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Research Article

Study to Assess the Diagnostic Yield of Bronchoalveolar Lavage Fluid in Sputum-Negative Suspected Pulmonary Tuberculosis Patients

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ABSTRACT

Background: Pulmonary tuberculosis (PTB) remains a major global health challenge, particularly in patients with sputum-negative disease, where conventional diagnostic methods may fail to detect Mycobacterium tuberculosis. Bronchoalveolar lavage (BAL) fluid analysis and post-bronchoscopy sputum testing offer enhanced diagnostic potential in these patients.

Objective: To assess the diagnostic yield of bronchoalveolar lavage fluid and post-bronchoscopy sputum examination using Acid-Fast Bacilli (AFB) smear and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) in sputum-negative suspected pulmonary tuberculosis patients.

Methods: This observational, cross-sectional study included 75 adult patients clinically and radiologically suspected of PTB but negative for sputum AFB smear and CBNAAT. Flexible fibreoptic bronchoscopy was performed to obtain BAL fluid, and post-bronchoscopy sputum samples were collected. BAL fluid and post-FOB sputum were analysed for AFB smear positivity, CBNAAT detection of M. tuberculosis, and rifampicin resistance. Data were analysed using descriptive statistics and expressed as frequency and proportion.

Results: The study population comprised 46 males (61.33%) and 29 females (38.67%), with the highest number in the 45–60 years age group (34.67%). Clinically, cough (80%) and fever (38.67%) were the most common symptoms. Chest X-ray showed unilateral lesions in 69.33% of participants, predominantly in the right upper zone (32%). The diagnostic yield of microbiological tests was highest for BAL CBNAAT (21.33%), followed by post-FOB sputum CBNAAT (14.67%), post-FOB sputum AFB (13.33%), and BAL AFB (10.67%). Rifampicin resistance was detected in 12.5% of BAL CBNAAT-positive cases. Chest X-ray exhibited a positivity rate of 90.67%.

Conclusion: BAL fluid CBNAAT provides the highest diagnostic yield in sputum-negative PTB patients, significantly outperforming conventional smear microscopy. Post-bronchoscopy sputum testing further enhances case detection. Early detection using these methods, along with rifampicin resistance assessment, enables timely initiation of therapy and improved tuberculosis control in high-burden settings.

Keywords*: Pulmonary tuberculosis, sputum-negative, bronchoalveolar lavage, CBNAAT, post-bronchoscopy sputum, diagnostic yield, rifampicin resistance.*

INTRODUCTION

Pulmonary tuberculosis (PTB) remains one of the leading infectious causes of morbidity and mortality worldwide, with an estimated 10.6 million cases and 1.6 million deaths reported globally in 2022 [1]. Early and accurate diagnosis of PTB is

crucial to reduce transmission and initiate timely treatment. Sputum smear microscopy for Acid-Fast Bacilli (AFB) remains the primary diagnostic tool in many high-burden countries; however, its sensitivity is limited, particularly in patients with low bacillary loads [2,3].

Sputum-negative PTB presents a diagnostic challenge, as patients exhibit clinical and radiological features suggestive of tuberculosis but fail to demonstrate Mycobacterium tuberculosis on routine sputum AFB smear or Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) [4,5]. Delayed diagnosis in such cases can result in disease progression and ongoing transmission.

Bronchoalveolar lavage (BAL) via fiberoptic bronchoscopy (FOB) has emerged as a valuable diagnostic approach in sputum-negative PTB. BAL allows for the collection of lower respiratory tract secretions directly from the affected bronchopulmonary segments, improving the likelihood of detecting mycobacteria [6,7]. Conventional AFB smear of BAL fluid has variable sensitivity, often limited by low bacterial load; in contrast, molecular methods such as CBNAAT offer rapid and more sensitive detection of Mycobacterium tuberculosis, including rifampicin resistance [8,9].

