Original Research Article

Comparison of real time PCR with phenotypic methods in bronchoalveolar lavage in diagnosis of sputum smear negative pulmonary tuberculosis patients

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ABSTRACT

Background: Early diagnosis of pulmonary tuberculosis is of utmost importance for proper control of the disease in the patient. Diagnosis of pulmonary tuberculosis is usually by acid fast bacilli (AFB) smear examination and culture *Mycobacterium tuberculosis* (MTB). In this study, we have employed polymerase chain reaction (PCR) for MTB in bronchoalveolar lavage (BAL) along with AFB smear and culture MTB for early diagnosis of pulmonary tuberculosis.

Methods: A prospective observational study was conducted in the Department of Pulmonary Medicine, Era's Lucknow medical college and Hospital, Lucknow over a period of two years. A total of 123 previously treated cases of pulmonary tuberculosis were enrolled for the study whose two sputum smear samples were negative for AFB. These patients underwent fibreoptic bronchoscopy and BAL was obtained which was sent for AFB smear, culture MTB and PCR for MTB.

Results: The examination of BAL revealed the highest sensitivity for culture MTB at 87.4% followed by PCR for MTB at 73.8% and then AFB smear at 61.2%. PCR for MTB helped in diagnosing an additional 12% patients of pulmonary tuberculosis which were negative on AFB smear and an additional 6.8% patients which were negative on culture MTB.

Conclusions: PCR for MTB is useful in making an early diagnosis of pulmonary tuberculosis especially in paucibacillary cases negative on AFB smear and also in some culture MTB negative patients.

Keywords: Bronchoalveolar lavage, Paucibacillary case, PCR for MTB, Pulmonary tuberculosis, Previously treated case

INTRODUCTION

India is amongst the high tuberculosis burden countries in the South-East Asian region (SEAR).[1] SEAR itself contributes about fifty percent of the globally occurring new tuberculosis cases.[1] India alone harbours one fourth of the global burden of tuberculosis patients.[2] There has been an increase in notification of new tuberculosis cases from India leading to an increase in notified cases by 37% in 2016 in comparison to that in 2013.[3] Hence, there are many unreported cases from India probably due to several undiagnosed cases here.We commonly use

phenotypic methods like AFB (acid fast bacilli) smear and *Mycobacterium tuberculosis* (MTB) culture for diagnosis of pulmonary tuberculosis in India.[2]

A high bacillary load of 5000 to 10,000 bacilli/ml of sputum specimen is required for the smear using Ziehl-Neelsen stain to be positive. This is in contrast to 10 to 100 bacilli/ml of sputum specimen for the culture and nucleic acid amplification test (NAAT) to be positive. The bacillary load in the specimen required for NAAT to be positive is much less in comparison to that for AFB smear and the results obtained with NAAT are much more rapid in comparison to the culture MTB. Thus NAAT favours an early diagnosis in paucibacillary tuberculosis patients negative on smear examination.

Early diagnosis of pulmonary tuberculosis is of utmost importance for proper control of the disease in the patient and to decrease the mortality due to tuberculosis.[3] Early diagnosis of pulmonary tuberculosis also helps in preventing the transmission of the disease from one person to another. The reports of both AFB smear and NAAT are available within a day but the sensitivity of smear is much less than that of NAAT especially in paucibacillary cases. MTB culture remains the gold standard for the diagnosis of tuberculosis due to its high sensitivity in comparison to the smear examination and NAAT but the results are available in about two weeks for liquid culture media and more than that for solid culture media. In this study we have incorporated real time PCR (Polymerase chain reaction) along with phenotypic methods like AFB smear and culture MTB for the diagnosis of pulmonary tuberculosis at the earliest in previously treated pulmonary tuberculosis cases.[4,5]

This study was undertaken in previously treated sputum smear negative pulmonary tuberculosis patients because these patients often have radiological abnormalities as a sequelae of previous pulmonary tuberculosis infection and a negative sputum smear result delays the diagnosis of active tuberculosis in these cases.

METHODS

A prospective study was conducted in the department of Pulmonary medicine at Era's Lucknow Medical College and Hospital, Lucknow over a period of two years from 2015 to 2017. A total of 123 patients with a presumptive diagnosis of pulmonary tuberculosis on the basis of chest x-ray and symptoms were enrolled in the study. Only previously treated patients of pulmonary tuberculosis were included in the study. The patients with high clinico-radiological suspicion of active pulmonary tuberculosis were initially subjected to sputum examination. Two sputum samples- one spot sample and one early morning sample were sent for AFB smear examination by Ziehl-Neelsen method. The patients were between the age group of 16 to 80 years of which 81 were males and 42 were females. The patients whose both sputum smears were negative were included in the study

and they underwent high resolution CT thorax. These smear negative patients were then posted for fibreoptic bronchoscopy and bronchoalveolar lavage (BAL) was taken from the involved segment of the lung. BAL was sent for AFB smear, culture MTB in Lowenstein Jensen (LJ) media and real time PCR for MTB where it was centrifuged at 6000rpm for ten minutes before being subjected to further examination. The MTB culture was done in the accredited Intermediate reference laboratory at the department of microbiology, King George's medical university, Lucknow. The LJ media was inoculated with processed BAL and was incubated at 37°C. The culture media was examined 72 hours after inoculation for gross contaminants. The culture media was examined weekly upto eight weeks for the appearance of typical colonies of *Mycobacterium tuberculosis* which usually appear at least after two weeks of inoculation of BAL into the culture media. The colony was confirmed by ZN staining.

