

DIAGNOSTIC YIELD OF BRONCHOALVEOLAR LAVAGE XPERT MTB/RIF ASSAY (GENE XPERT® IN SPUTUM SMEAR NEGATIVE PULMONARY TUBERCULOSIS PATIENTS – A ONE YEAR CROSS SECTIONAL STUDY

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ABSTRACT

Introduction: Tuberculosis remains a major public health issue and is one of the top ten causes of death worldwide. Seven countries worldwide account for 64% of the total incidence and India leading the count. Only about 50-60% of the cases are sputum smear positive, while rest are sputum scare or smear negative cases. Since December 2010, WHO has recommended the use of Xpert MTB/RIF assay due to its high-quality performance, as compared to microscopy in the diagnosis of tuberculosis. The present study was done to evaluate the early diagnosis of sputum smear negative pulmonary tuberculosis patients using Xpert MTB/RIF assay test in broncho-alveolar fluid and to evaluate prevalence of rifampicin resistance in these patients.

Methods: This was a one year cross section study conducted in a tertiary care hospital. A total of 143 suspected cases of pulmonary tuberculosis patients who were sputum smear negative were included in the study. All patients underwent bronchoscopy and the BAL specimen was subjected for Xpert MTB/RIF assay, smear by ZN staining and AFB Culture using MGIT 960 medium. The sensitivity and specificity of the Xpert MTB/RIF assay was compared to AFB culture which is considered as the gold standard method for diagnosing TB was calculated.

Results: Majority of the patients were males (74.83%) and under the age group of 30 years (26.57%). Out of 143 suspected, the final diagnosis of PTB was done in 93 patients. Out of 93 cases of PTB, 87(93.5%) patients were Xpert MTB/RIF assay positive, 80(86.0%) were AFB culture positive and 39(41.9%) patients were AFB smear positive. The sensitivity and specificity of BAL Xpert MTB/RIF assay was 92.5% and 79.37% respectively (p <0.0001).

Conclusion: The Xpert MTB/RIF assay is more sensitive and specific than smear microscopy for the diagnosis of PTB in sputum smear negative on specimens obtained from broncho-alveolar lavage (BAL).

Keywords: Xpert MTB/RIF assay, BAL, PTB, smear negative.

INTRODUCTION

Tuberculosis due to Mycobacterium tuberculosis remains a major public health issue. For the past 5 years, it has been the leading cause of death from a single infectious agent, ranking above

HIV/AIDS¹. India is one among the six countries that account for 60% of all new TB cases worldwide. Universal access to high-quality, patient-centered treatment for all TB patients is emphasized by WHO's Stop TB Strategy². However, in many areas of the world, TB diagnosis still relies on insensitive, poorly standardized sputum microscopy methods³. As much as 50-60% of AFB culture-positive clinical specimens may fail to reveal AFB on smear made from the specimen. Ineffective TB detection and the emergence and transmission of drug-resistant MTB strains increasingly jeopardize global TB control activities².

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Xpert MTB/RIF assay meets the demands as mentioned below in a remarkable manner³. It is a nucleic-acids amplification test for the detection of MTB complex in sputum or concentrated sputum sediment and the detection of RIF resistance-associated mutations of the *rpoB* gene. The Xpert MTB/RIF assay has the potential to bring standardized, sensitive and very specific diagnostic testing for both TB and drug resistance³. WHO recently has recommended Xpert MTB/RIF® assay test for the early diagnosis and rifampicin resistance and this has been incorporated in various national TB control programmes¹. Recent studies have found that when combined with Xpert MTB/RIF, Bronchoalveolar lavage fluid (BAL) could provide accurate results in detecting early-stage pulmonary TB, particularly in smear-negative pulmonary tuberculosis patients⁴. The present study was done to evaluate the role of BAL fluid for the early diagnosis of sputum smear negative pulmonary tuberculosis by using Xpert MTB/RIF assay test and to detect prevalence of rifampicin resistance in these patients.

