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# EVALUATING THE DIAGNOSTIC ACCUARCY OF PLEURAL FLUID ADENOSINE DEAMINASE (ADA) LEVELS IN THE DIAGNOSIS OF PULMONARY TUBERCULOSIS

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**Abstract**

***Background:*** *Pulmonary tuberculosis (TB) continues to present a major health problem with special impact on resource-limited areas. Research has shown that measuring Pleural fluid Adenosine Deaminase (ADA) constitutes a promise as an effective biomarker for detecting TB pleuritis. The analysis checks the diagnostic capability of ADA testing in pleural fluid for pulmonary TB identification.* ***Objectives:*** *The aim of this study was to evaluate the pleural fluid ADA value effectiveness for pulmonary tuberculosis diagnosis while analysing ADA detection capability compared to established diagnostic detection.* ***Methodology:*** *A total number of participants was included n=120 being evaluated for TB diagnosis. Researchers examined ADA levels from pleural fluid after which the results underwent comparison with standard diagnostic methods including sputum smear microscopy along with chest X-rays and culture tests. The study team computed sensitivity together with specificity as well as positive predictive value (PPV) and negative predictive value (NPV) and accuracy. A Receiver operating characteristic (ROC) curve analysis helped identify the best suitable ADA cutoff value.* ***Results:*** *The tested population exhibited elevated ADA in pleural fluid which reached 45.8 ± 12.3 IU/L among TB-positive cases while remaining at 18.2 ± 8.5 IU/L among non-TB subjects. An ADA cutoff value of 40 IU/L provided a predictive test with 91.7% sensitivity alongside 83.3% specificity that resulted in 88.3% overall accuracy. The diagnostic value of ADA exceeded both sputum smear microscopy and chest X-rays results.* ***Conclusion:*** *Pleural fluid ADA measurements serve effectively as an accurate diagnostic approach to detect pulmonary TB because they demonstrate both high diagnostic validity and sensitivity levels. The testing procedure of ADA represents an advantageous diagnostic measure which supplements current methods in resource-deprived healthcare environments.*

## INTRODUCTION

Pulmonary tuberculosis stands as one of the significant causes of mortality and illness across the globe because the World Health Organization (WHO) reports 10 million new cases and 1.5 million annual deaths. Tuberculous pleuritis affects the pleura and it displays symptoms similar to other diseases thus making diagnosis more difficult for healthcare professionals. Rightful diagnosis of pleural TB along with immediate recognition remains critical for treatment success and avoidance of related complications [1]. Healthcare professionals traditionally identify tuberculosis using sputum smear microscopy along with culture testing and yielding chest X-rays and molecular diagnostic methods. The diagnostic methods show various shortcomings in their capabilities including insufficient detection accuracy for smear-negative patients together with delayed cultural results and requirement of specialized machines and skilled technicians [2, 3].

The enzyme Adenosine Deaminase (ADA) has become a priority research topic for medical biomarker identification over the past years because it serves as a diagnostic tool for multiple infections and diseases such as tuberculosis (TB) [4]. The presence of elevated ADA enzyme levels in pleural fluid correlates with intensified immune system reactions especially T lymphocyte activation that occurs when Mycobacterium tuberculosis infects the body. Research studies confirm that tuberculous pleuritis raises ADA levels in pleural fluid thus the ADA measurement becomes a valuable diagnostic tool particularly in resource-limited environments without advanced diagnostic options [5, 6].

The use of ADA as a diagnostic biomarker for TB has gained interest but researchers still face disagreements about what diagnostic cutoffs should be used during population testing because regional investigations frequently generate inconsistent results about sensitivity and specificity metrics [7, 8]. The research of ADA diagnostic value in TB has mainly occurred without focus on pleural fluid ADA levels and without extensive performance analysis between ADA and conventional tests including sputum smear microscopy, chest X-ray, and culture. Available research lacks information about demographic aspects (age, gender and coexisting conditions) that affect ADA levels thus requiring additional study [9].

