Section: Respiratory Medicine

Original Research Article

RETROSPECTIVE ANALYSIS OF BRONCHO ALVEOLAR LAVAGE SAMPLE IN DIAGNOSIS OF TUBERCULOSIS BY USING XPERT®MTB/RIF ASSAY (CBNAAT), SMEAR MICROSCOPY AND MGIT LIQUID CULTURE AT TERTIARY CARE INSTITUTE

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ABSTRACT

Background: Diagnosing pulmonary tuberculosis (PTB) in patients with sputum scarcity or smear negativity is challenging. Bronchoalveolar lavage (BAL) obtained via fibre-optic bronchoscopy improves diagnostic yield. This study evaluated the diagnostic performance of CBNAAT (Xpert® MTB/RIF assay) and smear microscopy using BAL fluid, in comparison with MGIT liquid culture. **Materials and Methods:** This retrospective observational study included 102 patients who underwent BAL collection at a tertiary care centre between March 2024 and January 2025. BAL samples were tested using CBNAAT, smear microscopy, and MGIT culture. The diagnostic indices were calculated using culture as the reference standard. **Result:** Of the 102 patients, 71 (69.6%) were male and 31 (30.4%) were female. CBNAAT positivity was highest in the 41–60 years age group (56, 55%), followed by 21–40 years (27, 26%), 61–80 years (15, 15%), and 0–20 years (4, 4%). CBNAAT detected 53 culture-positive and 46 culture-negative cases, with 11 and 238 false and true negatives, respectively. The sensitivity, specificity, PPV, NPV, and accuracy of CBNAAT were 82.81%, 83.80%, 53.54%, 95.58%, and 83.62%, respectively. Smear microscopy detected 18 culture-positive and 3 culture-negative cases, with 46 false negatives and 281 true negatives. The sensitivity, specificity, PPV, NPV, and accuracy of smear microscopy were 28.13%, 98.94%, 85.71%, 85.93%, and 85.92%. **Conclusion:** CBNAAT demonstrated superior sensitivity and diagnostic accuracy over smear microscopy for BAL samples and should be considered a first-line tool for diagnosing PTB in patients with sputum scarcity or smear-negative results.

INTRODUCTION

Tuberculosis (TB) continues to be a major global health challenge, ranking among the top ten causes of death and remaining the foremost killer from a single infectious agent, surpassing HIV/AIDS.[1] According to the World Health Organization (WHO) Global Tuberculosis Report 2019, an estimated 10 million individuals (9.0–11.1 million) developed TB, with India alone contributing 27% of these cases. The WHO identifies India as one of the 30 countries with a high TB burden. Of the estimated global TB cases, only 7 million were enrolled in national programs and subsequently to the WHO. A significant portion of the 3 million unreported cases was concentrated in ten countries, with India accounting for the largest gap at 25%. This discrepancy is largely attributed to underreporting and underdiagnosis, where

individuals either do not access healthcare or are not accurately diagnosed.[1]

One of the key challenges in TB control is achieving prompt and precise diagnosis, which is critical for starting management early. Sputum remains the most commonly used specimen for detecting pulmonary tuberculosis (PTB). However, some cases of PTB could not produce sputum, a condition known as sputum-scarce pulmonary TB. Even in those who can expectorate sputum, 30%–60% of samples may yield negative results for acid-fast bacilli (AFB) on microscopy, complicating the diagnostic process.[2-4] In such cases, fibreoptic bronchoscopy (FOB) can be used to obtain bronchoalveolar lavage (BAL) fluid, which can aid in diagnosing both sputum-scarce and smear-negative PTB.[5]

A study observed that the Gene Xpert test demonstrated significantly higher sensitivity than smear microscopy in diagnosing smear-negative pulmonary TB using bronchoalveolar lavage (BAL) samples. Gene Xpert identified 93.5% of confirmed cases, whereas smear microscopy detected only 41.9%.[6] This underscores the value of the Gene Xpert test in cases who are unable to secrete sputum or have negative smear results. Gene Xpert detects Mycobacterium tuberculosis and determines rifampicin resistance through the use of three specific primers, providing high specificity. It is a cartridge-based, fully automated molecular test that can diagnose TB and detect rifampicin resistance within 2 hours, making it one of the fastest among the newer diagnostic technologies.[7] The objective of this study was to evaluate the diagnostic accuracy of CBNAAT, smear microscopy, and MGIT liquid culture using BAL samples in patients with sputum-scarce or smear-negative tuberculosis.

MATERIALS AND METHODS

A total of 102 patients were enrolled in this retrospective observational study, which was conducted in the Department of Respiratory Medicine over duration of 11 months, from March 2024 to January 2025. Ethical clearance was obtained from the Institutional Ethics Committee before the initiation of the study, and informed written consent was secured from all participants before undergoing any procedures.