In real time PCR, the DNA was extracted from the centrifuged BAL sample and was amplified followed by analyses for IS6110 DNA sequence to detect the presence of *Mycobacterium tuberculosis* in BAL. The results of real time PCR were obtained in about two to three hours. Informed consent was taken from all the patients enrolled in the study. The approval for the study was obtained from the institute's ethics committee.

RESULTS

All the 123 patients enrolled for the study were HIV seronegative. The most frequent age group in this study was (21-40) years (38.2%) followed by (41-60) years (34.1%). Males constituted almost two thirds of the study population in this study (Table 1).

Table 1: Demographic profile of the sputum smear negative cases of pulmonary tuberculosis included in the study (n=123).

Variable	Number of cases	Percentage (%)
Age group (years)		
Upto 20	13	10.6
21-40	47	38.2
41-60	42	34.1
>60	21	17.1
Gender		
Male	81	65.9
Female	42	34.1

The most common symptom was cough (95.6%) followed by fever (92.4%), dyspnea (87.2%), weight loss (84.7%), chest pain (35.4%) and haemoptysis (15.6%). Amongst the 123 patients, 81 patients (65.85%) had taken complete antituberculosis treatment while remaining 42 patients (34.15%) had defaulted the antituberculosis treatment due to several reasons (Table 2).

The most common radiological finding on high resolution CT thorax was bronchopneumonia (60.2%) followed by centrilobular nodular pattern (21.1%), cavitatory disease (14.6%) and bronchiectasis (4.1%). Fever and cough along with history of default antituberculosis treatment and cavitatory disease on radiological examination were most consistently associated with a diagnosis of pulmonary tuberculosis in this study (Table 2 and 3).

Table 2: Diagnosis of pulmonary tuberculosis (PTB) according to the previous antituberculosis treatment.

History of previous antituberculosis treatment	NO. of cases	Diagnosed with PTB	Percentage of diagnosed cases
Previous treatment taken	123	97	78.9%
Completed treatment	81	60	74.1%
Inadequate treatment	42	37	88.1%

Radiologically, unilateral disease was present in 45

patients (36.6%) while bilateral disease was present in 78 patients (63.4%). Extent of disease in the form of unilateral or bilateral disease was equally associated with a diagnosis of pulmonary tuberculosis (Table 3). BAL for AFB smear was positive in sixty three cases while culture MTB was positive in ninety cases. All AFB smear positive cases were also culture MTB positive (Table 4).

Table 3: Diagnosis of pulmonary tuberculosis (PTB) according to the radiological findings on high resolution CT Thorax.

Extent of disease	Number of cases	Diagnosed with PTB	Percentage of diagnosed cases
Unilateral	45	35	77.8%
Bilateral	78	63	80.7%
Radiological pattern			
Bronchopneumonia	74	60	81.1%
Centrilobular pattern	26	20	76.9%
Cavitatory disease	18	17	94.4%
Bronchiectasis	05	01	20.0%

Table 4: Diagnosis of all study cases and treatment given (n=123).

Number of cases	AFB smear	Culture MTB	PCR for MTB	Treatment
63	Positive	Positive	Positive	ATT
06	Negative	Positive	Positive	ATT
07	Negative	Negative	Positive	ATT
21	Negative	Positive	Negative	ATT
06	Negative	Negative	Negative	ATT
01	Negative	Negative	Positive	Antibiotics
19	Negative	Negative	Negative	Antibiotics

(ATT: Antituberculosis treatment)

Real time PCR for MTB was positive in seventy seven cases. In one case with history of previous antituberculosis treatment in the last one year and bilateral fibrocavitory disease, only PCR for MTB was positive and AFB smear was negative. This patient showed symptomatic improvement with antibiotic treatment only and later his culture MTB was also negative. Another twenty seven cases with negative results on both AFB smear and PCR MTB were put on antituberculosis treatment empirically due to high suspicion of active pulmonary tuberculosis and they responded to treatment. Out of these twenty seven cases, twenty one cases were culture MTB positive after 5-6 weeks of culture on LJ media (Table 4). Nineteen cases which were negative for all the tests of pulmonary tuberculosis were treated with antibiotics and they also responded to treatment. Hence, a total of 103 patients received antituberculosis treatment while the remaining 20 patients received antibiotic treatment (Table 4). Thus the sensitivity of AFB smear in BAL was 61.2% while

that of culture MTB in BAL was 87.4%. The sensitivity and specificity of real time PCR MTB in BAL was 73.8% and 96.3% (Table 5).

Table 5: Comparison of sensitivity of various diagnostic methods for the diagnosis of 103 cases of pulmonary tuberculosis.