Several studies have highlighted the utility of post-bronchoscopy sputum, which may contain mycobacteria dislodged during bronchoscopy, as an additional diagnostic specimen in sputum-negative PTB [10,11]. Combining BAL analysis with post-FOB sputum examination increases the overall diagnostic yield and provides an opportunity for early detection of drug resistance, crucial for guiding therapy [12].

Thus, this study was designed to assess the diagnostic yield of BAL fluid and post-bronchoscopy sputum in patients with clinical and radiological suspicion of PTB but negative sputum AFB and CBNAAT, aiming to identify the most effective strategy for early detection.

MATERIALS AND METHODS

Study Design and Setting

This observational, cross-sectional study was conducted in the Department of Respiratory Medicine at the Post Graduate Institute of Medical Education and Research (PGIMER) and Capital Hospital, Bhubaneswar, Odisha and the study period extended from **May 2023 to December 2024** (20 months).

Study Population

The study included patients attending the outpatient and inpatient departments of Respiratory Medicine who were clinically and radiologically suspected of pulmonary tuberculosis but were found to be negative for sputum Acid-Fast Bacilli (AFB) smear and Cartridge-Based Nucleic Acid Amplification Test (CBNAAT).

Sample Size

A total of **75 patients** were enrolled in the study using the formula:

$$n = \frac{Z^2 \times p(1-p)}{d^2}$$

Where:

- n = sample size
- Z = standard normal deviate corresponding to the desired confidence level
- p = anticipated diagnostic yield
- d = desired level of precision

A purposive non-probability sampling method, specifically consecutive sampling, was used to select the study participants.

Inclusion Criteria

1. Patients aged 15 years and above, irrespective of gender.
2. Patients with clinical and radiological features suggestive of pulmonary tuberculosis.
3. Chest X-ray and HRCT Thorax findings consistent with pulmonary tuberculosis.
4. Sputum smear and CBNAAT negative for Mycobacterium tuberculosis.

Exclusion Criteria

1. Sputum AFB smear-positive patients.
2. Sputum CBNAAT-positive patients.
3. Patients with extrapulmonary tuberculosis.
4. Severely dyspneic patients or those with unstable cardiovascular status (angina or arrhythmia).
5. HIV, Hepatitis B, or Hepatitis C seropositive patients.
6. Patients refusing consent or uncooperative during bronchoscopy.
7. Cases where bronchoscopy was contraindicated.

Study Parameters

1. Demographic details and detailed clinical history.
2. Clinical examination findings.
3. Chest X-ray and HRCT Thorax suggestive of pulmonary tuberculosis.
4. Sputum smear and CBNAAT for Mycobacterium tuberculosis.
5. Bronchoalveolar Lavage (BAL) fluid examination for:
    - AFB smear positivity.
    - CBNAAT positivity.
6. Post-bronchoscopy sputum AFB and CBNAAT results.

Bronchoscopy and BAL Fluid Collection Procedure

Flexible fibreoptic bronchoscopy was performed using a Fujinon bronchoscope in a well-ventilated bronchoscopy suite under strict aseptic precautions. The procedure was conducted through the transnasal route in the supine position under continuous pulse oximetry, ECG, and blood pressure monitoring.

Pre-Procedure Preparation

- Patients were instructed to remain **nil per oral** for at least 8 hours before the procedure.
- **Atropine (0.6 mg IM)** was administered 30–45 minutes before the procedure to reduce airway secretions.
- **Local anaesthesia** was achieved with **2% lignocaine gel** applied to the nasal mucosa and **10% lignocaine spray** to the oropharynx and vocal cords.
- **IV access** was established, and **oxygen** and **emergency equipment** were kept ready.
- **Informed written consent** was obtained from each participant.