Inclusion Criteria

The study included patients who underwent bronchoscopy with BAL collection and had complete results available for Xpert MTB/RIF, smear microscopy, and mycobacterial culture.

Exclusion Criteria

Patients were excluded from the analysis if they did not give informed consent, had missing laboratory results, or if BAL recovery was inadequate.

Methods

FOB was carried out using a standard method under proper sedation, with continuous monitoring of the patient's vital signs. The procedure was performed only after the clinician confirmed the patient was medically fit. A total of 200 ml of sterile saline was introduced into the targeted lung segment in 20 ml portions. The recovered bronchoalveolar lavage fluid was collected into a sterile container and promptly sent to the microbiology laboratory for testing.

In the laboratory, bronchoalveolar lavage samples were examined using smear microscopy with the auramine-O fluorochrome stain for acid-fast bacilli. The samples were then digested and decontaminated using the standard NaLC–NaOH method, with a final sodium hydroxide concentration of 1.5%. After processing, the sediment was mixed with 1 ml of phosphate-buffered saline (PBS) at pH 6.8. From this, 0.5 ml was used to inoculate the BACTEC MGIT 960 system for culture, following the manufacturer's instructions. Samples that tested positive in the MGIT 960 system were further evaluated to distinguish Mycobacterium tuberculosis complex from non-tuberculous mycobacteria. This included repeat fluorochrome staining, culture on brain heart infusion agar to rule out contamination, and confirmation with the MPT64 antigen-based immunochromatographic test (TBc ID, Becton and Dickinson, USA).

The Xpert MTB/RIF assay (CBNAAT) was conducted using the Cepheid GeneXpert® system, following the manufacturer's guidelines. The test results reported whether Mycobacterium tuberculosis was detected and whether rifampicin resistance was present. The findings were then used to assess and compare diagnostic accuracy.

Statistical Analysis

Continuous data's expressed as mean, standard deviation, while categorical data's shown as frequency and percentages. Group comparisons made using the unpaired t-test for continuous data and the chi-square test for categorical data. The diagnostic procedure of CBNAAT and smear microscopy was assessed by calculating sensitivity, specificity, and overall accuracy. significance was defined as a p-value < 0.05. Data analysis was performed using IBM SPSS Statistics (v24).

RESULTS

Out of the 102 cases, 71 (69.6%) were male and 31 (30.4%) were female. And the most of positive cases were in the 41–60 years age group, accounting for 56 (55%) cases, followed by 27 (26%) in the 21–40 years age. The 61–80 years had 15 (15%) cases, while the 0–20 year's group showed the least positivity with 4 (4%) cases [Table 1].

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Table 1: Gender and age-wise distribution of CBNAAT positivity

Characteristic	Category	N (%)
Gender	Male	71 (69.6%)
	Female	31 (30.4%)
Age (years)	0–20	4 (4%)
	21–40	27 (26%)
	41–60	56 (55%)
	61–80	15 (15%)

CBNAAT detected the organism in 53 of the culture-positive cases and in 46 of the culture-negative cases. It failed to detect the organism in 11 culture-positive samples but reported no detection in 238 culture-negative samples. Smear microscopy was positive in 18 culture-positive cases and 3 culture-negative cases, while it was negative in 46 culture-positive and 281 culture-negative samples. Based on these findings, the diagnostic performance of CBNAAT showed a sensitivity of 82.81%, specificity of 83.80%, positive predictive value (PPV) of 53.54%, negative predictive value (NPV) of 95.58%, and an overall accuracy of 83.62%. Smear microscopy demonstrated a sensitivity of 28.13%, specificity of 98.94%, PPV of 85.71%, NPV of 85.93%, and accuracy of 85.92% [Table 2 and 3].

Table 2: Diagnostic performance of CBNAAT and smear microscopy in comparison with culture results

		Culture Positive	Culture Negative
CBNAAT	Detected	53	46
	Not Detected	11	238
Smear microscopy	Positive	18	3
	Negative	46	281

Table 3: Sensitivity and specificity of CBNAAT and smear microscopy

	CBNAAT	Smear microscopy
Sensitivity	82.81%	28.13%
Specificity	83.80%	98.94%
PPV	53.54%	85.71%
NPV	95.58%	85.93%
Accuracy	83.62%	85.92%

