

## ORIGINAL RESEARCH

### **Study to Evaluate the Diagnostic Yield of Induced Sputum and Bronchial Washing by Zeihl Neelson technique and CB-NAAT in Clinico-Radiologically Suspected Sputum Smear Negative or unable to expectorate Pulmonary Tuberculosis Patients.**

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#### **ABSTRACT**

**Background:** Large number of pulmonary tuberculosis Patients (PTB) irrespective of their anti-tubercular treatment profile, presenting with symptoms of active pulmonary tuberculosis but their sputum smear for AFB by Zeihl Neelson technique is negative or who are unable to expectorate sputum in spite of best coughing technique. Such type of patients may require alternative methods to early diagnose and appropriate line of treatment to check further transmission of tuberculosis in the community.

#### **INTRODUCTION**

Tuberculosis (TB) is one of the most important public health problem worldwide and is declared as “global emergency” by the World Health Organization.<sup>1</sup> As per WHO Global TB Report, 2017, out of the estimated global annual incidence of 10.4 million TB cases, 2.8 million (About 27%) were estimated to have occurred in India.<sup>2</sup> A recent systematic review has shown that an average patient with TB is diagnosed after a delay of nearly 2 months, and after visiting three healthcare providers.<sup>3</sup> Detecting patients with active pulmonary TB (PTB) disease is an important component of TB control programs as early appropriate treatment renders these patients non-infectious and interrupts the chain of transmission of TB. Under the program conditions, diagnosis of PTB is based on demonstration of acid fast bacilli (AFB) in sputum smear by Zeihl Neelson method by light microscopy remains a fundamental tool of diagnosis, but may be negative up to 50% of active PTB.<sup>4</sup> In India, 1.41 million pulmonary tuberculosis (PTB) cases were notified in 2014 among which 45.5 lakh (34.11%) were sputum smear negative pulmonary tuberculosis (SSN-PTB).<sup>5</sup> These are referred as ‘smear not done’ cases according to WHO recommendations. The importance lies in identifying and treating these cases, as studies showed that more than 50% of these cases will need chemotherapy by end of one year and more than 17% of disease transmission could be attributed to them<sup>6</sup>. Every effort should be made to confirm these smear negative PTB cases bacteriologically confirm. There is no diagnostic guideline in NTEP for such type of patients. Alternative methods are frequently needed for obtaining sputum specimen insuch patients. These methods include induced sputum (IS), bronchial washing (BW), Bronchoalveolar lavage (BAL) and gastric washings samples. Each method has its advantages & disadvantages and had different diagnostic yield. Due to paucity of literature and guidelines for smear negative PTB cases, we planned this study.

## **AIMS & OBJECTIVES**

1. To know the additional yield of ZN staining in induced sputum (IS) and bronchial washing (BW) samples.
2. To know the diagnostic value of CB-NAAT over ZN Staining in induced sputum and Bronchial washings.

## **MATERIALS AND METHODS**

A prospective study was conducted in 12 months of period in clinico-radiological suspected active pulmonary tuberculosis (PTB) patients who fulfilled study criteria. Study was conducted in Department of Respiratory Medicine, MGUMST Jaipur. Patients with respiratory symptoms and chest roentgenogram suspicious of active Pulmonary tuberculosis (PTB) as per RNTCP guidelines<sup>2</sup> irrespective of history of anti-tubercular treatment (ATT) and two sputum smear negative for AFB by Ziehl Neelsen technique and who are unable to expectorate sputum were included in the study.

### **METHOD OF SPUTUM INDUCTION**

Sputum induction was done by using 10 ml of 3% hypertonic saline through ultrasonic nebulizer according to Ireda et al protocol.<sup>4</sup> After thorough mouth gargle, nebulisation by hypertonic saline will be given for maximum duration of 30 minutes or till adequate sputum (10ml) will be produced. If adequate sputum will not be produced, procedure will be termed 'failure'.

### **METHOD OF BRONCHIAL WASHING**

Bronchoscopy will be conducted using fiberoptic bronchoscope in endoscopy room of our department as per standard protocol by BTS.<sup>5</sup> Bronchial washings will be obtained using normal saline from both radiologically localized segments and also from areas of abnormal visible mucosa.

