Diagnostic Utility of Bronchoalveolar Lavage Xpert MTB/RIF Assay in Suspected Cases of Pulmonary Tuberculosis

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ABSTRACT

Introduction: Limited data on the diagnostic accuracy of the Xpert MTB/RIF assay using bronchoalveolar lavage fluid from patients with suspected pulmonary tuberculosis have been reported in India. The aim of this study was to evaluate Xpert MTB/RIF on bronchoalveolar lavage fluid in suspected PTB patients by comparing with AFB smear microscopy and culture

Material and methods: A cross sectional observational study was carried on a total of 450 bronchoalveolar lavage fluid were processed by Xpert MTB/RIF between January 2018 and December 2018. Culture results were considered as the gold standard for diagnosis of TB. Sensitivity, specificity, PPV, and negative predictive value (NPV) for the diagnosis of active PTB were calculated using the website http://vassarstats.net/clin1.html. SPSS statistics version 17.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

Results: Xpert MTB/RIF had a sensitivity of 97.05% (95% CI 88.8-99.4) while the specificity of 90.05% (95% CI 86.4-92.7) in culture confirmed cases and those for smear microscopy was 36.7% (95% CI 25.6-49.3) and 100% (95% CI 98.7-100) respectively.

Conclusion: The Xpert MTB/RIF assay is a rapid and simple technique with significantly higher sensitivity for diagnosing pulmonary TB compared to smear microscopy.($p < 0.005$)

Keywords: Gene Expert, Balf, MTB, Pulmonary TB, Sensitivity

INTRODUCTION

Tubercluosis is a global health problem of immense importance leading to significant morbidity and mortality in the developing world. TB caused an estimated 1.3 million deaths among HIV negative people and there were an additional 300,000 deaths from TB among HIV positive people.[1] Globally, the best estimate is that 10.0 million people developed TB diseases in 2017, two thirds were in developing countries in which india was leading with 27% of TB cases.[1] Deaths due to TB are intolerable because commonly and easily available anti tubercular drugs having greater than 90% of cure rates. The major task with TB is getting a quick and precise diagnosis to start treatment at a very early stage. In 40-60% of pulmonary TB cases sputum are negative for acid fast bacilli.[2] Since smear negative patients comprise the major portion and are still infectious so delay in diagnosis in these leads to increased morbidity and mortality. Bronchoscopy with bronchoalveolar lavage is routinely performed for these subset of patients of pulmonary tuberculosis.[3,4] Bronchoalveolar lavage is sent for ZN staining and mycobacterial cultures. ZN staining has very low sensitivity of 41% while culture considered as the gold standard with sensitivity of 86% but results takes 6-8 weeks.[5] Overcoming most of these shortcomings recently a new diagnostic assay known as Xpert MTB/RIF assay has been developed.

Xpert MTB/RIF assay is a hemi nested real-time PCR test that simultaneously identifies MTB/RIF resistance. Its diagnostic efficacy is comparable to culture in sputum samples and provides results within 2 hours.[6] In India, a high TB burden country there is scarcity of literature on utilization of MTB/RIF assay on BAL fluid for diagnosis of pulmonary tuberculosis.

The aim of this study was to measure diagnostic utility of Xpert MTB/RIF assay on BAL fluid for diagnosis of pulmonary tuberculosis in smear negative and sputum scarce cases in a tertiary care settings with high incidence of TB and compare it with traditional mycobacterial culture.

MATERIAL AND METHODS

This was an analytical retrospective study carried out in the Department of Microbiology, division Mycobacteriology, Government medical college and associated hospitals Srinagar on 450 patients with suspected pulmonary tuberculosis from January 2018 to December 2018. Approval from institutional ethical committee was taken.

Inclusion criteria

Patients aged above 18 years with clinical suspicion of pulmonary tuberculosis including symptoms of cough with or without expectoration for >2 weeks, weight loss, fatigue, haemoptysis and loss of appetite.

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Exclusion criteria

Smear positive cases, extra pulmonary TB cases

Patient with history of lung malignancies, fungal infections or evaluated for alternate diagnosis

Patients who had received more than 2 weeks of anti-tubercular therapy (ATT) in the past 90 days,

BAL specimens of 450 patients with suspected pulmonary tuberculosis were processed for ZN microscopy, Solid AFB culture and Xpert MTB/RIF assay.

