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Original Research Article

Evaluation of smear microscopy and geneXpert for the rapid diagnosis of pulmonary and extrapulmonary tuberculosis in a tertiary care hospital in North India: a descriptive prospective study

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ABSTRACT

Background: Tuberculosis is a global health problem associated with high morbidity and mortality. Rapid diagnosis of tuberculosis is essential for early disease management. Conventional methods like microscopy and culture are associated with low sensitivity and longer time to positivity respectively. The GeneXpert is an integrated device for the rapid detection of *Mycobacterium tuberculosis* and its sensitivity to rifampicin. We evaluated the performance of gene expert MTB/ RIF assay for the diagnosis of pulmonary and extrapulmonary tuberculosis.
Methods: A prospective cross sectional study was carried out in the Department of Microbiology. Samples were subjected to smear microscopy by ZN staining, culture on solid (LJ) and liquid media (BacT Alert) and GeneXpert assay.
Results: 122 pulmonary samples and 153 extrapulmonary samples collected from 275 patients were included in the study. Out of these, 48 samples were positive by both culture and Xpert assay and 2 samples were culture positive only. Out of 225 culture negative samples, 3 were positive by GeneXpert. The sensitivity for GeneXpert was much higher compared to smear micrscopy (96 Vs 46% respectively). The Xpert assay also detected 3 rifampcin resistant cases.
Conclusions: The test appeared to be as sensitive as culture for the detection of tuberculosis in smear positive, smear negative and extrapulmonary tuberculosis. We recommend the use of GeneXpert assay for the early detection of tuberculosis. We conclude that the test is simple and routine staff can perform the test with minimal training.

Keywords: GeneXpert, sensitivity, specificity, ZN staining

INTRODUCTION

Tuberculosis is one of the oldest diseases in the world associated with high morbidity and mortality. India accounts for one- fourth of the global TB burden with an estimated 2.79 million incident cases.[1] One of the significant reasons for the high prevalence of the disease is the difficulty in diagnosis. Traditionally, mycobacterial culture is the gold standard for the diagnosis of tuberculosis. This approach is relatively labor intensive, and takes about 2 months before results are available.[2] A rapid diagnosis of *Mycobacterium tuberculosis* is crucial for prevention and treatment of tuberculosis and to break the chain of transmission. Although, smear microscopy for acid fast bacilli is rapid and inexpensive method for diagnosis of tuberculosis, it lacks sensitivity and has poor predictive value.[3] Use of molecular diagnostic tests has led to incremental improvements in the detection and drug susceptibility testing of *M. tuberculosis*; however their use in low resource, high burden countries is limited

by the need for technical expertise, laboratory infrastructure and complexity of the test.[4]

In December 2010, the World Health Organization endorsed the use of GeneXpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA) for national tuberculosis programs in developing countries.[5] The Xpert assay is an automated nucleic acid amplification test for simultaneous detection of *Mycobacterium tuberculosis* complex (MTBC) and its resistance to rifampin directly from clinical samples. The assay does not require sample processing but can be used on chemically inactivated specimen and results are available within 2 hours. Therefore, it is simple, less time consuming and does not require special technical expertise and biosafety requirements.[6]

Rapid and accurate diagnosis of tuberculosis still poses a diagnostic challenge. The aim of this study was to evaluate the performance of GeneXpert assay for the direct detection of *M. tuberculosis* in pulmonary and extrapulmonary clinical specimens and to compare with conventional methods, viz a via, smear microscopy and culture.

METHODS

This descriptive and prospective study was conducted in department of microbiology, Government Medical College, Srinagar over a period of 1 year (June 2016 to May 2017). Clinical specimen both pulmonary (sputum, broncho alveolar lavage/aspirate and gastric lavage) and non-pulmonary (pleural fluid, tissue biopsy, pus, CSF, ascitic fluid, pericardial fluid, etc.) obtained for routine mycobacterial testing were included in the study. Two samples were collected from each patient whenever possible; one for GeneXpert and another for AFB smear and culture.

Sample processing

Non sterile specimens were processed by modified petroff method. After decontamination, sediment was dissolved in 2.5ml of distilled water for microscopy and inoculation in culture medium. Sterile specimens were concentrated by centrifugation and smear and cultures was inoculated from the sediment.