Bronchoscopy Procedure

- The bronchoscope was lubricated and gently advanced through the nasal passage under direct vision.
- The **epiglottis and vocal cords** were visualised, and additional lignocaine was instilled through the suction channel as needed.
- The bronchoscope was wedged into the **segmental or subsegmental bronchus** corresponding to the radiologically abnormal area.
- **Bronchoalveolar lavage** was performed by instilling **60 mL of sterile normal saline** in two aliquots of 30 mL each, followed by gentle suction to retrieve approximately **15 mL of BAL fluid**.
- The collected BAL fluid was transferred into a **sterile Falcon tube** and labelled appropriately.

Post-Procedure Care

- Patients were monitored for 24 hours for any complications such as cough, hemoptysis, fever, or arrhythmias.
- Oral intake was restricted for at least **two hours post-procedure**.
- **Post-bronchoscopy sputum samples** were collected for AFB and CBNAAT testing.

Laboratory Analysis

1. AFB Smear Microscopy

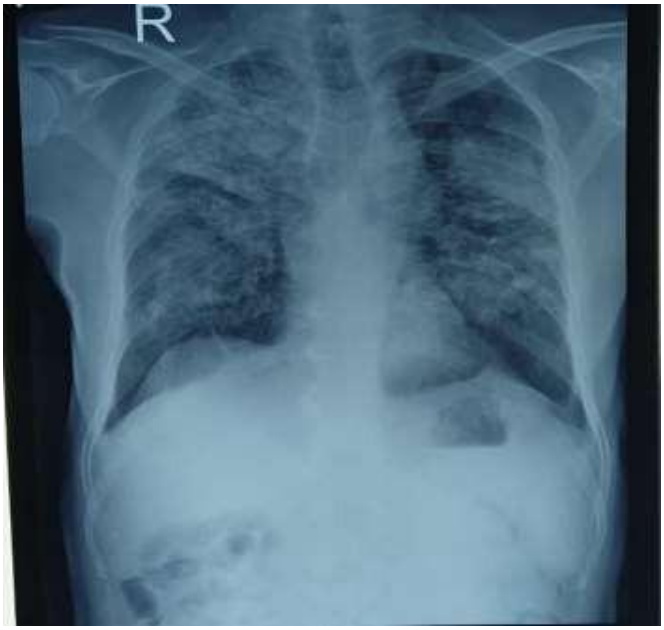
BAL fluid was centrifuged at **3000 rpm for 15–20 minutes**. The sediment was used to prepare smears, which were stained by **Ziehl-Neelsen (ZN) staining** and examined under an oil immersion microscope for the presence of acid-fast bacilli.

2. CBNAAT (GeneXpert)

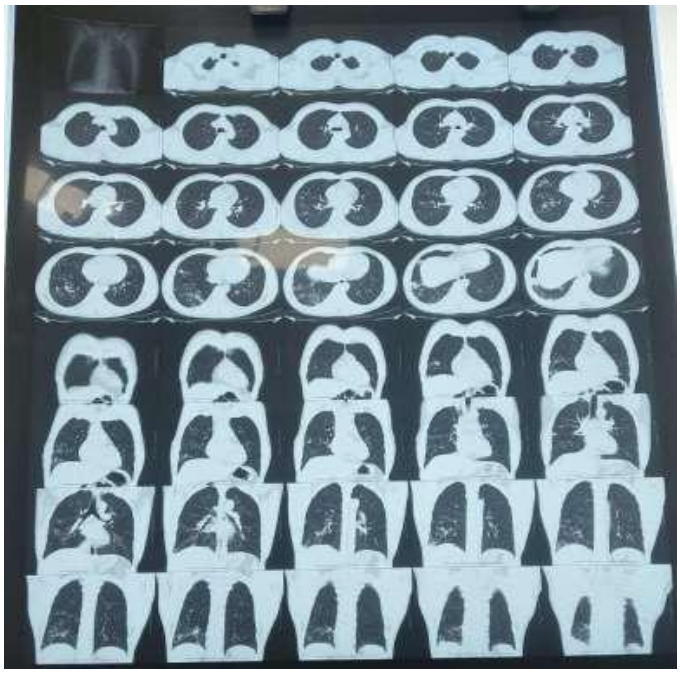
A minimum of **5 mL BAL fluid** was used for CBNAAT analysis. The sample was processed according to the manufacturer's instructions. If BAL fluid volume was <5 mL, **1 mL of the sample was mixed with 2 mL of CBNAAT reagent**. The GeneXpert system automatically performed **nucleic acid amplification** for the detection of **Mycobacterium tuberculosis** and **rifampicin resistance**. Results were available within **2 hours**.

