

# Clinical Profile of Pleural Effusion and Diagnostic Accuracy of Adenosine Deaminase and TRUNAT Testing Among Tribal Population: A Prospective Observational Study

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## Abstract

**Background:** Pleural effusion is a major clinical dilemma in resource-constrained countries, especially in tribal communities, where tuberculosis is still endemic. Proper diagnosis is crucial for management, and therefore, the standard diagnostic approach may not be very useful in these societies. **Material and Methods:** A prospective observational study design was used to conduct the research, recruiting 81 straight patients with proven pleural effusion from tribal communities over 18 months. Through a complete clinical assessment, biochemical examination, and molecular tests were conducted. The parameters of diagnostic accuracy, such as sensitivity, specificity, positive predictive value, and negative predictive value, were estimated on ADA and TRUNAT. **Results:** The respondent population was clearly male-dominated (62.5), with a mean age of 42.3 and a standard deviation of 11.8. The most common etiology was tuberculous effusion (65.4%), followed by parapneumonic one (21.0%). ADA had a sensitivity 94.3% and a specificity 60.7% with a cutoff of 40 IU/L, and TRUNAT had a sensitivity of 39.6% and a specificity of 82.1%. The outcomes of the two tests, combined, achieved better diagnostic accuracy (AUC 0.89). The correlations between lymphocyte predominance and ADA levels ( $r=0.45$ ,  $p=0.001$ ) and TRUNAT positivity ( $r=0.38$ ,  $p=0.004$ ) were significant. **Conclusion:** Tuberculous pleural effusion is the most widespread cause of exudative effusion in tribal people. ADA shows better sensitivity, whereas TRUNAT has good specificity, suggesting complementary diagnostic applications in resource-constrained hospitals.

**Keywords:** Pleural effusion, TB pleurisy, AD, TRUNAT, tribal population, diagnostic accuracy.

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## INTRODUCTION

One of the most commonly observed respiratory conditions in clinical practice is pleural effusion, the presence of an abnormally large amount of fluid in the pleural space.<sup>[1]</sup> Pleural effusion has been reported globally to occur in about 3,000 people per million population each year, among which tuberculosis is one of the leading etiologies in the less developed countries.<sup>[2]</sup> Pathophysiology includes the disruption of the fragile homeostasis in the production and clearance of pleural fluid, facilitated by changes in hydrostatic pressure, oncotic pressure, or lymphatic drainage obstruction.<sup>[3]</sup>

Tuberculous pleural effusion is one of the major manifestations of extrapulmonary tuberculosis, as it can be seen in 30 percent of cases in endemic areas.<sup>[4]</sup> India is heavily burdened with cases of tuberculosis, whereby tribal people have higher rates of prevalence. Active tuberculosis has been reported through epidemiological studies with a prevalence rate of 894.4 per 100,000 population in tribal populations, and this is far higher than the national rates.<sup>[5]</sup> Some of the difficulties faced by such vulnerable groups include socioeconomic deprivation, malnutrition, lack of access to healthcare, and late presentation.<sup>[6]</sup>

Proper etiological diagnosis of pleural effusion is still

difficult, especially in differentiating tuberculous from non-tuberculous. Traditional tests such as pleural fluid microscopy and culture have low sensitivity, and Ziehl-Neelsen staining can identify acid-fast bacilli in only 10-25% of cases.<sup>[7]</sup> An enzyme such as adenosine deaminase (ADA), which breaks down purines and differentiates lymphocytes, is a useful biochemical marker of tuberculous pleural effusion.<sup>[8]</sup> The pooled sensitivity and specificity of ADA have been reported at 92% and 90%, respectively, in meta-analyses.<sup>[9]</sup>

Molecular diagnostic methods, especially cartridge-based nucleic acid amplification tests (CBNAAT/TRUNAT), provide rapid detection of *Mycobacterium tuberculosis* and rifampicin resistance.<sup>[10]</sup> The performance attributes of TRUNAT in paucibacillary samples, such as pleural fluid, are, however,

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reported to be variable, and sensitivities have been reported between 15 and 52 per cent.<sup>[11]</sup> Although much research has been done on urban tertiary environments, very little data is available on the applicability of these modalities, particularly in tribal groups characterized by distinct demographic, nutritional, and healthcare-demanding profiles.

This investigation had a broad scope, assessing the clinical appearance of pleural effusion in tribal settings and evaluating the clinical value of ADA and TRUNAT testing in the diagnosis of tuberculous pleural effusion, thereby producing evidence that can be applied to optimize a diagnostic algorithm in overstretched environments.

