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A Comparative Study between Genexpert and Smear Microscopy for Diagnosis of Tuberculosis in Paediatric Patients

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Abstract: ***Diagnosis of tuberculosis (TB) in children is challenging due to insufficient specimen material and the scarcity of bacilli in specimens. In this study we compare the diagnostic yield of geneXpert and smear microscopy in detection of Tuberculosis in children. We have compared sensitivity, specificity, positive predictive value(PPV) and negative predictive value(NPV) of GeneXpert with smear microscopy in detection of Mycobacterium Tuberculosis in children. Out of the total 100 cases of suspected tuberculosis evaluated in a tertiary care hospital, 80 patients were clinically TB positive and 20 patients were clinically TB negative. Both genexpert and AFB smear microscopy showed 100% specificity and positive predictive value. However sensitivity of genexpert was 48.7 % & that of AFB smear was 16.3. Negative predictive value of genexpert was 32.8 % & that of AFB smear microscopy was 22.9%. Sensitivity of GeneXpert was approximately 3 times higher than AFB smear microscopy and Negative predictive value of GeneXpert is also higher as compared to AFB smear microscopy. Hence genexpert was found to be a better diagnostic test as compared to smear microscopy and can significantly reduce false negatives and the delay in treatment initiation, reducing premature death and ongoing transmission.***

Keywords: *GeneXpert, microscopy, sensitivity, specificity, positive predictive value, negative predictive value*

1. Introduction

TB remains one of the world's deadliest communicable diseases. If TB is to be eliminated as a global problem, earlier diagnosis, timely identification of rifampicin resistance as well as improved detection will be essential. TB can be identified under a regular microscope using Ziehl Neelsen stain on body fluid specimen like sputum. Traditionally, tuberculosis is mostly being diagnosed by a combination of chest X rays, the staining of sputum with special dyes followed by microscopy, growth of mycobacterium tuberculosis in culture and the Mantoux test. The diagnosis of tuberculosis (TB) in children is challenging due to insufficient specimen material and the scarcity of bacilli in specimens.

Sputum smear microscopy is easy to do and is very cheap and combined with chest X-rays has been used for a long time by TB control agencies worldwide. However the sputum smear microscopy (sputum AFB) test has some problems in HIV-positive patients and children. In patients with low bacterial load, the Xpert MTB/RIF test exhibits high sensitivity and specificity for detecting TB.

WHO has recently recommended a real time PCR test called CBNAAT (Cartridge Based Nucleic Acid Amplification Test)/GENEXPERT as a primary diagnostic modality for detection of TB due to its better accuracy. However there is paucity of Indian data on the same. GeneXpert is a cartridge based, automated diagnostic test that can identify Mycobacterium Tuberculosis (MTB) DNA and rifampicin (RIF) resistance by nucleic acid amplification technique [3].

2. Aims & Objectives

To compare *sensitivity, specificity, positive predictive value(PPV)* and *negative predictive value(NPV)* of GeneXpert with smear microscopy in detection of *Mycobacterium Tuberculosis* in children.

3. Materials and Methods

A Diagnostic Test Evaluation Study was conducted in a Tertiary Care Hospital in a metropolitan city. This study was conducted from February 2015 upto September 2016. During this period, **100 patients** with suspected Tuberculosis were included in this study.

Inclusion criteria:- Age group <12 years, Children with suspected Tuberculosis (Pulmonary/Extra pulmonary)

Exclusion criteria:- All patients > 12 years

Samples used for the study:- Sputum, Gastric lavage, Cerebrospinal fluid **,** Ascitic fluid, Pleural fluid**,** Tissue biopsy specimen, Pus.

4. Sample Processing

Samples were collected from patients with suspected TB in a sterile container and transported to the lab within 1 hour. Both GeneXpert and AFB smear microscopy were performed free of cost under RNTCP. All samples, except CSF, were decontaminated by Sputaprep (NaOH –NALC 2% i.e N-Acetyl L-Cysteine sodium hydroxide method) before testing. Briefly, an equal volume of NaOH-NALC was added to the sample tube and vortexed for 20 minutes. Sterile water was then added to reach a final volume of 45 ml. The tube was then centrifuged at 3000 g for 15 minutes, the supernatant discarded and the pellet used for testing. CSF was not decontaminated before centrifugation. All sample pellets (including CSF pellet) were then divided for smear microscopy and Xpert assay. Technicians interpreting these 2 results were blind to clinical data and to other test results. Both results were available within few hours.

Ziehl-Neelsen (ZN) smear:- Two drops of sample pellet (approximately 200 µl) were used for smear microscopy (ZN staining), according to the WHO standard protocol. Slides

showing red coloured acid fast bacilli were taken as positive and negative slides were those without any acid fast bacilli.

Xpert MTB/RIF:- Sample reagent was added to the specimen in a ratio of 2:1, manually agitated and kept for 10 min at room temperature, then shaken again and kept for 5 min; 2 ml of the inactivated material was transferred to the test cartridge and inserted into the test platform. Electronic results were available.