3. Post-Fibreoptic Bronchoscopy Sputum

Collected sputum samples were also examined for **AFB smear** and **CBNAAT positivity**.



Figure; 1 A picture of Chest Xray of one of our participants who was Sputum Negative but cameouttobe BAL (CBNAAT and **AFB) positive has been attached here with**



Figure; 2 HRCT Thorax of of one of our participants whowas Sputum Negative butcameouttobe BAL (CBNAAT and AFB) positive has been attached here with

Data Collection and Statistical Analysis

Data were entered into **Microsoft Excel** and analysed using **IBM SPSS version 26.0**.

- **Quantitative data** were expressed as **mean ± standard deviation (SD)**.
- **Qualitative data** were presented as **frequency and percentage**.
- The **association between categorical variables** was analysed using **Pearson's Chi-square test**.
- A **p-value <0.05** was considered **statistically significant**.

Ethical Considerations

Ethical approval was obtained from the **Institutional Ethics Committees** of PGIMER and Capital Hospital, Bhubaneswar.

- **Written informed consent** was obtained from all participants.
- **Confidentiality** of patient data was strictly maintained.
- **No vulnerable populations** were included, and **no external funding** was received for the study.

RESULTS AND OBSERVATIONS

Table 1: Demographic Profile of Study Participants (Age and Gender Distribution)

Variable	Category	Frequency (n)	Proportion (%)
Gender	Male	46	61.33
	Female	29	38.67
Total		**75**	**100.00**

Table 2: Age and Gender-wise Distribution of Study Participants

Age Group (Years)	Female (n = 29)	Proportion (%)	Male (n = 46)	Proportion (%)	Total (n = 75)	Overall Proportion (%)
18 – 29	10	34.48	8	17.39	18	24.00
30 – 44	8	27.59	11	23.91	19	25.33
45 – 60	9	31.03	17	36.96	26	34.67
Above 60	2	6.90	10	21.74	12	16.00
Total	**29**	**100.00**	**46**	**100.00**	**75**	**100.00**

Table 3: Distribution of Participants Based on History of Diabetes Mellitus (T2DM) and Chronic Kidney Disease (CKD)

Clinical Condition	Category	Frequency (n)	Proportion (%)
Diabetes Mellitus (T2DM)	Yes	19	25.33
	No	56	74.67
Chronic Kidney Disease (CKD)	Yes	3	4.00
	No	72	96.00
Total Participants		**75**	**100.00**

Table 4: Distribution of Participants According to Clinical Features and Physical Findings

Clinical Features / Physical Findings	Frequency (n)	Proportion (%)
Clinical Features		
Cough	60	80.00
Fever	29	38.67
Haemoptysis	28	37.33
Weight loss and loss of appetite	14	18.67
Breathlessness	10	13.33
Chest Pain	6	8.00
Physical Findings		
Coarse Crepitation	27	36.00
Fine Crepitation	14	18.67
Emaciated	11	14.67
Bronchial Breath Sound	9	12.00
Loss of Lung Volume	5	6.67
Absent Breath Sound	3	4.00
Coarse Crepitation + Bronchial Breath Sound	1	1.33
Decreased Breath Sound	1	1.33
Fine Crepitation + Bronchial Breath Sound	1	1.33
Within Normal Limits	3	4.00
Total Participants	**75**	**100.00**