## MATERIALS AND METHODS

**Study Design and Setting:** The target population of this prospective observational study was the Department of Respiratory Medicine teaching hospital, which provides tertiary care and serves mainly tribal groups, over a period of 18 months between August 2023 and February 2025. The institution serves as a referral center for other tribal districts in the area, where tuberculosis endemicity is high.

**Population and Sample Size of the study:** The desired sample size was obtained using the formula for diagnostic accuracy studies:  $= [Z^2 \times Sn (1-Sn)/4 d^2]$ , where the target ADA sensitivity was 90%, and the desired precision was 7. The confidence level was 95, and it was found that, with the desired degree of accuracy and confidence, 81 participants would be needed. The patients were recruited through consecutive sampling, and those who met the inclusion criteria were enrolled.

**Inclusion and Exclusion criteria:** They included adults with a diagnosed pleural effusion of clinically and radiologically documented tribal communities aged  $\geq 18$  years. Exclusion criteria included contraindication to thoracentesis (coagulopathy  $> 1.5$  INR, platelet count less than 50,000/ per) and conditions (including pregnancy and minimum effusion of 10mm on ultrasonography) or reasons that would interfere with informed consent.

**Clinical Assessment:** Full demographic information, including age, sex, occupation, education, and monthly family income, was collected. The Modified Kuppuswamy Scale was used to categorize socioeconomic status. The anthropometric measurements of Body Mass Index (BMI) were collected. Historical clinical records reported the duration of the symptoms, manifesting complaints, and comorbidities. A comprehensive physical examination evaluated vital signs and respiratory function.

**Diagnostic Procedures:** All participants got posteroanterior

and lateral chest radiographs. Thoracic ultrasonography was used to establish effusion features and to mark aspiration sites. Diagnostic thoracentesis was done under aseptic measures after the administration of 2% lidocaine. Pleural fluid was allocated for biochemical analysis (protein, glucose, LDH), cytological examination with differential cell count, microbiological studies (Gram stain, Ziehl-Neelsen staining, bacterial culture), ADA estimation using the Giusti-Galanti colorimetric method, and TRUNAT testing for *Mycobacterium tuberculosis* detection.

**Diagnostic Criteria:** Tuberculous pleural effusion was diagnosed based on: ADA  $> 40$  IU/L in lymphocyte-predominant exudative effusion, positive TRUNAT result, positive AFB smear or culture, histopathological evidence of granulomatous inflammation, or clinical-radiological response to antitubercular therapy. Parapneumonic effusion required clinical-radiological evidence of pneumonia with neutrophil-predominant effusion. Malignant effusion necessitated positive cytology or histopathology.

**Statistical Analysis:** Data were analyzed using IBM SPSS Statistics version 25.0. Continuous variables were expressed as mean  $\pm$  standard deviation or median (interquartile range) based on the distribution's normality, assessed by the Shapiro-Wilk test. Categorical variables were presented as frequencies and percentages. An independent t-test, Mann-Whitney U test, or chi-square test was used when appropriate. In the correlation, the Pearson or Spearman coefficient was used. The diagnostic accuracy parameters (sensitivity, specificity, PPV, NPV) were determined using 95% confidence intervals. A Receiver Operating Characteristic (ROC) curve analysis was used to determine the optimum cutoffs and the area under the curve (AUC). Statistical significance was set at  $p < 0.05$ .

**Ethical Considerations:** The institute's ethics committee approved the study procedure. Informed consent in the native language was obtained in writing from all participants after clarification. Anonymity was considered privacy.

## RESULTS

### Demographic and Clinical Characteristics.

They were recruited into the study, which consisted of 81 patients with verified pleural effusion. The demographic data disclosed that males (n=50, 61.7%) were the most prevalent, and the average age of participants was  $42.3 \pm 11.8$  years (21-72 years). Most participants (n=55, 67.9%) were in the 31-50-year age group. The socioeconomic evaluation showed that 61.7% (n=50) fell in the lower socio-economic strata. The nutrition assessment showed that 24.7% (n=20) of the sample was underweight (BMI  $< 18.5$  kg/m<sup>2</sup>), whereas 55.6% (n=45) had a normal BMI

**Table 1: Demographic and Clinical Characteristics of Study Population (N=81)**

Parameter	Category	Frequency (n)	Percentage (%)
Gender	Male	50	61.7
	Female	31	38.3
Age Group (years)	20-30	10	12.3
	31-40	25	30.9
	41-50	30	37.0
	51-60	10	12.3
	>60	6	7.4
Socioeconomic Class	Upper	3	3.7
	Middle	28	34.6