### **INCLUSION CRITERIA**

1. Patients with respiratory symptoms suspicious of active PTB such as cough for 2 weeks or more, evening rise of temperature, dyspnoea, haemoptysis loss of weight and loss of appetite as per NTEP.<sup>2</sup>
2. Sputum either not produced or sputum smear negative for AFB by Ziehl-Neelsen technique as per NTEP.
3. Chest radiology consistent with active PTB lesion.
4. Age more than 18 years.
5. Patients who will give informed consent.

### **EXCLUSION CRITERIA**

1. Patients with active haemoptysis, uncontrolled asthma or chronic obstructive pulmonary disease, unstable angina will be excluded from the study.
2. Pregnant females
3. HIV positive
4. Uncooperative patients

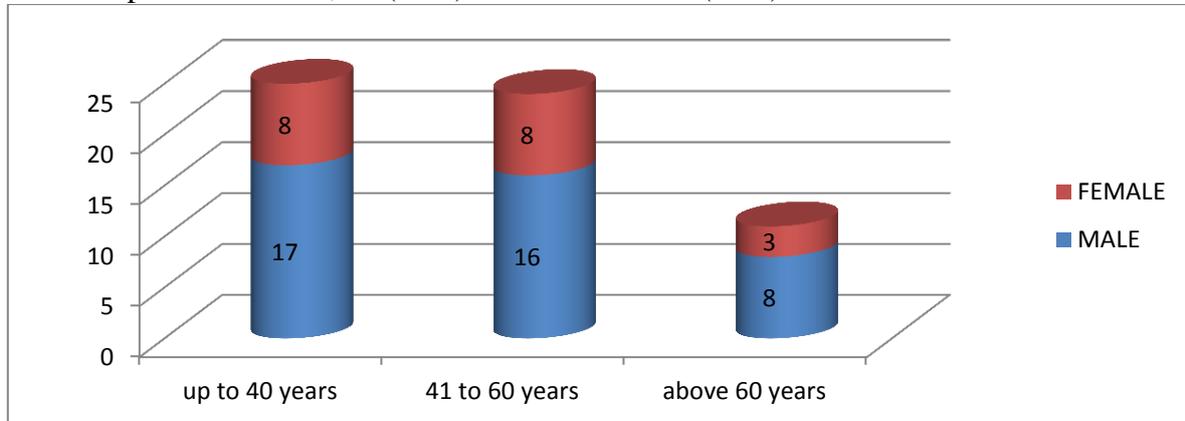
## **RESULT**

During study period 260 patients of presumptive PTB patients reported to department of respiratory medicine. Out of them, 80 patients (30.76%) were unable to expectorate sputum for microbiological confirmation of active tuberculosis so we offered them alternative

methods for sampling by induced sputum (IS) and bronchial washing (BW) as per protocol. Only 60 patients were fulfilled study criteria and enrolled for study.

**EPIDEMIOLOGY**

Out of 60 patients of PTB, 41 (68%) were males & 19 (32%) were females with 2.2:1 ratio.



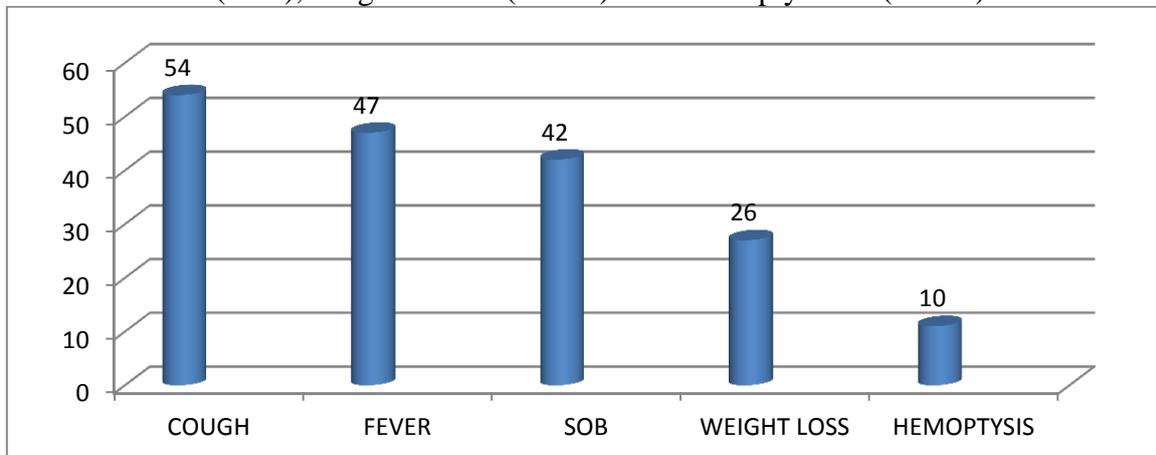
**Table 1: Chart of age and sex of study population (N= 60). As per age group 25 patients were within 40 years, 24 patients in 41 to 60 years and 11 patients were older than 60 years.**

