

Comparative Evaluation of GENE-XPRT MTB/RIF with AFB Smear Microscopy Methods for the Diagnosis of Tuberculosis in Sputum Specimens of Suspected Pulmonary Tuberculosis Patients.

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ABSTRACT

Background: Tuberculosis is a major public health problem worldwide and remains one of the most significant causes of death from an infectious agent. India contributes to 25.5% of the global new TB case detection. In recent times, emphasis has shifted from older phenotypically and biochemical methods of diagnosis to molecular methods such as GeneXpert MTB/RIF, a real time PCR that can detect MTB and rifampicin resistance simultaneously. **Methods:** This study aims at assessing the performance of acid fast bacilli (AFB) smear microscopy in comparison with GeneXpert MTB RIF, in the diagnosis of pulmonary tuberculosis. Study was a cross-sectional study carried out at the Department of Pulmonary Medicine, Muzaffarnagar Medical College, from January 2018 to April 2019. **Result:** The detection of MTB and rifampicin resistance using the Xpert MTB/RIF assay was assessed in 67 specimens from patients suspected of having pulmonary tuberculosis and compared with conventional smear microscopy. Out of these 67 sputum specimens, 38 samples were MTB positive by smear microscopy while 56 samples were MTB detected by Gene Xpert assay. Gene Xpert detected 18 additional tubercular cases and identify two cases of Rifampicin resistant MTB. **Conclusion:** Study show that there was no statistically significance in diagnostic value between GeneXpert and AFB smear microscopy in sputum samples however Gene Xpert MTB/RIF is useful method for rapid detection of MTB and Rifampicin resistance simultaneously.

Keywords: Direct smear microscopy, GeneXpert MTB/RIF assay, pulmonary tuberculosis.

INTRODUCTION

Tuberculosis a communicable diseases is major public health problem worldwide causing increased morbidity and mortality in developing countries. As per the Global TB report 2017 the estimated incidence of TB in India was approximately 28,00,000, accounting for about a quarter of the world's TB cases. There were an estimated 558 000 new cases of rifampicin resistant TB (RR-TB) globally, out of which India accounts 24%. Globally, 3.5% of new TB cases and 18% of previously treated cases had MDR/RR-TB.^[1] Poor health system, limited laboratory capacity for case detection, treatment barriers and complications make TB a major challenge for public health programs.^[2]

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For better patient management and successful patient outcomes, early diagnosis of disease is required. In lower-income countries, the lack of diagnostic infrastructure substantially aggravates the problem. There are number of tests available for the diagnosis of tuberculosis but TB control programmes uses conventional microscopy. Low sensitivity of conventional microscopy leads to false-negative results due to which misdiagnosis of TB suspects are common and multiple visits required for sputum collection leads to higher defaulter rate. Mycobacterial cultures for detection of MTB can be done either using solid (Lowenstein Jensen media) or liquid broth system (MGIT 320). Mycobacterial culture, on LJ media although considered as the gold standard is slow and usually takes 2-6 weeks time to yield a final result. Results by MGIT liquid culture medium come earlier (1-3 weeks) as compared to LJ medium.^[3,4,10,11]

In recent times, emphasis has shifted from older phenotypically and biochemical methods of diagnosis to molecular methods especially Polymerase Chain Reaction (PCR) technique.

Nucleic acid amplification techniques due to its rapidity and sensitivity provide the faster result. There are number of Nucleic Acid Amplification (NAA) methods that have been developed for rapid detection and identification of Mycobacterium tuberculosis (MTB) in clinical specimens of pulmonary cases.^[5-7] GeneXpert MTB/RIF assay is a rapid molecular test for diagnosis of TB recommended by WHO. The test has much better accuracy than sputum smear microscopy.^[5]

The Gene Xpert is based on real-time PCR (rT-PCR) and first fully automated cartridge based nucleic acid amplification (CB-NAAT) assay for TB detection and simultaneous detection of rifampicin resistance . It gives result within 2 hours. Diagnostic accuracy of Gene Xpert for pulmonary TB has been reported high. The Gene Xpert assay is a rapid diagnostic test with sensitivity of 98% in smear positive cases and 70% in smear-negative cases. Limited Indian data is available as this test is fairly recent to our country.^[9,10]

AIM

To compare sputum AFB smear report with Gene Xpert RIF/MTB assay in suspected cases of pulmonary tuberculosis patient.

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

MATERIALS AND METHODS

This study was a cross sectional prospective study conducted in the Department of Pulmonary Medicine, Muzaffarnagar Medical College, Muzaffarnagar from January 2018 to 30 April 2019. We reviewed the detection of MTB and RIF resistance using the Xpert MTB/RIF in 67 patients of suspected pulmonary tuberculosis and compared with conventional smear microscopy

Inclusion criteria

Patients with clinical suspicion of pulmonary tuberculosis including symptoms of cough for >2 weeks, weight loss, fatigue, haemoptysis and loss of appetite with in territory of Muzaffarnagar Medical College.

All sputum specimens from IPD and OPD were received with request of two tests: 1. AFB smear examination and 2. GeneXpert MTB/RIF assay. Exclusion criteria: Those specimens which had only one method, either GeneXpert or AFB smear examination were excluded from the study.

RESULTS

Among total no of 67 patients , 38 were AFB smear positive and 29 were AFB smear negative.

Table 1: Mycobacterium tuberculosis detected by AFB staining.