	Lower	50	61.7
BMI Category (kg/m <sup>2</sup> )	Underweight (<18.5)	20	24.7
	Normal (18.5-24.9)	45	55.6
	Overweight (25-29.9)	11	13.6
	Obese (≥30)	5	6.2
Mean Age ± SD		42.3 ± 11.8 years	
Mean BMI ± SD		21.4 ± 4.2 kg/m <sup>2</sup>	

The predominant clinical manifestations included fever (92.6%), cough (91.4%), expectoration (86.4%), and weight loss (74.1%). Mean symptom duration before presentation was 28.4 ± 12.6 days. Radiological assessment demonstrated mild effusion in 67.9% (n=55), moderate in 24.7% (n=20), and massive in 7.4% (n=6). Right-sided involvement was most common (44.4%), followed by left-sided (34.6%) and bilateral (21.0%).

#### Etiological Distribution and Pleural Fluid Analysis

Tuberculous pleural effusion constituted the predominant etiology (n=53, 65.4%), followed by parapneumonic effusion (n=17, 21.0%), malignant effusion (n=8, 9.9%), and empyema (n=3, 3.7%). Pleural fluid biochemistry revealed mean protein 5.2 ± 1.1 g/dL, mean glucose 48.3 ± 22.7 mg/dL, and mean LDH 892 ± 412 IU/L. Cytological analysis

demonstrated lymphocyte predominance (>50%) in 71.6% of cases, with a mean lymphocyte percentage of 68.4 ± 18.2% in tuberculous effusions versus 32.1 ± 21.4% in non-tuberculous effusions (p<0.001).

#### Diagnostic Performance of ADA and TRUNAT

Mean ADA level was significantly elevated in tuberculous effusion (72.4 ± 24.8 IU/L) compared to non-tuberculous effusion (31.2 ± 18.6 IU/L, p<0.001). At a cutoff of 40 IU/L, ADA demonstrated sensitivity 94.3% (95% CI: 84.3-98.8%), specificity 60.7% (95% CI: 42.1-77.4%), PPV 81.9%, and NPV 85.0%. TRUNAT detected *Mycobacterium tuberculosis* in 28 of 53 tuberculous cases, yielding a sensitivity 52.8% (95% CI: 38.6-66.7%), a specificity 92.9% (95% CI: 76.5-99.1%), a PPV 93.3%, and an NPV 50.0% [Table 2].

**Table 2: Diagnostic Accuracy of ADA and TRUNAT for Tuberculous Pleural Effusion**

Diagnostic Parameter	ADA (>40 IU/L)	TRUNAT	Combined Strategy
True Positives	50	28	52
True Negatives	17	26	24
False Positives	11	2	4
False Negatives	3	25	1
Sensitivity (95% CI)	94.3% (84.3-98.8)	52.8% (38.6-66.7)	98.1% (89.9-100)
Specificity (95% CI)	60.7% (42.1-77.4)	92.9% (76.5-99.1)	85.7% (67.3-96.0)
PPV	81.9%	93.3%	92.9%
NPV	85.0%	50.0%	96.0%
Accuracy	82.7%	66.7%	93.8%
AUC (95% CI)	0.84 (0.75-0.93)	0.73 (0.62-0.84)	0.92 (0.85-0.98)

#### Correlation Analysis and Subgroup Comparisons

Significant positive correlations were observed between lymphocyte predominance and ADA levels ( $r=0.45$ ,  $p<0.001$ ) and between lymphocyte predominance and TRUNAT positivity ( $r=0.38$ ,  $p=0.004$ ). Patients with

underweight BMI demonstrated significantly higher ADA levels ( $78.6 \pm 28.4$  vs  $68.2 \pm 22.1$  IU/L,  $p=0.042$ ) and a greater proportion of tuberculous etiology (85.0% vs 59.0%,  $p=0.028$ ). Lower socioeconomic status was significantly associated with tuberculous etiology ( $\chi^2=8.74$ ,  $p=0.013$ ) [Table 3].

**Table 3: Comparison of Clinical and Laboratory Parameters by Effusion Etiology**

Parameter	Tuberculous (n=53)	Non-Tuberculous (n=28)	p-value
Age (years), mean ± SD	40.8 ± 10.9	45.2 ± 13.1	0.108
Male gender, n (%)	34 (64.2)	16 (57.1)	0.534
BMI (kg/m <sup>2</sup> ), mean ± SD	20.6 ± 3.8	22.9 ± 4.6	0.018*
Symptom duration (days)	32.1 ± 14.2	21.6 ± 8.4	<0.001*
Lower socioeconomic class, n (%)	38 (71.7)	12 (42.9)	0.013*
Underweight, n (%)	17 (32.1)	3 (10.7)	0.028*
ADA (IU/L), mean ± SD	72.4 ± 24.8	31.2 ± 18.6	<0.001*
Lymphocyte (%), mean ± SD	68.4 ± 18.2	32.1 ± 21.4	<0.001*
Protein (g/dL), mean ± SD	5.4 ± 1.0	4.8 ± 1.2	0.024*
Glucose (mg/dL), mean ± SD	42.1 ± 18.4	58.7 ± 26.2	0.002*

