Original Research Article

Comparison of induced sputum and bronchial washings for CBNAAT in diagnosing sputum smear negative pulmonary tuberculosis

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ABSTRACT

Introduction: Tuberculosis is one of the most infectious public health problems for decades and has also become difficult to diagnose in recent times due to association with immunocompromised status. With the advent of the newer diagnostic technique like CBNAAT, can providing positive results even in less number of tuberculosis bacilli in specimen samples. The present study was aimed to compare induced sputum and bronchial washings for CBNAAT in diagnosing sputum smear negative tuberculosis.

Materials and Methods: A prospective, observational study was conducted in the Department of Pulmonary Medicine, Maharaja Institute of Medical Sciences (MIMS), Vizianagaram, Andhra Pradesh, India. A total of 43 patients with sputum negative tuberculosis were enrolled and induced sputum and BAL fluid for CBNAAT sent for analysis under RNTCP in district hospital.

Results: Male patients (67.45%) were more compared to the females (32.55%). Majority belongs to 51-60 years with mean age group of 49.18 years. Most common X-ray presentation was infiltration followed by consolidation. Induced sputum CBNAAT was positive in only 07 patients compared to bronchial washings CBNAAT in 27 patients.

Conclusions: As CBNAAT can diagnose rapidly and prevent transmission and mortality in smear-negative in pulmonary tuberculosis patients, all smear-negative and immunocompromised patients should be subjected to CBNAAT. CBNAAT detects pulmonary TB in PLHIV with greater efficacy than sputum microscopy, also helping in early diagnosis in less than 2 hours, so that early therapy can be started, thus decreasing the incidence of MDR-TB and associated its mortality.

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1. Introduction

In this decade of increasing immunocompromised diseases, Tuberculosis incidence is increasing. But it has become difficult for the physicians due to sputum negativity. With the advent of the newer diagnostic tools like CBNAAT, which are capable of providing positive results even in paucibacillary tuberculosis and helps in early diagnosis.

As per the Global TB report 2017 in India, the estimated incidence of TB was approximately 28,00,000, accounting for about a quarter of the world’s TB cases. In 2017 India re-estimated its national figures of the burden of Tuberculosis incorporating information from a broader range of sources.¹

In India, RNTCP was launched in 1993 and by 2006 whole country was covered. By 2007, RNTCP achieved a case detection rate of more than 70% and cure rate of >85% and aimed to achieve case detection and cure rates of least 90% by 2015.² The program aims to achieve ‘Universal Access’ for quality diagnosis and treatment for all TB patients in the country.

As per WHO recommendation, in suspected cases of pulmonary tuberculosis AFB smear examination is the initial diagnostic step for diagnosis.³ Sputum is the diagnostic tool for diagnosing TB in the directly observed treatment, short-course (DOTS) strategy in tuberculosis control programs in the world.⁴ Under RNTCP, two samples
of spontaneously obtained sputum (One early morning and one spot specimen) are examined through the fluorescent technique for diagnosing PTB. Sputum microscopy has less sensitivity and is of low value in patients who can not produce sputum or having bacilli less than 10000 bacilli/ml of sputum. More recently, the WHO endorsed the Genexpert (expert® MTB/Rif assay) for the diagnosis of TB. To improve the outcome, bronchial washings specimens were obtained using Fibreoptic bronchoscopy. In the present study we aimed to compare the results of induced sputum and bronchial washings for CBNAAT in patients with sputum negative pulmonary tuberculosis.

2. Aim of the Study
The study is aimed to compare the results of induced sputum smears and bronchial washings for CBNAAT in patients suspected to have sputum smear-negative epulmonary tuberculosis.

3. Objectives
To compare the results of sputum induction smears and bronchial washings CBNAAT in the diagnosis of sputum negative pulmonary tuberculosis.