Occupation	Male	Female	Total	%
Farmer	20	0	20	33 %
Household	0	14	14	23.3 %
Govt Job	10	0	10	16.6 %
Student	4	5	9	15 %
Stone Mining	7	0	7	11.6 %

**Table 2: showing that 1/3<sup>rd</sup> of study population were involved in farming related works & all were male and majority of females are involved in household works, 11% were involved in stone mining works.**

**CLINICAL PRESENTATIONS**

Main symptomatology of study group were cough 54 (90%), followed by fever 47 (78.3%), breathlessness 42 (70%), weight loss 26 (43.3%) and haemoptysis 10 (16.6%).



**Table 3: As per symptomatology cough was the dominant symptoms presented in 90% followed by fever episodes in 78%, shortness of breath 70%, and weight loss in 45% and haemoptysis in 18% of cases.**

#### **RADIOLOGICAL PRESENTATION**

Radiological distribution of pulmonary shadow was bilateral with multilobar followed by unilateral upper zone.

<b>Radiology</b>		<b>Total No</b>	<b>Percentage</b>
<b>Unilateral</b>		29	48.3%
<b>Bilateral</b>		31	51.7%
<b>Radiological Zone Involvement</b>	Upper Zone	20	33.3%
	Lower Zone	5	8.3%
	Multiple Zone	35	58.3%
<b>Radiological pattern</b>	Cavitation	22	36.7%
	Infiltrate	21	35%
	Consolidation	14	23.3
	Military	3	5%

**Table 4: Multilobar and bilateral radiological distribution of pulmonary infection was dominant followed by unilateral upper zone. Common radiological pattern of pulmonary infection was soft infiltrate/ nodularity in 21, cavitary lesion 22 and consolidation in 14 cases.**

#### **INDUCED SPUTUM (IS)**

Only 36 (60%) patients successfully induced sputum in sufficient amount and 21 detected tubercle bacilli by ZN stain & CBNAAT. So induced sputum technique confirmed PTB in 35% (21/60) of patients who are unable to expectorant sputum. ZN staining method detect Tubercle Bacilli in 16 and CBNAAT detected MTB in 19 (14 cases detect by both CBNAAT & ZN stain, isolated CBNAAT was positive in 5, isolated ZN stain in 2 cases).

<b>Sputum Induction N=60</b>	<b>Method</b>	<b>Number</b>	<b>Total</b>	<b>Percentage</b>
Successful SI (N=36)	Both CBNAAT/ ZN	14	21	60%
	Only ZN STAIN	2		
	Only CBNAAT	5		
	Unable to Detect	15	15	
Failed SI (N=24)		24	24	40%

**Table 5: Successful induction of sputum was in 60% (36) and diagnosis was confirmed in 35% (21) of study population by ZN stain and CBNAAT.**

#### **BRONCHIAL WASHING**

39 Patients those failed sputum induction (24) and didn't detect Mycobacteria in sputum induction (15), were further proceeded with Bronchial washing. Out of 39 patients, 25 were detected Tubercle Bacilli in BW samples by ZN Stain (5) and CBNAAT (24). One patients of BW was detected Tubercle Bacilli by ZN Stain only but not by CBNAAT, finally that patient detect NTM in culture.