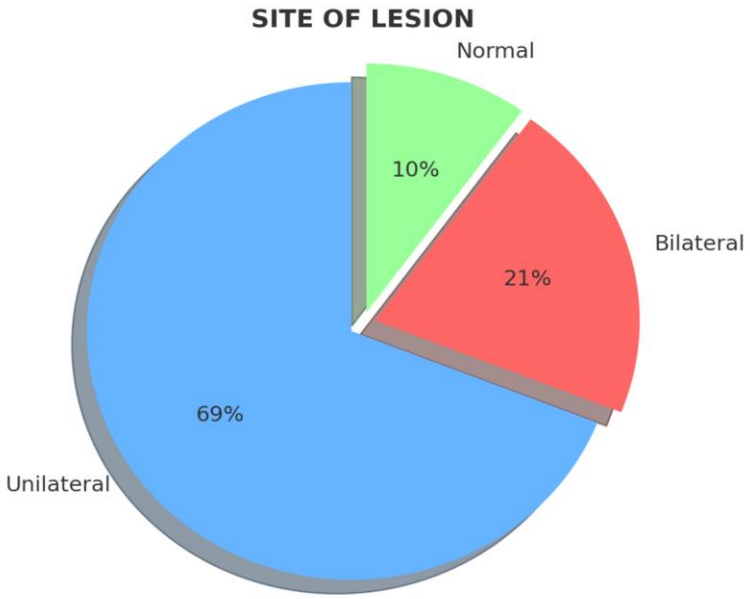
Table 5: Blood Investigations – Haemoglobin and Erythrocyte Sedimentation Rate (ESR)

Parameter	Mean	Standard Deviation (SD)	Minimum	Maximum	Median	Interquartile Range (IQR)
Haemoglobin (g/dL)	10.56	1.56	7.1	14.4	10.3	2.35
ESR (mm/hr)	55.75	20.73	10	126	53	27.5

Table 6: Chest X-Ray Findings – Site and Extent of Lesion

Chest X-Ray Findings	Category	Frequency (n)	Proportion (%)
Site of Lesion			
Unilateral	52	69.33	
Bilateral	16	21.33	
Within Normal Limits	7	9.33	

Extent of Lesion			
Right Upper Zone	24	32.00	
Diffuse	11	14.67	
Right Lower Zone	8	10.67	
Right Middle Zone	7	9.33	
Right Upper Zone + Left Upper Zone	5	6.67	
Left Lower Zone	4	5.33	
Left Upper Zone	4	5.33	
Left Upper Zone + Left Lower Zone	1	1.33	
Right Lower Zone + Right Middle Zone	2	2.67	
Right Middle Zone + Right Upper Zone	2	2.67	
Within Normal Limits	7	9.33	
Total Participants		**75**	**100.00**