4. Materials and Methods
This is a prospective study done in the Department of Pulmonary Medicine of a tertiary care institute between March 2017 through October 2018 done after Approval for the study has been taken by the ethics committee. Consecutive patients with newly suspected PTB were included in the study. All sputum negative cases were referred for induced sputum and BAL fluid for CBNAAT under RNTCP in district hospital.

4.1. Inclusion criteria
All cases were above 18 yr age of either sex with cough for more than two wee ks with two sputum smear negative for acid-fast bacilli were included.

4.2. Exclusion criteria
Refusal to give written inform consent was an important exclusion criterion. Patients with associated psychiatric problems, previous history of tuberculosis, HIV infection were excluded. Known asthmatics, patients with pneumothorax, active hemoptysis or history of cardiac disease particularly arrhythmias, hypotension SpO2<90%, past history of PTB were also not taken into the study. Those who failed to produce good quality sputum even after sputum induction were excluded out of the study. Patients suspicious of pulmonary tuberculosis were subjected to routine blood and urine examination. ELISA for HIV was done for all cases through ICTC of our hospital. Routine blood chemistry was also done and included testing for blood sugar, renal profile, and liver function tests. Sputum for AFB was ordered for all study patients as per RNTCP guidelines. All patients were instructed to submit the spot and spontaneously expectorated early morning sputum. Once both spúta are negative, then the patient is subjected to induced sputum and bronchoscopy for BAL fluid for CBNAAT. The results of sputum smears of above techniques were analyzed in total 43.

As the study was intended to know whether the induced sputum smear is better than BAL fluid for CBNAAT submitted under RNTCP, no sputum smear-negative specimens of the either spontaneously obtained or induced, or BAL fluid was subjected to culture examination as per the protocol of our study.

5. Results
5.1. Gender distribution
Out of 43 patients, 29 were male, and 14 were female. In 27 out of 43 BAL positive patients, male were 21, and female six patients were positive. In 07 out of 43 sputum induced patients, male were 6 and one female patient.

5.2. Symptoms
Out of 43 patients, cough is present in all 43 patients (100%) and fever in 40(93%) sputum in 40 patients (93%) loss of weight and appetite present in 27 patients (62%).

The chest radiologic manifestations of 43 patient of this study were presented as pulmonary infiltrations in 22 patients, consolidation in 12 patients, both consolidation and infiltrates in 02 patients, Cavity with infiltrates in 04 patients, collapse with consolidation seen in 03 patients as shown in Table 1.

Induced sputum CBNAAT positivity was seen in 07 cases amongst which cavity with infiltrations was seen in 03 cases, infiltrates in 02 cases, consolidation with infiltrations in 01 case and one with consolidation. All these cases also had BAL for CBNAAT positivity.

Out of 22 cases of pulmonary infiltrations, BAL for CBNAAT was positive in 13 cases, male 10 cases and female 3 cases. Out of 12 cases of consolidation, 08 were BAL positive, male were 07 and female 01, consolidation with infiltration in one male case, cavity with infiltrations in 03 male cases, 03 collapses with consolidation in 02 female and one male patient.

Out of 43 patients, in only 07(16.2%) patients induced sputum was CBNAAT positive even though adequate sputum was produced in 43 patients. Out of 43 patients who successfully completed the FOB procedure, 27(62.8%) patients were CBNAAT positive, and 16(37.2%) of patients were negative. The average volume of induced sputum obtained was 03 ml in all 43 patients and 20 ml of BAL
was collected in 43 cases. Post-procedure two hours of observation was carried out to know the complications.