<b>Bronchial washing (n= 39)</b>	<b>Method</b>	<b>Cases</b>	<b>Total</b>	<b>Percentage</b>
<b>Detect TB Bacilli</b>	Both ZN/CBNAAT	4	25	64.1%
	Only CBNAAT	20		
	Only ZN stain	1		
<b>Undiagnosed</b>	ZN/ CBNAAT	14	14	35.9%

**Table 6: Confirmation of Diagnosis of PTB in BW samples was significantly improved up to 64% (25/39) predominantly with CBNAAT technique.**

60 patients of suspected PTB, induced sputum and bronchial washing confirmed diagnosis in 46 cases by ZN stain and CBNAAT. Rest of 14 cases were remained undiagnosed after sputum induction and Bronchial washing sequentially.

Sample	Sensitive	Resistant	NTM	Total	Percentage
Induced Sputum N= 60	18	1	2	21	35%
Bronchial Washing N=39	23	2	0	25	64.1%
Remained Undiagnosed	0	0	0	14	23.3%

**Table 7: Diagnosis was confirmed in 76.7% (46/60) cases. In 35% (21/60) cases diagnosis confirmed among Induced sputum and in 64.1% (25/39) cases diagnosis confirmed among Bronchial Washing samples. MDR TB also confirmed in 3 and NTM TB in 2 cases respectively. 23.3% (14/60) of cases were remained undiagnosed by both samples.**

**YIELD of ZN Staining and CBNAAT**

Yield of ZN staining in induced sputum (SI) occurred 44.4% as compared to 12.8% in bronchial washing samples. Yield of CBNAAT in induced sputum (SI) occurred 52.7% as compared to 53.8% in bronchial washing samples.

Method	Induced sputum n=36	Bronchial washing n=39
ZN staining	16 (44.4%)	5 (12.8%)
CBNAAT	19 (52.7%)	21 (53.8%)
Diagnosis confirmed	21 (58.3%)	25 (64.1%)

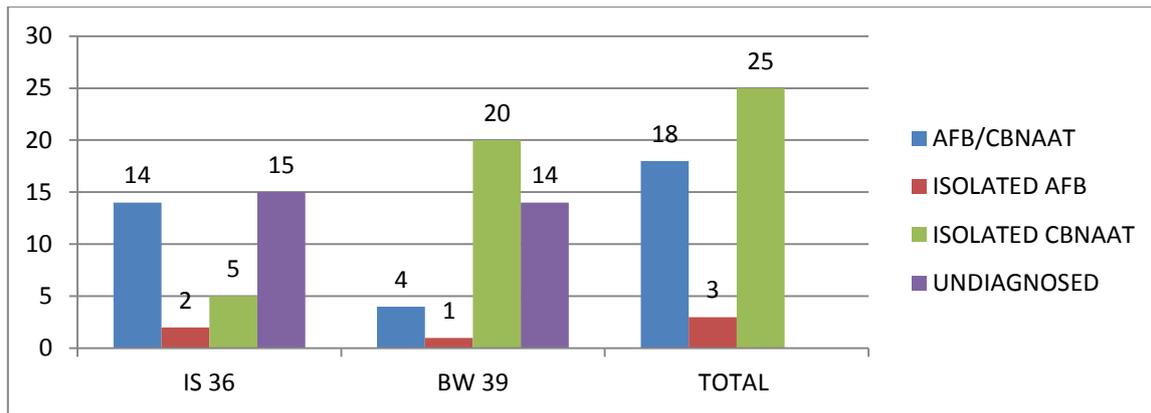
**Table 8: yield of ZN staining & CBNAAT in induced sputum & bronchial washing samples.**

**INDUCED SPUTUM (IS) EFFICACY**

Efficacy of sputum induction was 60% (36/60) of our study group of clinico-radiological suspected active PTB who were unable to expectorate. Diagnosis of PTB confirmed in 35% (21/60) of cases in IS samples. Efficacy of Ziehl Neelsen method and CBNAAT in IS samples were 44.4% (16/36) & 52.7% (19/36) respectively to detect TB bacilli. In more than 1/3<sup>rd</sup> of cases, sputum induction (SI) confirmed diagnosis of PTB.