MATERIALS AND METHODS

The present study was carried out in the KAHER's Department of Respiratory Medicine, Dr. Prabhakar Kore Hospital & Medical Research Centre, Belagavi, Karnataka between January 2017 to December 2017. All the patients with clinical and radiological suspicion of pulmonary tuberculosis with sputum smear negative for acid fast bacilli were included in the study. A total of 143 patients were included in the study.

Inclusion criteria: Patients of either gender aged 12 years and above, suspected cases of pulmonary tuberculosis, those who have not taken anti-TB medications or taking less than one week, HIV positive and immune compromised individuals.

Exclusion criteria: AFB smear positive cases, extra-pulmonary tuberculosis cases.

Bronchoscopy Procedure: Patients who were fulfilling the inclusion criteria were taken up for bronchoscopy after the informed written consent. Procedure was carried out electively after the patient being nil orally atleast for 4 hours.

Bronchoscopy procedure was done using Flexible fiberoptic video bronchoscope in dedicated suite under conscious sedation. Broncho-alveolar lavage was obtained by instilling aliquots of 20 ml of 0.9 % saline at room temperature into a segment of an affected lobe or segment and aspirating available fluid, maximum up to 100 ml of fluid was instilled. The obtained Broncho-alveolar lavage fluid was subjected to AFB smear by Ziehl-Neelsen staining, AFB culture using the MGIT 960 liquid medium and Xpert MTB/RIF assay test.

Bronchoalveolar lavage samples: A volume of one ml of BAL sample was transferred to the G4 version of Xpert® MTB/RIF (Cepheid, USA) cartridges without initial decontamination or centrifugation. The remaining BAL fluid was processed by the standard decontamination protocol, using NALC/NaOH method and centrifuged. AFB smear was done according to the standard protocol for Ziehl-Neelsen staining⁵. The centrifuged sample after decontamination was inoculated for liquid culture in BACTEC mycobacterium growth indicator tube (MGIT) 960 system (BD Diagnostics, USA)⁶. The BACTEC 960 TB System has been reported to yield 15-20% increased culture positivity on clinical specimens as compared to conventional solid media such as LJ medium, with an average time to detection of positive growth from 8 to 14 days as compared to 3 to 5 weeks on solid media⁶. Isolates were identified as *Mycobacterium tuberculosis* by immunochromatographic test kit (SD MPT64 TB Ag kit). Any diagnostic sample that was detected as nontuberculous mycobacterium (NTM) by culture method was considered as "non-TB."

Xpert® MTB/RIF Assay: It is an automated molecular test for *Mycobacterium tuberculosis* and its resistance to rifampicin, based on the Cepheid GeneXpert system. It uses hemi-nested real time PCR assay to amplify a specific sequence of the *rpoB* gene, which is then probed with molecular beacons for mutations within rifampicin-resistance determining region, providing a result within 2 hours. It is a single test that can detect both *Mycobacterium tuberculosis* complex and rifampicin resistance within 2 hours after starting the assay, with minimal hands on technical time⁷. The assay utilizes single-use plastic cartridges with multiple chambers that are preloaded with liquid buffers

and lyophilized reagents beads necessary for sample processing, DNA extraction and hemi-nested-PCR. Following sample loading all steps in the assay are automated and contained within the cartridge. The test procedure may be used directly on clinical specimens, either fresh sputum samples or sputum pellets, which are obtained after decontaminating and concentrating the sputum⁸. The test material is treated with a sodium hydroxide and isopropanol-containing sample reagent, mixed by hand or vortex, and incubated at room temperature for 15 minutes. After incubation. 2 ml of the treated sample is transferred to the cartridge, and the run is initiated. The test platform employs a sonic horn that inserts into the cartridge base to cause ultrasonic lysis of the bacilli and release of the genetic material⁹. The assay then amplifies a 192 bp segment of the *rp0B* gene using a hemi-nested rt-PCR reaction.

Final diagnosis: A final diagnosis of pulmonary tuberculosis was based on composite reference standard which included two criteria – AFB culture cases and probable PTB. “Culture confirmed PTB” were cases with MTB culture positive on MGIT culture. “Probable PTB” were cases without MTB on culture or alternate diagnosis, showing complete resolution in the clinical and radiological features of PTB to anti-TB drugs. The response to anti-TB drugs was monitored during follow up of patients every 2 months for a total of 6 months. Rests of the cases either with an alternate diagnosis or showing no improvement with anti-TB drugs were considered non-TB.