Figure; 1 Site of Lesion

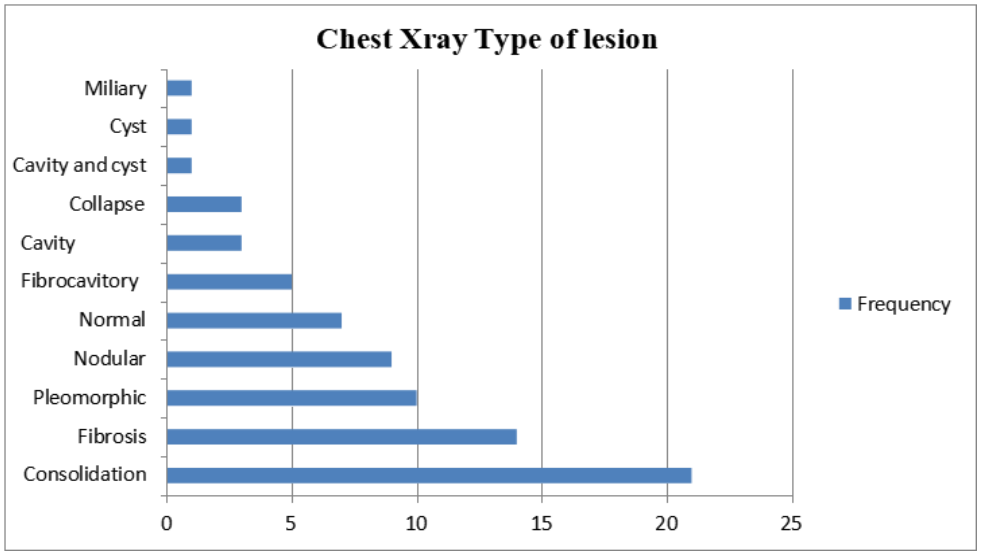


Figure 2: A bar graph showing types of lesions in chest x-ray of participants.

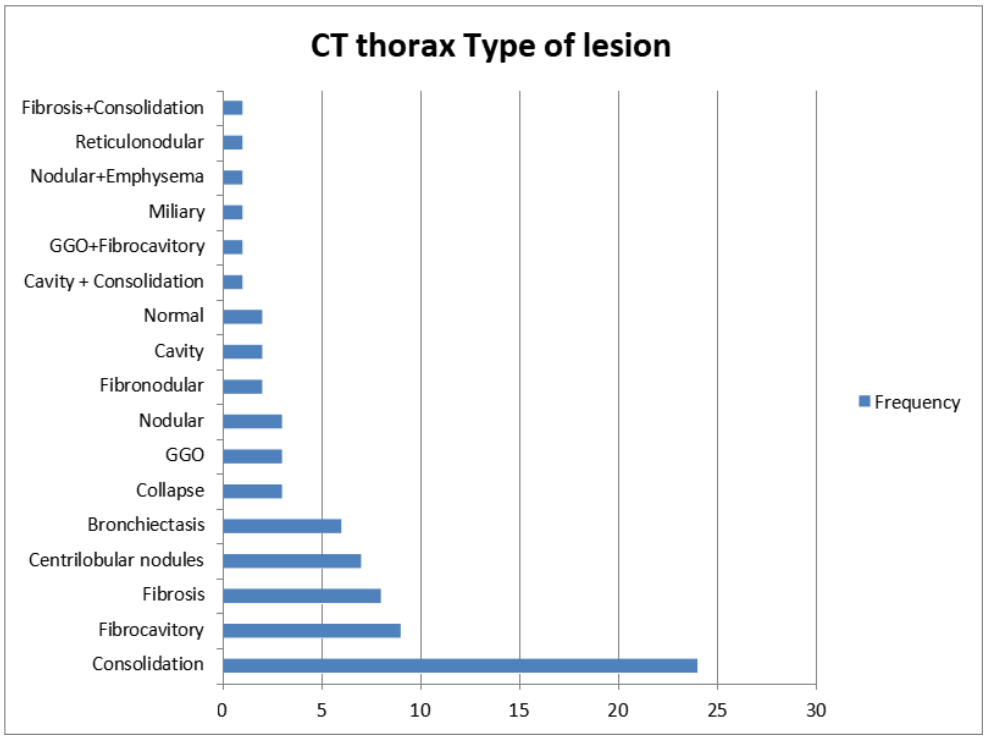
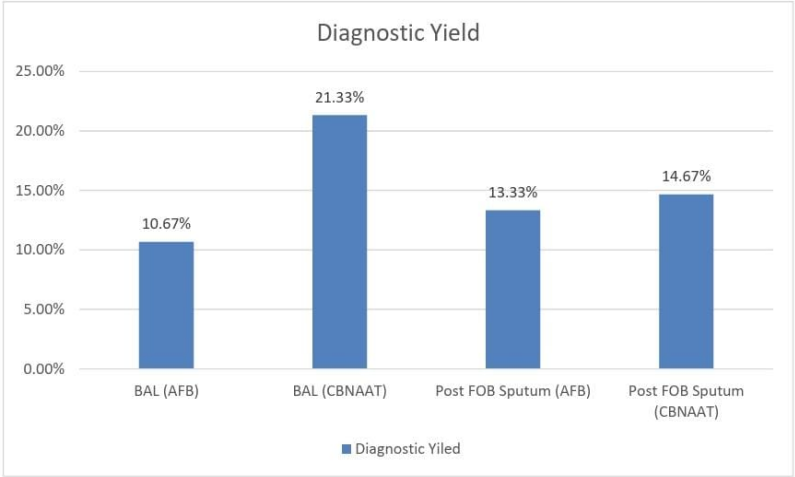