Children were suspected to have Tuberculosis in retrospect based on clinical, radiological and lab investigations. Clinical case definition categories for TB in children were determined retrospectively and taken from the standardised case definition recently published by Graham et al. as follows[1]:-

Confirmed TB cases: - were defined as children with at least 1 defined sign or symptom suggestive of TB **and** microbiologically confirmed TB, defined as at least one positive MGIT in any sample. A positive Xpert / smear was not considered as part of the 'Confirmed TB' case defintion because this was the research test under evaluation.

Probable TB cases: - were defined as children with at least 1 defined sign or symptom suggestive of TB **and** a CXR consistent with TB **and at least 1 of the following:**

1) Positive clinical response to TB therapy
2) Documented exposure to a household or close contact with a TB case
3) Immunological evidence of M. Tuberculosis infection.

Possible TB cases: - at least 1 of the signs and symptoms suggestive of tuberculosis **and** either 1 of the following: (a) A positive clinical response to anti-tuberculosis treatment (b) Documented exposure to M. tuberculosis (c) Immunological evidence of M. tuberculosis infection ***or*** Chest radiography is consistent with intrathoracic tuberculosis disease

TB Unlikely: - those who are symptomatic with symptoms other than the defined TB symptoms and who do not fit the above definitions with no alternative diagnosis confirmed.

Not TB: - cases were defined as those who fitted the diagnosis for 'TB unlikely' and also had an alternative diagnosis established (microbiologically or recovery without antituberculous therapy).

In this study, **Clinical Diagnosis** is taken as gold standard for detection of TB. Based on above definitions, patients were classified into 2 categories:

- **TB positive**:- "confirmed + probable + possible TB" cases combined together
- **TB negative**:- "TB unlikely + not TB" combined together

5. Ethical Consideration

Ethical clearance was taken from local Institutional Ethical Committee. No individual consent was required as archived patient records were collected and no patient identification was used. No additional specimen for genexpert and smear microscopy was taken for the purpose of this study.

6. Statistical Analysis

The data was tabulated in Microsoft excel spreadsheet in a master chart and studied for correlation. Stastical analysis of the data was conducted with Stastical Package for the Social Science System version (SPSS) 20.0. The sensitivity, specificity, PPV and NPV for the diagnosis of tuberculosis was calculated for AFB smear microscopy and the GeneXpert, using **clinical diagnosis as a gold standard.**

Clinical diagnosis is taken as gold standard as it is known that microbiological confirmation (i.e culture method) detects only approximately half of all paediatric TB cases when applied optimally and will therefore overestimate sensitivity and underestimate specificity.

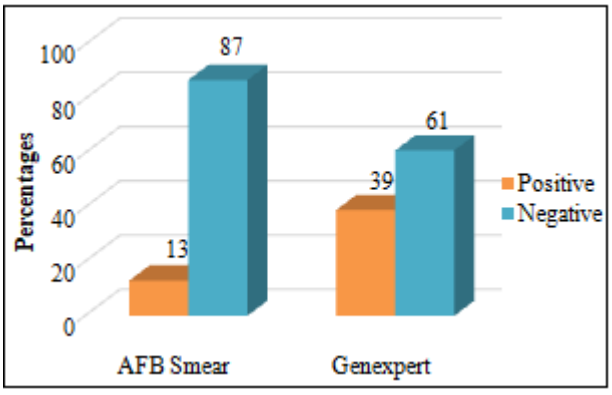
Conversely, a perfect clinical gold standard does not exist and therefore clinical gold standards are likely to underestimate sensitivity while overestimating specificity. This is a well-recognised problem in the evaluation of novel diagnostic tests for TB and particularly acute for paediatric TB and other paucibacillary manifestations.

7. Results

Table 1: Tuberculosis status of patients (Final diagnosis)

Findings	Frequency (no. of patients)	Percentage
TB Positive	80	80.0
Negative	20	20.0
Total	100	100.0

The clinically diagnosed gold standard was defined as all patients in the 'confirmed TB', 'probable TB' and 'possible TB' groups combined and termed as **TB positive**. 80 out of 100 patients satisfied the criteria for clinically diagnosed TB and were started on AKT and 20 patients were clinically **TB negative** ('TB unlikely' and 'not TB' combined together).



Graph 1: Results of AFB Smear and GeneXpert

There were total 13 AFB positive cases and 39 GeneXpert positive cases.

Table 2: AFB Smear Vs Final Diagnosis

AFB Smear	Tuberculosis		Total
	Positive	Negative	
Positive	13	0	13
Negative	67	20	87
Total	80	20	100

Out of 80 TB positive patients, AFB smear was positive in 13 patients and false negative in 67 cases against clinical diagnosis as gold standard. However out of 20 TB negative patients, AFB smear was negative in all of them with no false positives.