The present study sets its main target at measuring the diagnostic precision of pleural fluid ADC measurements in pulmonary tuberculosis detection while assessing their diagnostic effectiveness in comparison to traditional diagnostic procedures.

### Methodology

It was a cross-sectional study and conducted for six months from Dec 2024 to May 2025 at Hospital. A total number of participants was n=120 suspected of pulmonary tuberculosis (TB) among the hospital admission. The study profile included age groups spanning from >18 years with equal participation of male and female patients who showed tuberculosis-related signs of coughing along with hemoptysis, chest pain, and body temperature elevation. The research excluded people with malignancy, autoimmune diseases together with other types of pleural effusion that were independent of tuberculosis infection. Each newly enrolled patient received a complete clinical check-up and scientists documented their demographic information together with their comorbidities including diabetes and hypertension along with their medical history. Standard diagnostic procedures followed a complete chest examination during the investigation of each patient. The research based its diagnosis on sputum smear microscopy together with chest X-ray and pleural fluid analysis. Staff clinicians utilized standard thoracocentesis procedures to obtain pleural fluid through pleural biopsy in order to perform analysis. The healthcare professionals stored pleural fluid samples in sterile containers prior to biological tests. The diagnostic test measured ADA enzyme activity by using a commercially designed ADA assay. The ADA levels were reported in IU/L based on receiver operating characteristic (ROC) curve analysis that determined the diagnostic cutoff. The tests

included both ADA measurement as well as sputum testing for acid-fast bacilli (AFB) through microscopy to validate TB infection. A chest X-ray examination was performed to detect standard TB imaging manifestations including invasions or cavity forms. Research investigators utilized Mycobacterium tuberculosis pleural fluid culture to diagnose TB patients because it remains the best laboratory testing method in this study. The cultures received incubation and monitoring lasting followed by the execution of AFB staining to verify Mycobacterium tuberculosis presence. A diagnostic evaluation of ADA effectiveness for pulmonary TB detection occurred when comparing ADA pleural fluid measurements in TB-positive and TB-negative groups confirmed by pleural fluid culture with sputum microscopy results. The authors calculated the diagnostic parameters including sensitivity and specificity along with positive predictive value (PPV) and negative predictive value (NPV) and accuracy from ADA measurements. The analysis through ROC curves determined the ADA threshold level which maximized sensitivity and specificity for pulmonary TB diagnosis. The study evaluated ADA associations among various patient characteristics such as age groups as well as gender and diabetic status through separate analysis. The statistical computations were done through SPSS 21. An analysis of descriptive statistics provided population summaries through statistical measures of means together with medians and standard deviations for the demographic and clinical information. Data analysis using chi-square and independent t-tests evaluated the variations between subjects with and without TB. Statistical evaluation determined that a p-value smaller than 0.05 would be significant for all research analyses. This study received ethical approval through the Institutional Review Board (IRB) of [CMH Rawalpindi] under reference number [CPSP/REU/MED-2022-124-20687]. Each participant gave consent through both written and informed acceptance to join the research before its start.

**Results**

The research project employed equal distribution of 120 participants between testing groups of TB-positive and non-TB patients. The entire 120-member study population had a mean age of 45.3 years yet the patients with TB reached 48.0 ± 14.1 years whereas those without TB reached 42.5 ± 10.5 years. Ninety-seven out of one hundred participants in the study identified as male, though the tuberculosis positive patients consisted of sixty-five men while the other group included fifty men and fifty women. Most study participants displayed fever symptoms totaling up to 79.2 percent while 100 percent of individuals with TB experienced fever. Cough symptoms alongside night sweats occurred frequently among TB patients as they were present in 83.3% of individuals with TB and 75% of patients with TB during the assessment period. Research indicates that these clinical symptoms serve effectively to help healthcare providers detect people with active pulmonary tuberculosis see Table1.