Figure 3: A bar graph showing types of lesions in the CT Thorax of the participants

Table 7: Diagnostic Yield and Rifampicin Resistance

Test / Parameter	Positive / Detected	Negative / Not Detected	Frequency (%) Positive / Detected	Frequency (%) Negative / Not Detected
Chest X-Ray	68	7	90.67	9.33
BAL (AFB)	8	67	10.67	89.33
BAL (CBNAAT, M.tb)	16	59	21.33	78.67
BAL CBNAAT – Rifampicin Resistance	2	14	12.50	87.50



A bar graph showing diagnostic yield of BAL AFB,BAL CBNAAT,Post FOB Sputum AFB and Post FOB Sputum CBNAAT.

Figure 4: Abar graph showing diagnostic yield of BALAFB, BALCBNAAT, Post FOB Sputum AFB and Post FOB Sputum CBNAAT.

Table 8: Overall Diagnostic Yield of Microbiological Tests

Test / Sample	Positive / Detected	Negative / Not Detected	Frequency (%) Positive / Detected	Frequency (%) Negative / Not Detected
BAL (AFB)	8	67	10.67	89.33
BAL (CBNAAT)	16	59	21.33	78.67
Post-FOB Sputum (AFB)	10	65	13.33	86.67
Post-FOB Sputum (CBNAAT)	11	64	14.67	85.33

DISCUSSION

Pulmonary tuberculosis (PTB) remains a significant public health concern globally, particularly in high-burden countries such as India, where early diagnosis and timely initiation of treatment are crucial to reduce transmission and morbidity [1,2]. Sputum-negative PTB presents a major diagnostic challenge, as standard diagnostic tests—sputum AFB smear and CBNAAT—may fail to detect Mycobacterium tuberculosis in patients with low bacillary loads or atypical presentations [3]. In such cases, advanced diagnostic modalities like bronchoalveolar lavage (BAL) fluid examination and post-bronchoscopy sputum analysis play a vital role.

Demographic Characteristics

In this study, 61.33% of participants were male, consistent with previous epidemiological data showing higher PTB prevalence among males due to greater exposure to risk factors, such as smoking, occupational dust, and urban crowding, and potential gender-based immunological differences [3,4]. The largest age group was 45–60 years (34.67%), suggesting a higher susceptibility in middle-aged adults, which could be related to cumulative exposure, comorbidities, and age-related decline in immune function [5]. Among the participants, 25.33% had Type 2 Diabetes Mellitus (T2DM), a known risk factor for TB, as hyperglycemia impairs innate and adaptive immunity, facilitating mycobacterial survival and proliferation [6]. Chronic kidney disease was present in 4% of participants, a condition also associated with immune dysfunction and increased TB risk.

Clinical and Radiological Findings

Cough was the predominant symptom (80%), followed by fever (38.67%) and hemoptysis (37.33%), consistent with classical PTB presentations, although the lower prevalence of systemic symptoms in some participants reflects the early or paucibacillary nature of the disease [7]. Physical examination revealed coarse crepitations in 36% and fine crepitations in 18.67% of participants, indicating variable involvement of the lung parenchyma.

