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Original Research Article

A COMPARATIVE STUDY OF BRONCHO ALVEOLAR LAVAGE ADENOSINE DEAMINASE LEVELS vs CBNAAT IN PRESUMPTIVE PULMONARY TUBERCULOSIS PATIENTS

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ABSTRACT

Background: Tuberculosis (TB) is a considerable worldwide health concern, particularly in underdeveloped nations. Globally in 2024, there were an estimated 1.08 million TB deaths among HIV-negative people (95% uncertainty interval [UI]: 0.99–1.18 million) and an estimated 1,50,000 TB deaths among people with HIV (95% UI: 1,20, 000–1,83, 000)[1]. A significant percentage of tuberculosis cases are sputum-negative, challenging diagnosis and delays suitable treatment. BAL fluid analysis serves as a crucial diagnostic instrument when sputum samples are inadequate or consistently negative for acid-fast bacilli (AFB). **Aims and Objectives:** Aim of the study is to study the efficacy of bronchoalveolar lavage Adenosine Deaminase Levels, and CBNAAT in presumptive pulmonary tuberculosis patients.

Materials and Methods: The study was an Observational study, done at Government Hospital for Chest and Communicable Diseases, Visakhapatnam, over a period of 1year i.e November 2024 to October 2025. Study population includes Patients attended the Pulmonary Medicine department with symptoms of presumptive Pulmonary Tuberculosis. The Broncho alveolar lavage fluid was dispatched to the laboratory for liquid culture, ADA determination, and CBNAAT, while diagnostic investigations of BAL fluid and other specimens were submitted for examination based on clinical suspicion. Upon obtaining the analysis results, each test variable was compared with one another, leading to the formulation of conclusions. ADA cut off was obtained by using receiver operator curve. The liquid culture of Mycobacterium tuberculosis was utilized as the gold standard test.

Results: A significant association was found between ADA levels and culture results. CBNAAT showed strong concordance with culture results, supporting its diagnostic utility.

Conclusion: Our study concluded that the combination of BAL ADA + CBNAAT increases diagnostic confidence in smear-negative PTB and also early initiation of ATT without waiting for culture results.

Keywords: Tuberculosis, CBNAAT, Adenosine De Aminase

INTRODUCTION

Tuberculosis (TB) is a considerable worldwide health concern, particularly in underdeveloped nations. Globally in 2024, there were an estimated 1.08 million TB deaths among HIV-negative people (95% uncertainty interval [UI]: 0.99–1.18 million) and an estimated 1,50,000 TB deaths among people with HIV (95% UI: 1,20, 000–1,83, 000).[1] The disease

persists in spite of improvements in diagnosis and treatment techniques, mostly because of the intricate interactions between biological, medical, and socioeconomic factors. Pulmonary tuberculosis (PTB), the predominant variant, is generally identified using sputum smear microscopy.[2] A significant percentage of tuberculosis cases are sputum-negative, challenging diagnosis and delays suitable treatment. BAL fluid analysis serves as a crucial diagnostic instrument when sputum samples are inadequate or consistently negative for acid-fast bacilli (AFB). The procedure comprises the examination of bronchoalveolar lavage (BAL) fluid for adenosine deaminase (ADA) concentrations, cytological assessment, and sophisticated molecular methodologies such as GeneXpert MTB/RIF. Bronchoalveolar lavage (BAL) requires the infusion of saline into a lung, followed by the collection of the fluid for laboratory examination. This method facilitates the acquisition sample collection from the lower respiratory tract, potentially providing diagnostic insights absent in sputum. The BAL fluid may undergo multiple analyses, such as cytological evaluation, assessment of ADA activity, and nucleic acid amplification studies, including the GeneXpert MTB/RIF assay. Adenosine deaminase (ADA) is an enzyme involved in purine metabolism, and its activity is particularly elevated in diseases involving cellular immunity, such as TB. Measuring ADA levels in BAL fluid provides a useful, rapid, and cost-effective diagnostic adjunct for identifying TB in sputum-negative cases. Public health initiatives should focus on training healthcare providers in the use of ADA measurement and other advanced diagnostic techniques, particularly in high-burden settings. The present study was done to study the efficacy of bronchoalveolar lavage Adenosine Deaminase Levels, CBNAAT (Catridge Based Nucleic Acid Amplification Test), liquid culture in presumptive pulmonary tuberculosis patient.

Aims & Objectives

Aim of the study

To study the efficacy of bronchoalveolar lavage Adenosine Deaminase Levels, and CBNAAT in presumptive pulmonary tuberculosis patients.