Smears were prepared and stained with. Zeihl Neelson staining method and culture was done. The AFB smear was graded as per RNTCP guidelines: Scanty (1-9/100 fields), 1+ (10-99/100 fields), 2+ (1-10/ fields) and 3+ (>10/field). A person was taken as smear positive if at least one of the smears was graded scanty or higher.[7] Culture was done on either solid media (LJ media) or liquid media (BactT Alert) using standard protocol.

Specimen was inoculated on the LJ medium and incubated at 37ºC for growth. Cultures were incubated for 8 weeks in case of solid culture. Contamination by rapidly growing bacteria and those with morphologies inconsistent with MTBC were checked regularly. After the appearance of growth on LJ medium, identification of M. tuberculosis was done by morphological examination, ZN staining and biochemical tests.[8]

Culture in liquid medium: 0.5ml of the sample was added to the BactAlert MP bottles using manufacturers' instructions. As the instrument flagged the culture positive, an AFB smear was made and when positive; a subculture was made on LJ medium. Cultures were incubated for 6 weeks before being declared as negative.[8]

Analysis of samples by Xpert MTB/RIF assay

The assay was performed using version 4 cartridges according to the manufacturers' recommendations. Briefly the sample reagent (containing NaOH and isopropyl alcohol) was added at a 2:1 ratio to clinical specimen to kill the mycobacteria and liquefy the samples. For biopsy specimen, a 2:1 volume of sample reagent (SR) buffer was added to biopsy specimens after they had been chopped into very small pieces with a sterile blade in a sterile petri dish.

Fluids were processed directly by the addition of a 2:1 volume of SR buffer, except for CSF (usually <1ml), which was raised to 2ml by the addition of SR buffer. The sample-SR mixture was shaken vigorously and incubated for 10 minutes before being shaken again and kept at room temperature for another 10 minutes. Two ml of the digested material was transferred to the cartridge. The cartridge was subsequently loaded in the GeneXpert instrument where all subsequent steps occurred automatically. In case the results were reported as invalid, error or no result, the sample was reprocessed and rerun, if sufficient material was available.

Data collection

The data collected included the patients' demographics, semi quantitative bacillary load by AFB microscopy and past history of TB treatment

Statistical analysis

The patients were characterized using simple descriptive statistics. Sensitivity, specificity, positive predictive value, and negative predictive value of smear microscopy and the Xpert assay for detecting MTBC was done using phenotypic culture as the reference standard.

RESULTS

Out of 282 samples were received during the study period, 4 samples were contaminated and 3 yielded non tubercular bacteria and were thus excluded from the study. A total of 275 samples were included in the study. Of these, 122 of the samples were pulmonary and 153 were extrapulmonary (Table 1). 141 samples were

obtained from male patients while 134 samples were from female patients. Male to female ratio was 1.05.

Table 1: Details of pulmonary and extrapulmonary samples included in the study.

Pulmonary	No. of sample
sputum	32
BAL	85
Gastric lavage	5
Total	122
Extrapulmonary	
Pleural fluid	23
Ascitic fluid	21
Pus	18
CSF	34
Urine	35
Synovial aspirate	6
Endometrial curettege	3
Lymph node aspirates/ Biopsy	11
pericardial fluid	2
Total	153
Total samples	275

Of the 275 samples, 48 were positive for *Mycobacterium tuberculosis* by both culture and GeneXpert assay (Table 2). An additional 2 samples were positive by culture but negative on Xpert analysis. 3 samples that were culture negative were detected as positive on Xpert analysis.

Table 2: Comparison of GeneXpert results with smear microscopy and culture.

Parameter	Xpert positive	Xpert negative	Total
Smear positive, culture positive	23	0	23
Smear positive, culture negative	1	0	1
Smear negative, culture positive	25	2	27
Smear negative, culture negative	2	222	224
Total	51	224	275

23 of the culture positive samples were smear positive while the other 27 samples were smear negative (Table 2). However, 1 samples positive by smear microscopy were culture negative.

However, the isolate was positive on gene expert assay. The sensitivity, specificity, positive predictive value and negative predicative value of smear microscopy when compared to culture are shown in Table 3.

On comparison of GeneXpert with smear microscopy, all 25 smear positive samples were geneXpert positive. In addition, GeneXpert detected 27 of the smear negative cases. Increased detection of *Mycobacterium tuberculosis* by gene expert compared to culture and smear microscopy was 5.88% and 47.06% respectively.