Chest X-ray analysis demonstrated unilateral lesions in 69.33% of participants, predominantly affecting the right upper zone (32%). Bilateral involvement was less common (21.33%), while 9.33% had normal chest radiographs. These findings are consistent with previous studies indicating that the upper lobes, particularly the right upper lobe, are preferentially involved in pulmonary tuberculosis due to higher oxygen tension favorable for mycobacterial growth [8]. HRCT imaging provided further localization of lesions and helped in targeting bronchoscopy, particularly in cases with subtle or non-specific radiographic abnormalities.

Diagnostic Yield of BAL and Post-FOB Sputum

The diagnostic yield of microbiological tests in this study showed BAL CBNAAT had the highest yield (21.33%), followed by post-FOB sputum CBNAAT (14.67%) and post-FOB sputum AFB (13.33%). BAL AFB had the lowest yield (10.67%). This hierarchy aligns with prior evidence that molecular tests like CBNAAT significantly outperform conventional smear microscopy in detecting Mycobacterium tuberculosis, especially in paucibacillary or smear-negative patients [9,10].

The lower yield of BAL AFB (10.67%) can be attributed to the requirement for a higher bacterial load for smear positivity, while CBNAAT can detect even minimal mycobacterial DNA. BAL CBNAAT also provides the additional advantage of detecting rifampicin resistance, which was found in 12.5% of positive BAL samples, emphasizing the importance of rapid molecular diagnostics for early initiation of appropriate therapy and prevention of multidrug-resistant TB [11].

Post-bronchoscopy sputum examination contributed significantly to case detection, with 11 participants (14.67%) testing positive on CBNAAT. This suggests that bronchoscopy dislodges mycobacteria from the bronchial mucosa, which are then captured in the sputum, enhancing diagnostic yield. Several studies have reported similar findings, highlighting that combining BAL and post-FOB sputum testing increases the sensitivity of detecting PTB in sputum-negative patients [10,12].

Comparison with Previous Studies

The diagnostic yield observed in this study is comparable to previous reports. Lamprecht et al. reported BAL CBNAAT yield of 20–25% in sputum-negative PTB, while Kim et al. found BAL CBNAAT detected M.tb in 22% of cases [6,7]. Post-bronchoscopy sputum CBNAAT yield of 14.67% in our study is also in line with prior meta-analyses, which report a

range of 10–18% [10,12]. BAL AFB yields in the literature vary from 8–12%, consistent with our finding of 10.67%, reflecting the limited sensitivity of smear microscopy in paucibacillary cases [9].

Clinical and Public Health Implications

The study highlights the critical role of BAL CBNAAT and post-bronchoscopy sputum CBNAAT in enhancing diagnostic accuracy for sputum-negative PTB. Early detection through these methods can reduce delays in treatment initiation, limit disease progression, and prevent ongoing transmission, particularly in high TB burden settings. The ability to rapidly detect rifampicin resistance allows for prompt initiation of appropriate therapy, which is essential in controlling multidrug-resistant TB.

Limitations

This study has several limitations. First, it was conducted at a single tertiary care center with a relatively small sample size (n=75), which may limit the generalizability of the findings. Second, culture-based confirmation, which is considered the gold standard, was not performed due to resource constraints. Third, long-term follow-up to assess treatment outcomes based on BAL and post-FOB sputum results was not included. Future multicenter studies with larger sample sizes, culture correlation, and longitudinal follow-up are needed to validate these findings.

CONCLUSION

Bronchoalveolar lavage fluid analysis using CBNAAT provides the highest diagnostic yield in sputum-negative PTB patients, followed by post-bronchoscopy sputum CBNAAT and AFB smear. Conventional smear microscopy of BAL fluid yields the lowest sensitivity. Chest X-ray remains an important initial screening tool. Combining BAL and post-FOB sputum testing enhances early detection of tuberculosis and identification of rifampicin resistance, supporting timely clinical management and improved TB control in high-burden settings.

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