Objectives of the study

- To study the bronchoalveolar lavage Adenosine Deaminase Levels in presumptive pulmonary tuberculosis patients.
- To study the efficacy of bronchoalveolar lavage CBNAAT in presumptive pulmonary tuberculosis patients.
- To compare the bronchoalveolar lavage Adenosine Deaminase levels, with bronchoalveolar lavage CBNAAT.

MATERIALS AND METHODS

The study was an Observational study, done at Government Hospital for Chest and Communicable Diseases, Visakhapatnam, over a period of 1year i.e November 2024 to October 2025. Study population includes Patients attended the Pulmonary Medicine department with symptoms of presumptive Pulmonary Tuberculosis. As per the calculation by using the formula (n) = (z1– α/2)2pq/d2, sample size was calculated to be 50. All the patients underwent Chest X ray PA and lateral view, CBP, RFT, LFT, Serum Total Protein, Serum Albumin, Sr. LDH, Viral markers, CT, BT, ECG, 2d echo, Sputum CBNAAT, AFB stain, BAL fluid ADA, BAL CBNAAT, BAL liquid culture, HRCT scan chest.

Inclusion Criteria

1. Age more than 18 Years
2. All the patients with clinical and radiological signs of presumptive pulmonary tuberculosis
3. Sputum CBNAAT/sputum smear Afb negative cases

Exclusion Criteria

1. Patients not given consent
2. Sputum positive cases
3. Cases who are unfit for bronchoscopy
4. Extra pulmonary tuberculosis patients

Methodology

The study was performed following the acquisition of Institutional Ethics Committee approval and consent was taken from subjects. Demographic information, encompassing age, gender, education, occupation, socioeconomic status, marital status, and address were collected. Patients attending to the outpatient department with negative sputum CBNAAT and AFB stain underwent bronchoscopy following consent and regular examinations. Fiberoptic bronchoscopy was administered via the trans nasal or oral route following adequate lubrication with xylocaine spray. Repeated instillations of 50 ml normal saline totaling 100–150 ml, or the recovery of 50 ml of recovered fluid, were deemed sufficient for lavage. The lavage fluid was dispatched to the laboratory for liquid culture, ADA determination, and CBNAAT, while diagnostic investigations of BAL fluid and other specimens were submitted for examination based on clinical suspicion. The BAL fluid was centrifuged at 6000 rpm for 10 minutes, and the ADA activity of the supernatant was quantified using the ADA assay kit. The culture was performed via the BACTEC mycobacteria growth indicator tube method. All tubes were examined for positive till 42 days. Positive cultures were further validated via ZN staining, fluorescence staining, and immunological chromatography assays for the identification of MTB antigen MPT 64. CBNAAT was conducted in accordance with NTEP recommendations. Upon obtaining the analysis results, each test variable was compared with one another, leading to the formulation of conclusions. The liquid culture of Mycobacterium tuberculosis was utilized as the gold standard test.

Statistical Analysis

Data was analyzed with Microsoft Excel and SPSS software version 26.0. Mean and standard deviation of the quantitative variables was measured. For categorical variables, association was estimated by

using the chi-square test or Fisher's exact test. P value ≤0.05 was taken as significant.

RESULTS

The mean age of the cases was 46.76 ± 13.63 years. Of the total, 33(66%) were males and 17(34%) were females (Table 1). CBNAAT was positive in 24(48%) cases (Table 2). Culture was positive in 22(44%) cases (Table 3). On X ray findings, Consolidation seen in 20(40%) cases predominantly followed by cavity which was seen in 12(24%) cases (Table 4). Based on the ROC curve, cutoff level of ADA was >4 (fig 1). Of the ADA ≥4 cases, in 18 (81.8%) cases, CBNAAT was positive, and in 4 (18.2%) cases CBNAAT was negative. While in ADA < 4, in 6 (21.4%) cases, CBNAAT was positive, and in 22 (78.6%) cases CBNAAT also negative (Table 5). The association between ADA and CBNAAT was significant. Culture was positive in 17(68.00%) cases of ADA levels of ≥4, and 5(20.00%) cases of ADA<4; culture was negative in 8(32.00%) cases of ADA levels of ≥4, and 20(80.00%) cases of ADA <4(Table 6). The association between ADA levels and culture was significant. CBNAAT was positive in 21(87.50%) cases of culture positive cases, and 3(12.50%) cases of culture negative cases; CBNAAT was negative in 1(3.80%) case of culture positive cases, and 25(96.20%) cases of culture negative cases (Table 7). The association between CBNAAT and culture was significant.

