. Thus ESR is not a strong indicator of any diagnostic value in this study in diagnosing TB. Similar findings were reported earlier study.[23] The tuberculin test has sensitivity and specificity proximity to genexpert.

The results of the current study are also in line with a recent study had stated that CXR in diagnostic work of TB among HIV plays an unprecedented role while ESR has little clinical significance in the evaluation of TB. This was from cohort of TB-HIV co-infection of which around 53.3% were confirmed by Gene Xpert, and 46.7% by clinical judgment, CXR and ESR. The sensitivity, specificity, PPV and NPV of CXR were 67.9%, 77.3%, 43.8%, and 90.3%, and that of ESR were 49.4%, 55.1%, 22.1%, and 83.0%, respectively. The overall agreement between CXR and Gene Xpert® was good while that of ESR and Gene Xpert® was poor.[24] The value of xpert testing is better understood, form another study, that had reported that Xpert detected a large proportion of TB cases missed by microscopy. The CXR was useful in greatly reducing the number of diagnostic tests needed even among presumptive TB patients.[25] Likewise, a study showed that the majority of the sputum smear-negative patients did not have TB on single Xpert testing. The CXR gave an overestimate of sputum smear-negative TB cases (i.e. CXR was suggestive of pulmonary TB in 15 (71.4%) of the 21 patients with a positive Xpert test).[26]

A large scale study was done comparing Xpet testing with clinical tests with sample of 1690, had stated that using CXR prior to culture or Xpert testing reduces the number needed to screened. Thus, applying the CXR to triage for culture or Xpert testing reduces the number of missed cases and increases the efficiency of culture and Xpert testing.[27] A recent multicenter study was reported The sensitivity of xpert-Ultra (a better version of xpert) in bacteriologically confirmed TB and probable TB cases was 87.5% (42/48) and 44.4% (36/81), respectively.[28] The specificity of Ultra was high (99.4%, 172/173). The sensitivity of Ultra is 80.0% (40/50) in children aged <4 years, which is significantly higher than that in older children (48.1%, 38/79) ($p<0.001$). Ultra conducted using gastric aspirate (GA) samples can provide faster results, allowing an early and accurate TB diagnosis, especially in younger children with difficulty producing sputum.[28] The screening via CXR for suspected populations and conformation of children for TB via gene xpert (gastric lavage) based rapid diagnosis is recommended for clinical practice. The TT and sputum AFB have specific implications but not ESR as per the study and existing reviewed literature. A constant updating of guidelines (by regulatory bodies like RNTCP/ WHO) is also recommended for amendments of clinical diagnosis of children with TB.

The small sample size is the limitation of the study. The participants under each age group were reduced owing to same reason. The future directions include validating the genexpert based diagnostic accuracy form larger samples and multiple centres on specific paediatric age groups.

CONCLUSION

The xpert based diagnosis of gastric lavage samples after a TT had high sensitivity and specificity, followed by chest x ray while the ESR had lower clinical accuracy. The 'gene xpert analysis' is highly useful rapid tool for diagnosis of children with TB with identification of Rifampicin resistance.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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