Out of 43 patients who underwent both induced sputum and FOB, pulmonary tuberculosis is diagnosed in 27 cases, pneumonia in 16 cases. Of the total 43 cases, male were 29 (67.45%), and female were 14 (32.55%)

### Table 1: Radiological profile of sputum negative pulmonary TB patients

<table>
<thead>
<tr>
<th>Chest x-ray findings</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infiltrations</td>
<td>22</td>
<td>51.16</td>
</tr>
<tr>
<td>consolidation</td>
<td>12</td>
<td>27.90</td>
</tr>
<tr>
<td>Consolidation with infiltrations</td>
<td>02</td>
<td>04.66</td>
</tr>
<tr>
<td>Cavity with infiltrations</td>
<td>04</td>
<td>09.31</td>
</tr>
<tr>
<td>Collapse with consolidation</td>
<td>03</td>
<td>06.97</td>
</tr>
</tbody>
</table>

Most common radiological presentation on chest x-ray are infiltrations (51.16%) followed by consolidation (27.9%), cavity with infiltrations (9.31%), collapse with consolidation (6.97%), consolidation with infiltrations (4.66%).

Of the total 43 patients induced sputum CBNAAT positive in 07 (16.27%) and BAL fluid for CB NAAT positive in 27 (62.79%) as shown in Table 2.

### Table 2: Results (induced sputum and BAL fluid for AFB)

<table>
<thead>
<tr>
<th>Results</th>
<th>Positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induced sputum for CBNAAT</td>
<td>07</td>
<td>16.27</td>
</tr>
<tr>
<td>BAL fluid for CBNAAT</td>
<td>27</td>
<td>62.79</td>
</tr>
</tbody>
</table>

### 6. Results

Most common symptomatic presentation was cough followed by fever and sputum as shown in Table 3.

### Table 3: Clinical profile of sputum negative pulmonary TB patients

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Number of patients suffering</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>cough</td>
<td>43</td>
<td>100</td>
</tr>
<tr>
<td>fever</td>
<td>40</td>
<td>93</td>
</tr>
<tr>
<td>sputum</td>
<td>40</td>
<td>93</td>
</tr>
<tr>
<td>Loss of weight</td>
<td>27</td>
<td>62</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>27</td>
<td>62</td>
</tr>
</tbody>
</table>

Of the total 43 patients, Majority belongs to the age group 51–60 Yrs consisting of 12 patients. The mean age group was 49.18 Yrs.

Of the 43 patients, results of CBNAAT induced sputum and BAL fluid were compared in with the radiological findings as shown in Table 4.

### 7. Discussion

Sputum microscopy for AFB is having high specificity investigation to diagnose pulmonary TB with low cost. In 22-61% of the patients with sputum AFB smear-negative, culture was positive, especially in immune-compromised hosts. Conventional methods of mycobacterial cultures take 6-8 weeks for results, resulting in a delay in starting the treatment. DNA fingerprinting studies showed that 17% of the disease transmission could be from the smear-negative culture-positive patients. In these cases, induced sputum and bronchial washings obtained using Fibreoptic bronchoscopy may provide confirmation of a diagnosis of sputum negative pulmonary TB by obtaining specimens from specific sites in the lungs with infiltrates which can be sent for CB-NAAT.

The present study aims to investigate the diagnostic yield of induced sputum CBNAAT and bronchial washings specimen for CB-NAAT test in patients suspected to have sputum smear-negative pulmonary tuberculosis. The present study BAL for CBNAAT positive in 27 (62.79%), which was higher than to those of L.Saglam et al., bronchial washings smear was positive in 53% of cases. It may be due to CBNAAT, which can detect even 100 AFB bacilli/ml. In the present study induced sputum smear CBNAAT yield was 16.2% only which is similar to 19.34% in Andersen et al., Parry et al., Li et al. studies but very low compared to previous studies by L. Saglam et al. with 47% positivity and Hartung et al study with 42% positivity. This study shows that induced sputum smear positivity (n=07) is lower compared to bronchial washings (n=27) for detecting active PTB. If the clinical probability of PTB is high, then early FOB may be carried out in such patients.