Table 1: Age and Genderdistribution

Age	Gender		Total
	Male	Female	
21-30	4(57.10%)	3(42.90%)	7(14.00%)
31-40	4(36.40%)	7(63.60%)	11(22.00%)
41-50	8(66.70%)	4(33.30%)	12(24.00%)
51-60	9(75.00%)	3(25.00%)	12(24.00%)
61-70	8(100.00%)	0(0.00%)	8(16.00%)
Total	33(66.00%)	17(34.00%)	50(100.00%)

Table 2: CBNAAT

CBNAAT	Frequency	Percentage
Positive	24	48
Negative	26	52
Total	50	100

Table 3: Culture

Culture	Frequency	Percentage
Positive	22	44
Negative	28	56
Total	50	100

Table 4: Distribution of X-Ray findings

X ray findings	Frequency	Percentage
Cavity	12	24
Collapse	2	4
Consolidation	20	40
Cystic changes	2	4
Miliary pattern	4	8
Nodules	9	18
Normal	1	2
Total	50	100

Table 5: Comparison of BAL ADA and BAL CBNAAT: Chi square test 18.1; P value 0.0001(significant)

ADA	CBNAAT positive	CBNAAT negative	Total
≥4	18 (81.8%)	4 (18.2%)	22 (44%)
<4	6 (21.4%)	22 (78.6%)	28 (56%)
Total	24 (48%)	26 (52%)	50(100%)

Table 6: BAL ADA levels and Culture: Chi-Square value: 11.69; P value:0.001 (significant)

ADA levels	Culture		Total
	Positive	Negative	
≥4	17(68.00%)	8(32.00%)	25(50.00%)
<4	5(20.00%)	20(80.00%)	25(50.00%)
Total	22(44.00%)	28(56.00%)	50(100.00%)

Table 7: CBNAAT and Culture: Chi-Square value: 35.44; P value: 0.001(significant)

CBNAAT	Culture		Total
	Positive	Negative	
Positive	21(87.50%)	3(12.50%)	24(48.00%)
Negative	1(3.80%)	25(96.20%)	26(52.00%)
Total	22(44.00%)	28(56.00%)	50(100.00%)

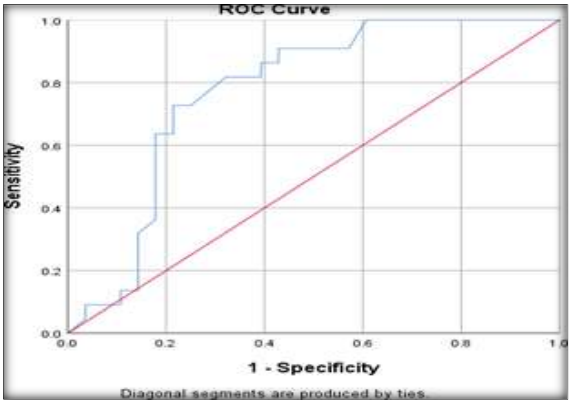

Figure 1: Receiver operator curve for ADA: ADA cut off was obtained by using receiver operator curve

DISCUSSION

India accounted for one fourth of the world's tuberculosis (TB) cases in 2024, topping the list of countries with the highest TB burden, according to the World Health Organization's (WHO) Global TB Report 2025.[1] Offer of upfront NAAT for diagnosis of TB has been prioritized by the National Tuberculosis Elimination programme in India.[3] Response to DS-TB treatment is monitored by using sputum smear microscopy whereas Liquid Culture is used for monitoring treatment response to DR-TB. The country continued the consistent trend of improvement in case finding in 2023, as demonstrated by the notification of 25.52 lakh TB cases and a total Annualized TB case notification rate of 178.8 per lakh population which are the highest ever achieved by India.[3]

In the present study, the mean age of the cases was 46.76 ± 13.63 years , out of the total 66% cases were males and 34% cases were females which is similar to studies done by Dubey S et.al,[4] where mean age was 47.31±12.29 years and Over half of the research participants were men and their M:F ratio was 1.2:1.In the present study, out of the total 66% cases were males and 34% cases were females, indicating males are more involved in study population and are often attributed to increased exposure, occupational risk and lifestyle habits such as smoking which make them more prone to tuberculosis disease development. Eshwaramma et.al,[5] did a study on Predictive Role of ADA in Bronchoalveolar Lavage Sample in Sputum Negative Pulmonary Kochs. In this study, the male to female ratio among tuberculous cases was 1.9:1, with 28 men and 15 females.