Table 3: Sensitivity, specificity, positive predictive value and negative predictive value of AFB smear microscopy and GeneXpert when compared with culture.

	Sensitivity	Specificity	PPV	NPV
Xpert	96	98.67	94.12	99.11
Smear microscopy	46	99.56	95.83	89.24

Rifampicin resistance was detected in 3 clinical isolates by Xpert assay. All the three isolates were previously treated pulmonary TB cases.

DISCUSSION

Rapid and accurate diagnosis of tuberculosis is a challenge in developing countries.[9] Laboratory confirmed diagnosis of tuberculosis is pivotal for management of disease and reduce the transmission of infection. Improved detection of tuberculosis is considered a priority by World health organization.[10] However the current frontline diagnostic test, smear microscopy, lacks sensitivity. Due to the slow growth of *Mycobacterium tuberculosis* and need for sophisticated lab facility, culture is available in reference laboratories. The Xpert MTB/RIF assay has been introduced with the aim to increase the detection of tuberculosis especially in smear negative, extrapulmonary and pediatric age groups. This study was aimed at assessing the effectiveness to various diagnostic modalities available at our centre for the detection of tuberculosis.[11,12]

A large number of cases remain undiagnosed by traditional sputum microscopy. Therefore, diagnostic delays in detection of smear negative pulmonary samples is of major concern. Also, the diagnosis of extrapulmonary samples represents a challenge due to their paucibacillary nature. In the absence of alternative tests, such cases would remain undetected and unreported. In our study, ZN stained smears had a sensitivity of 46% compared to culture. Thus, sputum microscopy could detect only half of the cases detected by GeneXpert. For a sample to be positive, a bacterial load of 10^4 organisms is required while as identification of TB bacilli by GeneXpert require 131CFU/ml of sample.[13,14] Studies have shown that GeneXpert increased TB detection rate by 23 to 60% among culture confirmed cases while as in our study it was 47%. Culture methods like LJ medium and liquid culture are the gold standard tests for the detection of *Mycobacterium tuberculosis*.

However, these tests are time consuming and need a good laboratory infrastructure. The average time for the isolates to grow on LJ medium was after 3 weeks to 8 weeks. Liquid cultures tend to grow more rapidly than

solid cultures. The average turnaround time was 12 days +/-5 days.[9,14] In our study, overall sensitivity of GeneXpert for the detection of tuberculosis was 96%. Compared to culture, the sensitivity of GeneXpert for smear positive and smear negative samples were 100% and 92.6% respectively.[15]

Some recent evaluations have demonstrated that the test has an overall pooled sensitivity of 90.4% (95% CI 89.2%- 91.4%). Studies have shown that the test accurately detects 98.2% of smear positive and 72.5% of smear negative cases.[16,17] In contrast, our data shows a better sensitivity for smear negative cases compared to other studies. False negative results were seen in 2 specimens (1 endometrial curetting and pleural fluid). The possible explanation for this could be the paucibacillary nature and uneven distribution of bacilli in the specimen and the presence of PCR inhibitors in the sample.

GeneXpert detected 1 sample which was smear positive but culture negative. This discrepent result could be attributed to the previous anti tubercular treatment of the patient. Excretion of the residual DNA from the dead bacilli explains the positive Xpert and negative culture result.[18] Invalid and error results were seen in 8/275 (2.9%) of all specimen. These samples were reevaluated and all gave negative results on retesting.

Early determination of rifampicin sensitivity is important for the timely detection of multidrug resistant tuberculosis and timely initiation of appropriate therapy in order to reduce the risk of spread and poor outcome. Xpert MTB/ RIF assay has a pooled sensitivity of 95% (95% CI 90-97%) and pooled specificity of 98% (95% CrI 97-99%).

In our study, rifampicin resistance was detected in 3 (5.88%) cases. All patients with rifampcin resistance were previously treated cases who had completed their course of treatment suggesting relapse in these cases. Rifampicin resistance was not evaluated against the phenotypic modified proportion method which is the main limitation of the study.[15,19,20]

CONCLUSION

In conclusion, although GeneXpert and smear microscopy are comparable in specificity GeneXpert is a more sensitive test for the rapid diagnosis of tuberculosis. Our study reconfirms the utility of GeneXpert in the diagnosis of tuberculosis especially smear negative pulmonary tuberculosis and extrapulmonary tuberculosis.

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