Table 3: Genexpert Vs Final Diagnosis

Genexpert	Tuberculosis		Total
	Positive	Negative	
Positive	39	0	39
Negative	41	20	61
Total	80	20	100

Out of the 80 TB positive patients, genexpert was positive in 39 cases and falsely negative in 41 cases against clinical diagnosis as gold standard. And out of 20 TB negative patients, all 20 were genexpert negative with no false positives. Based on above 2 tables, sensitivity, specificity, positive predictive value and negative predictive values were deduced for the 2 tests that are being compared in this study.

Table 4: Diagnostic evaluation of AFB smear Vs Genexpert

Statistic	AFB Smear	Genexpert
Sensitivity	16.3% (8.9% - 26.2%)	48.7% (37.4% - 60.2%)
Specificity	100.0% (83.1% - 100.0%)	100.0% (83.1% - 100.0%)
PPV	100.0% (75.3% - 100.0%)	100.0% (90.9% - 100.0%)
NPV	22.9% (14.6% - 33.2%)	32.8% (21.3% - 46.0%)

Both genexpert and AFB smear microscopy showed 100% specificity and positive predictive value as both tests did not show any false positives.

However sensitivity of genexpert was 48.7 %(37.4% - 60.2%) which was three times higher as compared to AFB smear which was 16.3 %(8.9%-26.2%).

Negative predictive value of genexpert was 32.8 %(21.3% - 46.0%) which is also higher as compared to AFB smear microscopy which was 22.9% (14.6% - 33.2%).

8. Discussion

The most common way for diagnosing TB worldwide is through sputum smear microscopy using the fluorescence microscope (Auramine) or the Ziehl-Neelsen method (gold standard). However this method is susceptible to human error and other factors beyond control that can result in false negatives. The impact of false negative in TB diagnosis can have far reaching consequences and is very detrimental to the global initiative as it may mean further spread of TB infections from untreated cases.

The GeneXpert (Xpert MTB/RIF) is a cartridge based automated diagnostic test that can identify Mycobacterium tuberculosis (MTB) DNA and resistance to rifampicin (RIF) by nucleic acid amplification technique (NAAT) [2].

The Xpert MTB/RIF assay is based on hemi- nested real-time PCR amplifying the ***rpoB*** gene target. Basically, target detection and characterization is performed in real time, using a six-colour laser detection device. Molecular beacons using novel fluorophors and quenchers are used to detect hybridization to each of the five amplified target regions of the gene. ***Bacillus globigii***, a spore-forming soil organism, is used as a full process control, acting as quality check for bacterial trapping, bacterial lysis, DNA extraction, amplification, and probe detection.

Adoption of Xpert MTB/RIF does not eliminate the need for conventional TB microscopy, culture and DST (Drug Sensitivity test) capacity.

In our study the sensitivity and specificity of MTB/RIF assay to detect Rifampicin resistance was not evaluated and not included in our objective.

Both AFB smear microscopy and GeneXpert are rapid diagnostic tests and results are available within few hours.

This study confirms that Xpert is a suitable, rapid and specific method for the diagnosis of childhood TB with approximately thrice the sensitivity of smear microscopy.

CDC updated guidelines for Nucleic Acid Amplification Test (NAAT) - 2009:[3]

Revised Interpretation of NAA test results in correlation with the AFB smear results:-

- If both the results are positive, presume the patient has TB and begin anti-TB treatment while awaiting culture results. The positive predictive value of FDA-approved NAA tests for TB is >95% in AFB smear-positive cases .
- If the NAA result is positive and the AFB smear result is negative, use clinical judgment whether to begin anti-TB treatment while awaiting culture results and determine if additional diagnostic testing is needed.
- If the NAA result is negative and the AFB smear result is positive, a test for inhibitors should be performed and an additional specimen should be tested with NAA. Sputum specimens (3%-7%) might contain inhibitors that prevent or reduce amplification and cause false-negative NAA results.

1) *If inhibitors are detected*, the NAA test is of no diagnostic help for this specimen. Use clinical judgment to determine whether to begin anti-TB treatment while awaiting results of culture and additional diagnostic testing.
2) *If inhibitors are not detected*, use clinical judgment to determine whether to begin anti-TB treatment while awaiting culture results and determine if additional diagnostic testing is needed. A patient can be presumed to have an infection with non tuberculous mycobacteria if a second specimen is smear positive and NAA negative and has no inhibitors detected.
   - If the NAA result is negative and the AFB smear result is negative, use clinical judgment to determine whether to begin anti-TB treatment while awaiting results of culture and additional diagnostic tests.

9. Conclusion

Sensitivity of GeneXpert is (48.7%) approximately three times higher than AFB smear microscop(16.3%).

Negative predictive value of GeneXpert is (32.8%) also higher as compared to AFB smear microscopy (22.9).

Both GeneXpert and AFB smear microscopy have 100% specificity and positive predictive value with no false positives in this study.

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