Diagnostic method	True positive	False negative	False positive	Sensitivity (%)
AFB smear	63	40	0	61.2
Culture MTB	90	13	0	87.4
PCR MTB	76	27	1	73.8

DISCUSSION

The 2020 milestones of the end TB strategy have set a target of reducing mortality rate due to tuberculosis by 35% and decreasing the incidence of tuberculosis by 20%

in comparison to the figures in 2015.[3] Early diagnosis of pulmonary tuberculosis will play a pivotal role in the achievement of this target utilizing diagnostic tests with high sensitivity and specificity. CBNAAT (Cartridge Based Nucleic Acid Amplification Test) which was earlier mainly approved to diagnose multidrug resistant (MDR) tuberculosis has now been approved by RNTCP (Revised National Tuberculosis Control Programme) in India for diagnosis of tuberculosis in pediatric patients along with HIV positive patients. It has also been approved for diagnosis of smear negative pulmonary tuberculosis and for diagnosis of extra pulmonary tuberculosis.[2] A significant increase in number of CBNAAT laboratories across India has led to an increased diagnosis of MDR TB in additional five thousand cases in 2016 in comparison to 2015.[2] Xpert MTB/RIF has now been recommended to be used as the initial diagnostic test instead of smear and culture MTB in presumptive cases of tuberculosis including MDR tuberculosis and in HIV positive patients.[6] Authors have also utilized the real time PCR for MTB in the early diagnosis of sputum smear negative cases of pulmonary tuberculosis.The real time PCR helped us in clinching the diagnosis of pulmonary tuberculosis in about 12% of extra cases in comparison to the smear microscopy and that too within one day of obtaining the bronchoalveolar lavage.This early diagnosis significantly helped us in treating our patients more effectively.The real time PCR also helped us in excluding tuberculosis in nineteen cases which were treated with antibiotics and they all responded to treatment clinico-radiologically. Xpert MTB/RIF has also been recommended to be used in follow up of smear negative tuberculosis patients except in MDR tuberculosis and in HIV positive patients.[6] The sensitivity of real time PCR has been found to be much higher than AFB smear in this study thereby favouring the use of real time PCR in the early diagnosis of pulmonary tuberculosis especially in paucibacillary cases which are negative on sputum smear examination. High sensitivity and specificity of real time PCR in diagnosing pulmonary tuberculosis has been reported by several studies although the sensitivity of PCR is lower in smear negative cases in comparison to the smear positive cases.[7-9] Seven of our cases (6.8%) which were AFB smear and culture MTB negative but were positive on PCR for MTB were treated with antituberculosis drugs with good clinico-radiological response thereby suggesting that PCR is also useful in some culture MTB negative patients to diagnose pulmonary tuberculosis (Table 4). PCR for MTB was falsely positive in only one patient with history of antituberculosis treatment in the last one year and this may have occurred due to cross contamination or due to the presence of dead bacilli in the patient. PCR for MTB has also been used in diagnosis of extrapulmonary tuberculosis including abdominal tuberculosis and tuberculous meningitis.[10-12] PCR for MTB has been very useful in differentiating *Mycobacterium tuberculosis* (MTB) infection from Nontuberculous mycobacteria due to use of DNA sequence IS6110 specific to MTB. PCR based NAAT has also been found to be a better diagnostic tool than microscopy and culture in pediatric pulmonary tuberculosis.[13-15] Recently, PCR for MTB has been utilized in endobronchial ultrasound guided transbronchial needle aspiration specimens to diagnose tuberculous mediastinal lymphadenopathy and to differentiate sarcoidosis from tuberculosis in mediastinal lymphadenopathy.[16,17]

The role of fibreoptic bronchoscopy in sputum smear negative patients is well established now.[18-20] Hence, patients with sputum smear negative for AFB can be subjected to fibreoptic bronchoscopy to obtain BAL from specific involved segments of the lung which can be processed for AFB smear, culture MTB and PCR for MTB where facilities are available to prevent any delay in diagnosis.

Limitation of the study was use of liquid culture media rather than Lowenstein Jensen solid media could have increased the sensitivity of culture media especially in those seven cases which were diagnosed as pulmonary tuberculosis only on the basis of real time PCR for MTB and in those six cases which were negative for AFB smear, culture MTB and PCR for MTB but responded to antituberculosis treatment.

CONCLUSION

Bronchoalveolar lavage can be sent for examination in sputum smear negative cases of pulmonary tuberculosis. AFB smear is the cheapest and the most rapid test to confirm the diagnosis of pulmonary tuberculosis but its sensitivity is low. Culture MTB remains the gold standard for diagnosis of pulmonary tuberculosis because of its high sensitivity and its ability to detect viable bacilli but its results are available only after a few weeks. However, PCR for MTB is a rapid test to confirm the diagnosis of pulmonary tuberculosis whose results are available within a few hours but it does not differentiate between dead and viable bacilli. PCR for MTB is positive in paucibacillary cases which are often negative on AFB smear and culture MTB thereby assisting in the diagnosis of these cases.

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