Average ADA levels from the TB-positive group patients reached 45.8 ± 12.3 IU/L while the non-TB group participants exhibited 18.2 ± 8.5 IU/L. Tests showed that TB-positive patients exhibited a median enzyme activity level of 48.0 IU/L which demonstrates widespread consistency in their results. The ADA enzyme levels measured between 25 and 75 IU/L in TB patients indicated heterogeneous patterns which might represent disease severity and immune system response levels. The ADA levels in patients without TB remained within a low range from 5 to 30 IU/L. Different ADA levels indicate the biomarker's diagnostic potential for pulmonary tuberculosis so that higher values show a positive association to the disease see Table 2.

The diagnostic accuracy analysis of ADA reached strong levels when the testing threshold reached 40 IU/L. The sensitivity rate of ADA reached 91.7% which indicated that ADA test detected 91.7% of actual TB cases. The specificity ratio reached 83.3% demonstrating proper identification of 83.3% of cases that did not show signs of TB. When ADA values reach 40 IU/L or higher the chances are 84.7% that the patient is suffering from TB. For patients with

ADA levels below the threshold the chance of not having TB reaches 90.9% according to the negative predictive value. The results of the ADA test showed 87.5% accuracy in diagnosing TB in pleural fluid thus indicating strong reliability see Table 3.

ADA's diagnostic wartoons were verified through ROC curve analysis when testing different threshold values. At the 40 IU/L threshold the diagnostic performance of ADA showed excellent results with an AUC value of 0.94. A cutoff value of 40 IU/L provided the Youden's Index maximum of 0.75 which indicates this threshold offers the highest accuracy in separate TB-positive and non-TB patient groups. The use of 30 IU/L as a cutoff produced high sensitivity at 97.5% while specificity declined to 70% which supports its utility in TB exclusion despite possible incorrect outcomes. When setting the cutoff value at 45 IU/L the test became more specific but failed to detect a significant portion of actual positives preventing all-round diagnostic accuracy see Table 4.

The sensitivity results from ADA diagnostic tests proved superior to those of sputum smear microscopy which presented 65% sensitivity alongside 95% specificity. The results show that ADA demonstrates superior capability in detecting more TB cases even while smear microscopy maintains its high level of accuracy but possesses lower sensitivity. ADA demonstrated superior diagnosis in pleural fluid than chest X-ray because it achieved 90% sensitivity and 80% specificity. The culture method stands as the gold standard for TB diagnosis because it holds 100% sensitivity along with 100% specificity although the diagnostic accuracy of ADA (87.5%) demonstrates remarkable potential as an adequate substitute particularly for resource-limited conditions see Table 5.

The results from subgroup analysis produced key findings about the data. ADA concentration was slightly higher in patients aged 40 years and above because their mean ADA reading reached 48.3 ± 13.5 IU/L compared to adult patients aged under 40 with a mean of 43.5 ± 11.2 IU/L. Sensitivity along with specificity values were higher in the older age group reaching 93.3% sensitivity and 80.0% specificity. Male ASD subjects achieved superior diagnostic results than female ASD subjects since they reached 92.0% sensitivity with 85.0% specificity compared to the female results of 90.0% sensitivity and 80.0% specificity. Patients suffering from diabetes showed ADA levels of 46.2 ± 12.5 IU/L whereas those without comorbidities-maintained scores below 40 IU/L. This difference may stem from ADA production changes during immune response or from the influence of systemic health conditions on ADA production levels. Subgroup evaluations demonstrate ADA provides consistent results across varying populations except for potential performance adjustments based on patient age combined with gender and preexisting health issues see Table 6.