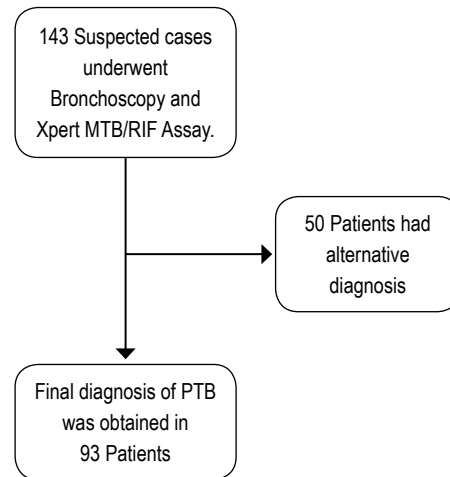
Before the commencement of the study the Ethical clearance was obtained from the Ethical and Research Committee, Jawaharlal Nehru Medical College, Belagavi.

Statistical analysis: Data analysis was performed using SPSS 20.0 software and Excel 2010 software. The demographical features like age, gender, clinical and radiological findings, past history of tuberculosis and history of contact with PTB patients were recorded. The sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) of Xpert MTB/RIF and smear microscopy, with culture as the

reference standard was calculated. A “p” value of <0.05 was considered to be statistically significant.

RESULTS AND OBSERVATION

A total of 143 patients underwent BAL Xpert MTB/RIF assay, the final diagnosis of PTB was obtained in 93 patients (Method of achieving final diagnosis has been explained in Methods).



A total of 143 patients who were suspected to have pulmonary tuberculosis were included in the study, out of which 107(74.83%) were males and 36(25.17%) were female patients. Most of the patients included were <30 years of age and the mean age was 46.38 ± 17.11 yrs. The cough was the most common symptom in 117 (81.82%) the patients followed by fever in 81(56.64%), weight loss in 67(46.85%), decreased appetite in 41(28.67%), breathlessness in 27(18.88%) and hemoptysis in 25(17.48%) patients. 12(8.39%) of patients had history of contact with the TB patients and 67(46.85%) of patients had previous history of tuberculosis. The predominant findings on chest x-ray was infiltrations in 69(48.25%)patients, followed by cavity-27(18.8%), fibrosis-16(11.19%), consolidation-14(9.79%), destroyed lung-10(6.99%), pleural effusion-7(4.9%), pneumothorax- 1(0.7%) and chest x-ray was normal in 4(2.8%) patients. A total of 41(28.67%) patients were diabetics, 7(4.9%) patients were HIV positive and 12(8.3%) of the patients had other predisposing conditions.

Table 1: Percentage of positive results				
Diagnostic tests	Present	%	Absent	%
Xpert MTB/RIF	87	60.84	56	39.16
Smear method	39	27.27	104	72.73
AFB culture method	80	55.94	63	44.06

Out of 143 suspected cases of pulmonary tuberculosis, BAL Xpert MTB/RIF assay was positive in 87(60.84%) patients, BAL AFB Smear was positive in 39(27.27%) patients and AFB culture using the MGIT 960 medium was positive in 80(55.94%) patients.

Table 2: Sensitivity and specificity of Xpert MTB/RIF assay as compared to AFB culture	
Sensitivity	92.50%
Specificity	79.37%
Positive predictive value	85.06%
Negative predictive value	89.29%

The Xpert MTB/RIF assay was 92.5% sensitive and 79.37% specific as compared to the AFB culture which is the gold standard test for the diagnosis of tuberculosis with a p value of 0.0001 which is statistically significant, where as the sensitivity and specificity of BAL AFB smear as compared to the AFB culture was of 47.5% and 98.41% respectively.