Total	AFB smear positive	AFB smear negative
67	38 (56.71%)	29 (43.28%)

All smear positive and negative patients were subjected to GeneXpert MTB/ RIF assay.

Table 2: Mycobacterium tuberculosis detected by Gene Xpert.

Total	Gene Xpert MTB detected	Gene Xpert MTB NOT detected
67	56(83.58%)	11(16.41%)

Out of the 67 samples examined, 56 samples (83.58%) were MTB detected by GeneXpert assay. Among 38 smear positive patients,33 patients were GeneXpert MTB positive and 05 were negative. Among 29 smear negative patients, 23 patients were GeneXpert positive and 06 were negative.

Table 3: GeneXpert MTB/RIF assay result among AFB smear positive and AFB smear negative patients.

	AFB smear positive	AFB smear negative	Total
Gene Xpert MTB not detected	5	06	11
Gene Xpert MTB detected	33	23	56
Total	38	29	N=67

With the help of chi square test in this table p value is 0.4096 (>0.05)

Table 4: Observation of AFB staining report & GeneXpert MTB/RIF assay report in patients

AFB smear + GeneXpert +	33
AFB smear - GeneXpert +	23
AFB smear + GeneXpert -	05
AFB smear - GeneXpert -	06
Total	67

Only 05 sample showed negative result for GeneXpert but smear positive. The probable reason for false negative result would be PCR inhibitors present in the assay.

Out of the 67 samples examined, 38 samples (56.71%) were MTBC positive by smear microscopy while 56 samples (83.58%) were MTB detected by GeneXpert assay. GeneXpert indeed detected 18 additional positive cases as compared to smear microscopy. Only two clinical isolates of the entire patients were resistant to Rifampicin .There was no statistically significance in diagnostic value between GeneXpert and AFB smear microscopy in sputum samples. (p>0.05). Among 29 smear negative

patients, 23 patients were GeneXpert positive and 06 were negative that means smear negative are the most likely to be benefited from GeneXpert.

DISCUSSION

WHO have started the largest roll-out of GeneXpert, as detecting TB rapidly and identifying drug resistance on the spot is an essential step to improve care of the infected people and avoid transmission in the community.^[12] In a survey conducted among prisoners, in Malaysia, it was documented that a single GeneXpert assay proved to get 53% sensitivity, 100% specificity and concluded that single test yields low screening sensitivity.^[13]

Sharma et al show Sensitivity and specificity of GeneXpert in sputum assay 96.9% and 99.8% respectively.^[14] In MTB, mono resistance to rifampicin is rare and almost 90% of resistant cases to rifampicin are also resistant to isoniazid, so a positive result of rifampicin is a strong surrogate of MDR-TB, and in resistant isolates it has been shown that from 95% to 98% RIF resistance is caused by mutations in the proB gene encoding the RNA polymerase B-unit.^[15,16]

Since 2010, the WHO recommended the Xpert MTB/RIF assay as a diagnostic tool, being reported in a multi-country study to have sensitivities of 98.2% among smear-positive, culture positive patients and 72.5% among smear negative, culture positive patients on a single direct Xpert MTB/RIF test compared to repeated smears and culture results.^[17] Other researchers reported 100% sensitivity and specificity of 86%,^[18] and 86.9% sensitivity and 99.7% specificity.^[19] In India, the government has provided GeneXpert equipment under the Revised National Tuberculosis Control Program (RNTCP) to recognised medical centers and services can be availed free of cost for rapid detection of rifampicin resistance tuberculosis.

Out of 67 sputum specimens, 38 were AFB smear positive and 56 were GeneXpert positive. 05 samples were found to be AFB staining positive and GeneXpert negative. Even though Gene Xpert MTB/RIF test detect 18 additional cases the difference was not statistically significant ($P > 0.05$) that means GeneXpert and AFB smear microscopy share almost same diagnostic value in sputum samples. Therefore GeneXpert does not eliminate the need of conventional microscopy to diagnose and monitor the progression of treatment. Rifampicin resistance was seen in only two cases of pulmonary specimens in this study. Rifampin resistance was uncommon in our study (4.1%). Although GeneXpert detect Rifampicin resistance rapidly it does not eliminate the need of culture to detect resistance to drugs other than Rifampicin.

CONCLUSION

There is a great need for better TB diagnostics primarily in developing countries. Basic test to diagnose pulmonary tuberculosis by AFB smear microscopy has poor sensitivity which leads to false-negative. Mycobacterial cultures either using solid or liquid broth system take time for final result and simultaneous detection of Rifampicin resistance is not possible with it. Our study show that there was no statistically significance in diagnostic value between GeneXpert and AFB smear microscopy in sputum samples. Therefore GeneXpert does not eliminate the need of smear microscopy to diagnose and monitor the progression of treatment. On other side GeneXpert can be a useful diagnostic method in patients of suspected MDR pulmonary tuberculosis due to its rapidly (2 hrs) detection of Rifampicin resistance but it does not eliminate the need of culture to detect resistance to drugs other than Rifampicin and therefore test results must always be confirmed by culture and DST due to rising incidence of XDR cases. The early diagnosis of MDR TB by MTB/RIF assay and to initiate correct regimen with regular follow up would be helpful in management of MDR tuberculosis patients.

Limitations

As number of sputum samples present in this study is less, further studies with more number of samples need to be done.

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