Each BAL samples received in the lab from the associated hospitals as per the collection and transportation policy of the laboratory, were divided into three parts; one part was immediately tested using Xpert MTB/RIF assay, second part used for ZN smear microscopy and third part for Solid culture on lowenstein Jenson (LJ) medium and performed on same day. GeneXpert testing was performed according to the manufacturer's instructions.[7] Sample reagent was added to untreated BAL at 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. Direct Smear microscopy was performed to investigate presence of acid fast bacilli with the second part of the specimen using conventional ZN staining method. Slides showing red coloured acid fast bacilli were taken as positive and negative slides were those without any acid fast bacilli.[8] Third part was processed using the N-acetyl-L cysteine- sodium hydroxide method (NALC-NaOH) as per the manufacturer's instructions, cultured on LJ medium. Sodium hydroxide (NaOH) is a decontaminating agent and also acts as emulsifier and NALC acts as a mucolytic agent and also reduces the concentration of NaOH required.[8] Positive culture tubes were confirmed by rapid immunochromatography test kit using MPT 64 antigen (SD MPT64TB Ag kit) according to the manufacturer's instructions. Any diagnostic sample that was detected as non-tuberculous mycobacterium (NTM) by culture method was considered as "non-TB."

Data were obtained using both paper and electronic documentation systems of the laboratory. The socio-demographic and clinical data of each patient were collected. By taking culture method as reference, samples that were positive and negative in culture were considered true positive and true negative. Culture negative and Xpert MTB/RIF assay positive samples were taken as false positive samples. Xpert MTB/RIF assay negative and culture positive samples were considered false negative.

STATISTICAL ANALYSIS

The sensitivity, specificity, PPV, and negative predictive value (NPV) for the diagnosis of active PTB were calculated using the website http://vassarstats.net/clin1.html. The 95% confidence intervals (CIs) were estimated according to exact binomial distribution. Sensitivity and specificity values were compared using McNemar's test. All CIs were two-sided. A p-value of <0.05 was considered statistically significant. SPSS statistics version 17.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis.

RESULTS

Data of total of 450 presumptive TB patients was analysed in the study. Out of 450 BAL fluid samples MTB was detected in 104 (23.1%) by Xpert MTB/RIF assay. The mean age of the patients was 38 ± 20 years. About 250 patients (55.5%) were females while the rest were male patients. The common presenting complaints included cough with or without sputum (44.4%), fever (42.2%) and hemoptysis (13.3%). Radiological features compatible with the diagnosis of tuberculosis included cavity (38.6%), consolidation (33.3%), nodulo-striate opacities (17.7%) and others (10.2%). The demographic features of these patients are presented in Table I. Out of 450 patients who were tuberculosis suspect,

Age	Mean 38 ± 20 years
Gender	
Male	200
Female	250
Symptoms	
Cough	200
Fever	190
Hemoptysis	60
Radiological features	
Cavitation	174
Consolidation	150
Nodulo-straite opacities	80
Others	46

Table-1: Demographic and clinical features of the cases

ZN Staining	No. of samples	Xpert MTB/RIF assay Positive (n= 104)	Negative (n=346)
Positive	25	20	5
Negative	425	84	341
Culture			
Positive	68	66	2
Negative	382	38	344

Table-2: Frequency of BAL AFB smear and Gene xpert as compared to mycobacterial cultures

	Sensitivity % 95% CI	Specificity % 95% CI
Xpert MTB/RIF assay	97.05 (88.8-99.4)	90.05 (86.4-92.7)
Smear microscopy	36.7(25.6-49.3)	100 (98.7-100)

Table-3: Sensitivity and specificity of the Xpert MTB/RIF assay, smear microscopy relative to the culture for the diagnosis of pulmonary tuberculosis

	PPV % 95% CI	NPV % 95% CI
Xpert MTB/RIF assay	63.4 (53.3-72.5)	99.4 (97.6-99.9)
Smear microscopy	100 (83.4-100)	89.8 (86.5-92.5)

Table-4: PPV and NPV of Xpert MTB/RIF assay, smear microscopy relative to the culture for the diagnosis of pulmonary tuberculosis

68 (15.1%) cases were confirmed as pulmonary tuberculosis on mycobacterial cultures which was taken as gold standard. Out of 104 BAL fluid which were positive by Xpert MTB/RIF assay 66 (63.4%) were positive by culture including 25 (24.03%) positive on microscopy for acid fast bacilli. The correlation of Xpert MTB/RIF assay with other diagnostic modalities is given in Table II.