Table 3: Correlation between Xpert MTB/RIF assay and AFB culture method						
Xpert MTB/RIF Assay	AFB culture				Total	%
	Present	%	Absent	%		
Present	74	85.06	13	14.94	87	60.84
Absent	6	10.71	50	89.29	56	39.16
Total	80	55.94	63	44.06	143	100.00

Thirteen patients were Xpert MTB/RIF assay positive but AFB culture negative. Four patients with Xpert MTB/RIF assay positive with previous history of PTB had AFB culture negative. In the present study 13 patients with Xpert MTB/RIF positive and AFB culture negative were started on anti-tubercular treatment based on clinico-radiological grounds and 3 were lost to follow up and 10 were started on ATT and all of them showed clinico-radiological improvement.

Table 4: Distribution of patients by Rifampicin resistance		
Rifampicin resistance	No of patients	% of patients
Present	16	11.19
Absent	127	88.81
Total	143	100.00

Table 4 Rifampicin resistance was found in 16 (11.19%) out of 87 patients who were Xpert MTB/Rif MTB/RIF positive and all (100%) were AFB culture positive. A total of 10(62.5%) patients who were Xpert MTB/RIF assay positive with previous history of PTB had rifampicin resistance.

Table 5: Final diagnosis by BAL Analysis		
Final diagnosis	No of patients	% of patients
Bacterial Pneumonia	7	4.89
Lung cancer	13	9.09
Non tubercular Mycobacterial Infection(NTM)	1	0.70
Pneumocystis Pneumonia	1	0.70
Post tubercular sequelae	28	19.58
Pulmonary Tuberculosis	93	65.03
Total	143	100.00

A total of 93(65.03%) patients had a final diagnosis of pulmonary tuberculosis by BAL analysis, 7(4.89%) - bacterial pneumonia, 13(9.09%) – lung cancer, 1(0.70%) –non-tuberculous mycobacterium infection, 1(0.70%) – pneumocystis pneumonia, and 28(19.58%) patients were diagnosed with post-tubercular sequelae.

DISCUSSION

All the patients in the present study were sputum smear negative and clinically suspected cases of pulmonary tuberculosis. Almost 50-60% of the cases are found to sputum scarce or smear negative for the tuberculosis¹⁰. Although the relative transmission rate of smear negative tuberculosis is lower than that of smear positive cases, it is still responsible for 17% of tuberculosis transmission¹¹. When the patients present with the

signs and symptoms of TB and if the sputum is found to be negative then the further investigation is required. The other respiratory specimens like the bronchial washings, bronchial aspirate, BAL, TBLB and gastric lavage in children examinations helps to achieve the diagnosis. Fiberoptic bronchoscopy constitutes an interesting alternative for TB diagnosis in smear-negative or sputum-scarce patients^{12,13}. Paludet al¹² and Robert et al¹⁴ observed that the use of bronchoscopy with BAL allows for better diagnosis and understand the inflammatory nature of many diseases and in patients with infectious granulomatous disease. In this study we have confirmed that bronchoscopy supplemented with lavage was useful in diagnosing TB¹⁴.

The concentration of *M. tuberculosis* bacteria in clinical sputum samples can vary from over 10⁷ to less than 20 CFU/ml¹⁵. The AFB smear requires 10³-10⁵ bacilli /ml of sputum; it is therefore difficult to diagnose the patients who are paucibacillary like in HIV individuals. Recent diagnostic tests like the Xpert MTB/RIF and Xpert Ultra can detect upto 131cfu/ml and 31cfu/ml in the given specimen, thus increasing the diagnostic rate of the disease¹.

Xpert MTB/RIF test integrates sample decontamination, hands free operation, onboard sample processing, and is an ultrasensitive hemi nested PCR for the simultaneous detection of Mycobacterium tuberculosis and rifampicin resistance, either in expectorated sputum or concentrated sputum sediments, in approximately two hours¹⁶. Testing is standardized and requires only moderate laboratory infrastructure and training. The conventional diagnostic methods for Mycobacterium tuberculosis are slow and/or lack sensitivity¹⁵. A number of new diagnostic approaches like Xpert MTB/RIF assay have thus brought incremental improvements to detection and drug susceptibility testing.