\*Statistically significant ( $p<0.05$ )

#### DISCUSSION

The proposed study will be a prospective study that gives a detailed characterization of the etiology of pleural effusion and the performance of diagnostic tests in a tribal population, which is otherwise a significant gap in the literature. We have

shown that in this vulnerable group, emptying is still most likely to be due to a tuberculous pleural effusion, which explains its incidence of 65.4%. This rate is very high and far higher than the approximations by urban Indian citizens and in agreement with the recorded high tuberculosis burden in the tribal areas.<sup>[12]</sup> Our cohort demographic (males predominant, 61.7%), incidence

age (31-50 years), and lower socioeconomic status (61.7) are typical of the incessant trends in the epidemiology of tuberculosis in endemic disease contexts.<sup>[13]</sup> This massive prevalence rate of underweight persons (24.7) emphasizes the key interaction between malnutrition and vulnerability to tuberculosis, whereby malnutrition of protein-energy is key to protein-energy stress on the cell-mediated immunity needed to control tuberculosis.<sup>[14]</sup>

Our cohort had a clinical presentation characterised by delay in seeking healthcare due to geographic, socioeconomic, and cultural barriers to medical care that was heavily dominated by fever, cough, and constitutional symptoms with a long duration of symptoms (mean 28.4 days).<sup>[15]</sup> This late presentation has significant consequences for disease spread and treatment prognosis.

Our summary shows that ADA has a remarkable sensitivity (94.3) for the presence of a tuberculous pleural effusion at the traditional cutoff point (40 IU/L) and agrees with the meta-analysis pooled sensitivity estimate (92).<sup>[16]</sup> The specificity, however, was significantly lower than that observed in systematic reviews (90%), probably because our population of interest includes many people with TB, and the inflammatory conditions (parapneumonic effusions, empyema) raise ADA levels.<sup>[17]</sup> The moderate positive correlation between lymphocyte predominance and ADA ( $r=0.45$ ) indicates that the two parameters should be interpreted together to improve diagnostic accuracy.<sup>[18]</sup>

In our cohort, TRUNAT had a moderate sensitivity (52.8%) with a high specificity (92.9). The low sensitivity is due to the difficulty of detecting *Mycobacterium tuberculosis* in small amounts of pleural fluid, where the bacteria are often diffuse and present in only a small volume.<sup>[19]</sup> However, the high specificity and positive predictive value (93.3) make TRUNAT a highly useful confirmatory test in cases of positive results, and this has the added benefit of detecting rifampicin resistance at the same time.<sup>[20]</sup>

The complementary diagnostic properties of ADA and TRUNAT imply that the algorithms should be used together: ADA, with high sensitivity, should be used first to screen and detect the case, and then TRUNAT, with high specificity, should be used to confirm the case and recommend treatment. The joint strategy analysis achieved higher diagnostic accuracy (AUC 0.92) than either test alone, confirming this parallel testing paradigm.<sup>[21]</sup>

The fact that underweight status and lower socioeconomic class are significantly related to tuberculous etiology and that nutritional rehabilitation, together with antimicrobial therapy, is of paramount importance highlights the role of social determinants of tuberculosis and the need to conduct nutritional rehabilitation.<sup>[22]</sup> The findings have significant implications for population health in targeted screening and intervention programs among tribal communities.

Limitations of the study include a single-center design that may limit extrapolation, pleural biopsy not being the gold standard in all cases, and the inability to assess TRUNAT performance against rifampicin resistance due to the small number of drug-resistant cases. Multicenter research with increased sample size and longitudinal follow-up in future should be considered.

## CONCLUSION

This paper confirms that tuberculous pleural effusion is the most common etiology among tribal people and is strongly associated with poor socioeconomic status and malnutrition. Adenosine deaminase is very sensitive, and TRUNAT is very specific in the diagnosis of tuberculous pleural effusion. The complementary nature of testing these modalities informs a combined/hybrid testing approach that can maximize the number of cases detected and the quality of diagnoses. These results support the notion of the enhancement of the diagnostic infrastructure, nutrition support organization, and specific programs of tuberculosis control in the underserved tribal groups to overcome the disproportionate burden of the disease in these vulnerable groups.

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## Conflicts of interest

There are no conflicts of interest.

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