Radiologically consolidation (40%) and cavity formation (24%) were the most frequent findings in our study which are similar to the study conducted by Dubey S et al,[4] where consolidation (24%) and cavity (20%) indicating consolidation is more common radiological feature of sputum negative TB though Cavity is more typically seen in sputum positive cases due to high bacillary loads. The sputum negativity may be attributed to paucibacillary status, immunocompetent individuals.

In our study BAL CBNAAT was positive in 48% of the cases, among them 87.5% are culture positive indicating the high sensitivity of CBNAAT. Remaining12.5% cases, the CBNAAT was positive and culture negative. CBNAAT can produce false positive result primarily due to the detection of DNA from non-viable bacilli from previous infections and laboratory contamination. Correct sampling technique and prevention of sample contamination while handling increases the sensitivity and diagnostic accuracy of CBNAAT particularly in facilities with high TB load. In developing countries like India, where many patients with PTB who are co-infected with HIV and present during the late stages of HIV disease (CD4+ count <200 per mm3) and those who are severely immunosuppressed (non-HIV) are more likely to be sputum smear-negative.[6] However, in spite of best efforts at sputum collection, processing and examination, some patients with active PTB do not produce adequate sputum, while others who produce adequate sputum also remain smear-negative for reasons that are as yet unknown.[7]

ADA increases in Tuberculosis, as a result of mycobacterial antigens stimulating T-cell lymphocyte activity. The mean ADA value was 4.09 ± 2.55 U/L. ROC analysis identified >4 U/L as the optimal cutoff which is similar to the study done by Eswaramma et al.5 where ADA cut off was 3.84 U/L in sputum CBNAAT negative cases and 3.5 U/L in a study conducted by Naga Basava Raju et al.[8] ADA ≥4 U/L showed a strong and statistically significant association with both CBNAAT and Culture. In our study among patients with ADA ≥4, 81.8% were CBNAAT positive and 68% were culture positive. In studies conducted by Kothari et al,[9] and Dr. Krutesh Tripathi et al,[10] BAL ADA had a sensitivity of 75% and 76.47% respectively. This strong correlation supports the role of ADA as an effective, inexpensive, and rapid adjunctive biomarker for identifying patients with high probability of tuberculosis, particularly in cases where bacillary load is low and direct smear tests and sputum CBNAAT are negative.

ADA activity has been studied as a valuable marker for differentiating tuberculous pleural effusion from other causes of exudative pleural effusions,[11,12] and as a marker for diagnosing tuberculosis in various body fluids. However, a few studies are there to

depict the role of ADA in BAL fluid in differentiating tuberculous cases and non-tuberculous cases. Some studies have reported that ADA activity in BAL fluids of patients with pulmonary TB is higher than in other non-tuberculous pulmonary disorders.[13-15] The discrepancies between different studies usually result from differences in reported ADA levels and sensitivities and specificities. The discrepancies may be caused by different methods used for measuring ADA, diseases present, or differing techniques for bronchoalveolar lavage fluid collection. There may also be differences between humans in regards to the activity of ADA in BAL fluid.[16] Therefore, studies in similar populations using similar methods should be done to determine the ADA cut-off value. Additionally, it is important to understand TB epidemiology. Apart from its sensitivity and specificity, ADA's predictive value depends on the local prevalence as well as its sensitivity and specificity. The PPV increased with increasing prevalence.[17]

The study was conducted with a relatively small sample size of 50 patients, which may limit the generalizability of the findings to the wider population. As this was a single-center observational study, the results may reflect local patient characteristics and healthcare practices and may not be universally applicable.

CONCLUSION

In the present study a strong and statistically significant relationship was observed between CBNAAT results and culture results. This suggests good agreement between these two diagnostic methods. A significant association was found between ADA levels and culture results. CBNAAT showed strong concordance with culture results, supporting its diagnostic utility. CBNAAT from BAL fluid had high sensitivity and specificity, nearly matching culture results, and provided rapid results within 2 hours. ADA $\geq$ 4 U/L was significantly associated with both CBNAAT and culture positivity, making it a useful, inexpensive, supportive test in resource limited settings. The combination of BAL ADA + CBNAAT increases diagnostic confidence in smear-negative PTB and also early initiation of ATT without waiting for culture results.

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