In our study, out of 143 suspected cases of tuberculosis, BAL Xpert MTB/RIF assay was positive in 87 (60.84%) patients, BAL AFB smear was positive in 39 (27.27%) and AFB culture using the MGIT 960 medium was positive in 80 (55.94%) patients. This result indicates BAL Xpert MTB/RIF assay is more sensitive and faster method for detection of tuberculosis as compared to AFB culture. However, in a retrospective study

conducted by Paludet al¹², BAL Xpert MTB/RIF assay was 60% sensitive and culture was 66 % sensitive, but culture took longer time for diagnosis as compared to Xpert MTB/RIF assay.

So, the Xpert MTB/RIF assay was 92.5% sensitive and 79.37% specific as compared to the AFB culture which is the gold standard test for the diagnosis of tuberculosis (p value <0.0001). The results of our study are comparable with studies conducted by Barnard et al¹⁷ which showed Xpert MTB/RIF assay sensitivity of 92.3% and specificity of 87.7%. Palud et al¹² included 162 patients and observed the sensitivity of the Xpert MTB/RIF assay to be 80% and specificity to be 98.6%. A study conducted by Khalifaet al¹⁸ in Pakistan where the prevalence of TB is high showed that the Xpert assay helped in diagnosing the disease in 93 patients with a sensitivity of 91.86% and specificity of 71.42%.

Cochrane systematic review¹⁹ done in 2013 has observed that Xpert MTB/RIF assay test to be highly accurate. When compared to culture, Xpert has about 88% sensitivity and 98% specificity for pulmonary TB in adults. In smear-negative patients with TB, Xpert had a sensitivity of 67%. For rapid detection of rifampicin resistance, the sensitivity is 94% and specificity is 98%¹⁹.

As the incidence and prevalence of TB is high in India there is requirement of high suspicion and early diagnosis of TB so as to decrease the transmission rates, early initiation of treatment and also the early detection of MDR TB. There are several studies conducted in India which show the similar results. Study conducted by Hazarikaet al²⁰ showed that 88 out of 162 patients had Xpert assay positive. The sensitivity of Xpert was 78.89% and specificity was 95.83%.

The patients who have been treated for pulmonary tuberculosis can reveal Genexpert test positivity due to the presence of dead bacilli and their DNA gets amplified by this test²¹. One study has shown that up to 27 % of patients have been reported to remain sputum Xpert MTB/RIF positive 26 weeks after successful anti-tuberculous treatment was initiated²². Due to the nature of the polymerase chain reaction test, Xpert MTB/RIF amplifies any DNA whether it originates from alive or dead bacilli. Therefore, it cannot be assumed, solely on the

basis of the test that a positive result equates to active disease²³. In this study 13(9.1%) patients were Xpert positive but culture negative out of which 4(30.7%) patients had previous history of tuberculosis, Xpert detects DNA from nonviable cells that are not intact, thereby suggesting that free DNA—and not just DNA from intact cells—is detected by Xpert, and this free DNA is non culturable²⁴. This might be a possible cause of false positivity.

Six cases (10.71%) were Xpert Negative but culture positive. This might be due to the reason that the Xpert assay can detect upto 131cfu/ml of bacilli and the AFB culture can detect 10-100 bacilli/ml and hence in paucibacillary condition like in HIV positive individuals and immunocompromised conditions culture plays an important role in the diagnosis²⁵. One patient was diagnosed to have non-tubercular mycobacterium infection based on the culture report and the patients Xpert assay was negative for MTB, the Xpert MTB/Rif assay does not detect DNA from nontuberculous mycobacteria, and almost all positive results likely reflect the true detection of *M. tuberculosis* complex DNA²⁴.

CONCLUSION

The findings of our study shows that the Xpert MTB/RIF assay is more sensitive and specific than smear microscopy for the diagnosis of pulmonary tuberculosis in sputum smear negative on specimens obtained from bronchial washing, and can be routinely utilized in patients with a high clinical suspicion of pulmonary tuberculosis. With increasing incidence and prevalence of MDR tuberculosis Xpert MTB/RIF assay can be a useful tool for early diagnosis, and the other major advantage is that it simultaneously detects Rifampicin resistance. Patients with Xpert MTB/RIF assay positive but AFB culture negative remains a conflict area and should be read cautiously taking the clinical and radiological findings into consideration before starting the treatment.

CONFLICT OF INTEREST

None

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