DISCUSSION

In our study, of the 102 patients who underwent bronchoalveolar lavage, 71 (69.6%) were male and 31 (30.4%) were female. Following our findings, Archana et al. also reported a higher number of male patients, with 76 out of 112 (67.8%) being male and 36 (32.1%) being female.[8] Consistent with our findings, Kim et al. reported that 67.4% of the cases in the BAL and 62.5% in the bronchial washing cases were male.[9]Kandi et al. also reported a male predominance, with 60% of the 200 samples collected from male patients.[10] Dubey et al. reported a similar male predominance, with 55% males and 45% females among 100 patients.[11]

CBNAAT positivity in our study was most frequent in the 41–60 years age group, accounting for 56 cases (55%), followed by 27 (26%) in the 21–40 years, 15 (15%) in the 61–80 years, and 4 (4%) in the 0–20 years group. In line with our findings, Archana et al. observed a mean age of 41 years, ranging from 22.5 to 60 years old.[8] Similarly, Kim et al. observed an average age of 50.9 years (±14.9) in the BAL group.[9] Barnard et al. reported an average age of 44.4 years, and Dubey et al. found that 59% of their patients were within the 41–60 years range.[11]

Compared with the MGIT culture, CBNAAT in our study detected 53 true positives and 46 false positives, with 11 false negatives and 238 true negatives. The test concluded a sensitivity of 82.81%, specificity of 83.80%, PPV of 53.54%, NPV of 95.58%, and an overall diagnostic accuracy of 83.62%. Consistent with our findings, Archana et al. reported a higher sensitivity (96.51%) and specificity (92.31%), with a PPV of 97.65% and an NPV of 88.89%.[8] Similarly, Kim et al. observed PCR positivity in 57.1% of BAL samples and Xpert MTB/RIF positivity in 75% of culture-positive cases.[9]

Bhatia et al. found that the sensitivity, specificity, PPV, and NPV of CBNAAT were 76.5 %, 91.1%, 48.2%, and 97.3 %, respectively.[13] Likewise, Kandi et al. reported sensitivity and specificity of 79.2% and 89.5%, respectively, with both PPV and NPV at 79.2% and 89.5%.[10] Dubey et al. observed a sensitivity of 78.85%, specificity of 93.02%, PPV of 64.71%, and NPV of 96.39%.[11] Barnard et al. showed even higher performance, with a sensitivity of 92.3%, specificity of 87.7%, PPV of 80%, and NPV of 95.5%.[12] Saini et al. said a comparable CBNAAT sensitivity of 81.8% and NPV of 88.2%. However, their specificity was lower at 50%, and PPV was 37.5%.[14]

In our study, smear microscopy identified 18 true positives and three false positives, with 46 false negatives and 281 true negatives. This yielded a sensitivity of 28.13%, specificity of 98.94%, PPV of 85.71%, NPV of 85.93%, and diagnostic accuracy of 85.92%. Similarly, Archana et al. observed a higher sensitivity of 76.67% and lower specificity of 86.36%, with a PPV of 95.83% and NPV of

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47.50%.8 In comparison, Kim et al. found smear positivity in 28.6% of BAL samples, closely matching our sensitivity.[9]

Fakey Khan et al. noted that 92% of CBNAAT false-negative cases were also smear-negative, indicating low sensitivity of smear microscopy in their cohort.[15]Kandi et al. observed higher sensitivity of 41.5% and similar specificity of 98.2%, with PPV and NPV both at 41.5% and 98.2%.[10] Barnard et al. said a smear sensitivity of 41.0% and a specificity of 98.6%, which were also close to our values.[12] Similarly, Saini et al. mentioned very low smear positivity in BAL fluid, only 9.7% highlighting its limited utility in their paediatric cohort.[14] Likewise, Sun et al. reported low sensitivity for brushing smear microscopy (35.2%) and very high specificity of 98.9%. Their PPV was 98.4%, and their NPV was 42.6%.[16]

Overall, the comparisons suggest that our study findings align well with existing evidence, particularly in demonstrating the diagnostic value of CBNAAT in smear-negative and sputum-scarce pulmonary TB. The observed male predominance, age distribution, and high specificity of smear microscopy are also in agreement with most of the reviewed studies, reinforcing the credibility and clinical relevance of our findings.

Limitations: Since this was a retrospective study, there is a chance of selection bias and less ability to control confounding factors. Additionally, as the research was done in a single tertiary care centre with low cases, the findings may not be easily generalizable to other populations or healthcare settings.

CONCLUSION

Xpert MTB/RIF (CBNAAT) is advised for routine use in patients strongly suspected of having pulmonary TB, due to its superior sensitivity compared to smear microscopy and its high specificity when detecting TB from bronchial washings. It also offers rapid information on rifampicin resistance. However, positive CBNAAT results with concurrent negative cultures should be carefully assessed in the clinical context and ideally verified through further testing or follow-up evaluations.

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