In this study, Mean age is 49.18 years with a prominently male patient. Out of 43 patient’s males were 29 (67.45%) and female were 14 (32.55%) with a male: female ratio 2.07. Most cardinal symptoms were cough (100%) and fever (93%). Most radiological findings were infiltrates in 51.16% and consolidation in 27.90%. Out of 43 sputum smear-negative patients, Induced sputum smear was positive for CBNAAT in 07 (16.2%) patients, and bronchial washing CBNAAT was positive for AFB in 27 (62.8%) patients.

Submission of spontaneously produced sputum is the only method of passive case finding of suspected PTB under RNTCP. In suspected PTB, sputum smear examination is the cheap and very useful diagnostic tool. The WHO recommends detection of AFB in the sputum as the initial approach to the diagnosis of tuberculosis.

Sputum induction was demonstrated to have given good diagnostic yield than spontaneous sputum or gastric aspirate more than 50 years ago, but it fell into disuse after FOB was introduced. Sometimes
Table 4: Induced sputum and BAL for CB NAAT results in relation to radiological findings

<table>
<thead>
<tr>
<th>Chest x-ray</th>
<th>Number of patients with a percentage</th>
<th>PTB</th>
<th>Induced sputum CBNAAT positive</th>
<th>BAL for CBNAAT positive</th>
<th>Gender significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
</tr>
<tr>
<td>Infiltrations</td>
<td>22( 51.16% )</td>
<td>13</td>
<td>02</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Consolidation</td>
<td>12( 27.90% )</td>
<td>08</td>
<td>01</td>
<td>08</td>
<td>07</td>
</tr>
<tr>
<td>Consolidation with</td>
<td>02( 4.66% )</td>
<td>01</td>
<td>01</td>
<td>01</td>
<td>01</td>
</tr>
<tr>
<td>infiltrations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cavity with infiltrations</td>
<td>04( 9.31% )</td>
<td>03</td>
<td>03</td>
<td>03</td>
<td>03</td>
</tr>
<tr>
<td>Collapse with consolidation</td>
<td>03( 6.97% )</td>
<td>02</td>
<td>00</td>
<td>02</td>
<td>00</td>
</tr>
</tbody>
</table>

sputum smears may be negative even when adequate sputum is submitted particularly in countries experiencing dual TB/HIV epidemic.25

The positive diagnostic yield with properly collected first sputum sample is high. In every DOTS center, sputum smears are examined by a well trained and experienced laboratory technician. In our DOTS center, sputum smears were made with fluorescent microscopy technique to examine the sputum samples submitted under RNTCP.

In our study, the reasons for lesser positivity reported may be due to more number of patients presented with infiltrations and very less cavitatory lesions who have less bacilli load. Application of the molecular methods such as CBNAAT and PCR may be helpful as an adjunctive method for the diagnosis of TB in this individual patients. In developing countries like India, the facilities of Bronchoscopy may not be available in all circumstances, in such places induced sputum using hypertonic saline plays an important role adding to the diagnostic yield in patients with SSN-PTB. But the yield is low compare to BAL for CBNAAT.

8. Conclusion

Out of 43 sputum smear-negative pulmonary tuberculosis patients, induced sputum CBNAAT positive in 07 patients (16.2%) and bronchial washings in 27 patients (62.8%). Induced sputum detected fewer cases as compared to bronchial washings, but this difference is statistically significant as p > 0.001. When kappa coefficient was used to know the degree of agreement between the two tests, it was found that there is fairly good agreement (k =0.5). The reason may be due to obtaining sufficient bacilli in BAL than sputum induction.

Patient with only consolidation or infiltrations and collapse with consolidation should be referred to the tertiary care center for bronchoscopy guided procedure for BAL CBNAAT to diagnose and exclude another alternate diagnosis like pneumonia and malignancy.

CBNAAT was also useful in early diagnosis of carcinoma which has consolidation with air bronchogram appearance in CT scan, thus helping in an early stage of carcinoma detection in non-resolving pneumonia.

9. Source of funding

None.

10. Conflict of interest

None.

11. Acknowledgement

None.

References

1. India TB report 2018.

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