**EFFICACY OF BW**

Out of 39 BW samples, diagnosis of PTB was confirmed in 64.1% (25/39). While Ziehl Neelsen staining was able to detect mycobacterium tuberculosis in 12.8% (5/39) in BW sample. Efficacy of CBNAAT to detect TB bacilli in BW samples was 61.5% (24/39).



**Table 9: Among induced sputum, AFB detect TB bacilli in 16 and CBNAAT detect in 19. Overall 21 cases confirmed PTB by sputum induction. 25 cases confirmed PTB among 39 bronchial washing samples.**

## DISCUSSION

Pulmonary tuberculosis is still one of the most common infectious diseases in the world. It is seen that among new cases, the ratio of smear-positive pulmonary tuberculosis is 57% and rest of patients are SNPTB. In India, 1.41 million pulmonary tuberculosis (PTB) cases were notified in 2014 among which 45.5 lakh (34.11%) were sputum smear negative pulmonary tuberculosis (SSN-PTB).<sup>5</sup> Historical studies observed that household contacts of smear-positive patients were between 2 and 12 times more likely than household contacts of smear-negative patients to be infected with *Mycobacterium tuberculosis*.<sup>14</sup>

Smear-negative pulmonary tuberculosis SNPTB occurs less frequently than smear-positive pulmonary tuberculosis (SPPTB), but it is an infectious communicable form of tuberculosis. In a cohort study conducted in Holland, it was revealed that smear-negative culture-positive pulmonary tuberculosis was responsible for 13% of the transmission of tuberculosis.<sup>15</sup> In another study conducted by Tostmann A et al in San Francisco, it was detected that at least 17% of the transmission of tuberculosis was due to SNPT.<sup>16</sup>

So there are extra efforts and procedure need in smear negative PTB to confirm early diagnosis and put on adequate treatment earliest to cut the chain of transmission. In our study out of 60 cases, cough 54 (90%) were dominated symptoms followed by fever 47 (78.3%) and SOB 42 (70%) among smear negative clinic-radiological active PTB.

In our study, 35% (21/60) of cases confirmed diagnosis of tuberculosis in induced sputum samples. Ziehl Neelsen method and CBNAAT in IS samples were detected tubercle bacilli 44.4% (16/36) & 52.7% (19/36) respectively. Efficacy of CBNAAT was slight higher as compared to ZN staining method in IS sample. Similar result of study done by Luhadia A et al<sup>13</sup> found that sputum induction has produced positive yield for tuberculosis in 45% patients. These detection rates are comparable to our study. **Another study done by L. Saglamet al<sup>17</sup>** found 47% positivity and Hartung et al<sup>18</sup> study with 42% positivity also correlate with our study results. In Nageswar Rao Gopathiet al<sup>11</sup> study, induced sputum smear positivity of 63.3% is higher than our study that was possibly due to method of case selection and severity of disease.

In our study, bronchoscopy and bronchial washing samples were performed in 39 cases and diagnosis of tuberculosis confirmed in 64% (25/39). In 39 bronchial washing samples, Ziehl Neelsen staining was detected *Mycobacterium tuberculosis* in 5 (12.8%) cases. CBNAAT method detected TB bacilli in BW samples in 24 (61.5%). These results are comparable with study done by Nageswar Rao Gopathiet al<sup>11</sup>, in which bronchial washings smear results were positive in 78.5% of cases. In another studies by Charoenratanakul et al., Fujii et al., and Chawla et al., positive BW/BL results ranged from 25-72% [19-21].

Yield of ZN staining was 44.4% in induced sputum (SI) and 12.8% in bronchial washing samples. Our study shows that diagnostic yield of ZN staining is much higher in induced sputum as compared to bronchial washing but there was no difference in yield of CBNAAT in induced sputum (SI) & bronchial washing samples.

### RECOMMENDATION OF STUDY

1. Those presumptive PTB cases are unable to submit quality expectorant for smear examination, should be offered for sputum induction that is possible even remote health facilities. Sputum induction is easy, cost effective, outdoor procedure and need less expertise.
2. Those cases of presumptive PTB failed induction may be subjected for bronchial washing sampling at appropriate centre.

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