With the culture method as the reference standard, the Xpert MTB/RIF assay with BALF specimens showed positive results for 66 of the 68 patients with culture-positive TB; the sensitivity of the Xpert MTB/RIF assay was 97.05% (95% CI 88.8-99.4) while the specificity was 90.05% (95% CI 86.4-92.7) (Table III). All of the samples were also tested by smear microscopy, which had a sensitivity of 36.7% (95% CI 25.6-49.3) and a specificity of 100% (95% CI 98.7-100). With culture as the reference standard, the overall PPV and NPV for the Xpert MTB/RIF assay were and 63.4% (53.3-72.5) and 99.4% (97.6-99.9), respectively. The corresponding values for smear microscopy were 100% (83.4-100) and 89.8% (86.5-92.5) respectively (Table IV).Only one patient was detected as rifampicin resistance by Xpert MTB/RIF assay while all other were rifampicin sensitive

DISCUSSION

To date, sputum smear microscopy remains the first microbial analysis for both the diagnosis of TB and the assessment of patient infectiousness. However, the limited sensitivity of this method hinders its widespread application for TB diagnosis. In addition, smear microscopy shows limited specificity, because AFB staining cannot distinguish between MTB and non-tuberculosis mycobac-teria (NTM). Although culture is the 'gold standard' for TB diagnosis, it is slow and may take up to 2–8 weeks to yield results. The Xpert MTB/RIF assay is a rapid, automated molecular test with good sensitivity for PTB on sputum samples. However, with regard to its utility on BALF samples, especially in smear-negative and sputum scarce cases only a few studies have been conducted so far. In this study, we evaluated Xpert MTB/RIF on BAL fluid in sputum smear-negative and sputum-scarce PTB patients in a high incidence setting. In this study, the Xpert MTB/RIF assay was found to have a sensitivity of 97.5% when compared to culture, which was higher than the sensitivity of smear microscopy (36.7%). The results for the sensitivity of the Xpert MTB/RIF assay are consistent with those of previous studies conducted by Dewald et al., who reported a value of 92.3%, and a study conducted by Khalil et al., who reported a sensitivity of 91.86%.[9,10] while specificity was 90.05% which is comparable to studies by Pierrae et al. (98.6%) and Lee et al. (100%).[11,12] PPV and NPV of 63.4% (53.3 -72.5) and 99.4% (97.6-99.9) in our study is comparable to a study by Aggarwal et al.[13]

In comparison with culture used as gold standard, sensitivity, specificity, PPV and NPV for Smear microscopy for BAL sample were recorded as 36.7%, 100%, 100% and 89.8% respectively, which is in line with other studies.[9,10,11] Xpert MTB/RIF compared to smear microscopy showed significantly higher sensitivity (97.5 vs 36.7%, $p < 0.005$) but slightly lower specificity (90.05 vs 100%, $p < 0.005$)

In this study, there were also 2 cases that tested culture-positive and Xpert MTB/RIF-negative; two cases were finally confirmed to have MTB infection. A possible explanation for this discrepancy could be the presence of PCR inhibitors or insufficient nucleic acid material in some specimens. Another reason could be that BALF samples in this study were contaminated with a little blood, which could have affected detection using the Xpert MTB/RIF assay. In support of this speculation, a previous study reported that the sensitivity of Xpert MTB/RIF on bloodstained sputum is lower because blood is a known inhibitor of DNA amplification.[14]

Thirty eight cases were identified to be Xpert MTB/RIF-positive but culture-negative, a similar situation has also been reported in previous studies.[15,16] One explanation for these differences in the results between Xpert MTB/RIF and culture may be the nature of the PCR test. The Xpert MTB/RIF assay amplifies any DNA whether it originates from live or dead bacilli; therefore, it cannot be assumed that a positive result equates to active disease. Other reason for Xpert positive culture negative results could have been the inclusion of cases which had received <14 days of ATT which could have resulted in negative cultures. Furthermore in our clinical setting, patients with clinical and radiological features of pulmonary infection receive non-TB antibiotics when symptoms are present <2 weeks. Beta-lactams are reported to have early antitubercular activity comparable to the conventional ATT drugs other than isoniazid.[17] We hypothesize these reasons for Xpert positive culture negative results.

Limitations

There are some limitations to this study. First, the study was performed at a single site, which may limit generalization of the results. Second, the study was performed retrospectively, and studies with a retrospective design are generally prone to bias. One of the important strength of the Xpert assay is its ability to detect the presence of Rifampicin resistance. The sensitivity and specificity of MTB/RIF assay to detect Rifampicin resistance in our study was not evaluated and not included in our objective as facilities for detection of rifampicin sensitivity by phenotypic method were not available.

CONCLUSION

GeneXpert and AFB smear microscopy share almost same

specificity but sensitivity of GeneXpert is much higher than AFB smear microscopy in BAL fluids. Although culture is considered as a gold standard method but as it takes days to come positive and simultaneous detection of Rifampicin resistance is not possible with it. On other side GeneXpert can be a useful diagnostic method in patients of suspected pulmonary tuberculosis either AFB smear negative or positive due to its rapidity and simultaneous detection of Rifampicin resistance. Positive GeneXpert, but culture negative results need to be read cautiously and should be well correlated with clinical and treatment history of the patient.

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