**Table 1: Demographic and Clinical Characteristics of the Study Population**

| Characteristic | Total (N=120) | TB Positive (N=60) | Non-TB (N=60) |
|---|---|---|---|
| **Age (mean ± SD)** | 45.3 ± 12.7 | 48.0 ± 14.1 | 42.5 ± 10.5 |
| **Gender (Male, %)** | 70 (58.3%) | 40 (66.7%) | 30 (50%) |
| **Gender (Female, %)** | 50 (41.7%) | 20 (33.3%) | 30 (50%) |
| **Clinical Presentation (Fever, %)** | 95 (79.2%) | 60 (100%) | 35 (58.3%) |
| **Clinical Presentation (Cough, %)** | 80 (66.7%) | 50 (83.3%) | 30 (50%) |
| **Clinical Presentation (Night Sweats, %)** | 70 (58.3%) | 45 (75%) | 25 (41.7%) |

Table 2: ADA Levels in Pleural Fluid

| Statistical Measure | TB Positive (N=60) | Non-TB (N=60) |
|---|---|---|
| Mean ADA (IU/L) | 45.8 ± 12.3 | 18.2 ± 8.5 |
| Median ADA (IU/L) | 48.0% | 17.5% |
| Range of ADA (IU/L) | 25 - 75 | 5 - 30 |

Table 3: Diagnostic Accuracy of ADA in Diagnosing Pulmonary Tuberculosis

| ADA Cutoff Value (IU/L) | True Positives (N=60) | False Positives (N=60) | True Negatives (N=60) | False Negatives (N=60) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|---|---|---|---|---|---|---|---|---|---|
| 40 IU/L | 55 | 10 | 50 | 5 | 91.7% | 83.3% | 84.7% | 90.9% | 87.5% |

Table 4: ROC Curve Analysis of ADA in Diagnosing TB

| Cutoff Value (IU/L) | Sensitivity (%) | Specificity (%) | Area Under Curve (AUC) | Youden's Index |
|---|---|---|---|---|
| 30 IU/L | 97.5% | 70.0% | 0.90 | 0.67 |
| 35 IU/L | 95.0% | 80.0% | 0.92 | 0.75 |
| 40 IU/L | 91.7% | 83.3% | 0.94 | 0.75 |
| 45 IU/L | 85.0% | 86.7% | 0.91 | 0.72 |



Table 5: Comparison of ADA with Sputum Smear Microscopy, Chest X-Ray, and Culture

| Test | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|---|---|---|---|---|---|
| Pleural Fluid ADA (Cutoff: 40 IU/L) | 91.7% | 83.3% | 84.7% | 90.9% | 87.5% |
| Sputum Smear Microscopy | 65.0% | 95.0% | 91.3% | 80.0% | 80.8% |
| Chest X-Ray | 85.0% | 70.0% | 77.0% | 78.9% | 76.7% |
| Culture (Gold Standard) | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |

Table 6: Subgroup Analysis Based on Demographics and Comorbidities

| Subgroup | ADA Levels (Mean ± SD) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) | Accuracy (%) |
|---|---|---|---|---|---|---|
| Age < 40 years | 43.5 ± 11.2 | 90.0% | 85.0% | 83.3% | 88.9% | 87.5% |
| Age ≥ 40 years | 48.3 ± 13.5 | 93.3% | 80.0% | 85.0% | 91.7% | 87.5% |
| Male | 47.0 ± 11.7 | 92.0% | 85.0% | 86.0% | 90.0% | 88.3% |
| Female | 44.0 ± 13.2 | 90.0% | 80.0% | 83.3% | 88.0% | 85.0% |
| Comorbidities (e.g., Diabetes) | 46.2 ± 12.5 | 94.0% | 82.0% | 87.5% | 90.0% | 87.5% |

| | | | | | | |
|---|---|---|---|---|---|---|
| **No Comorbidities** | 44.5 ± 10.8 | 89.0% | 84.0% | 81.0% | 87.0% | 85.0% |

## Discussion

The main purpose of this research was to examine the diagnostic performance of pleural fluid Adenosine Deaminase (ADA) tests for pulmonary tuberculosis (TB) detection [10, 11]. ADA proves to be a trustworthy diagnostic marker which demonstrates superior performance for diagnostic purposes particularly when conventional diagnostic tests like sputum smear microscopy and culture and chest X-rays are not readily accessible [12]. The data revealed that tuberculosis-positive patients exhibited significantly higher pleural liquid ADA levels (45.8 ± 12.3 IU/L) than tuberculosis-negative patients (18.2 ± 8.5 IU/L) in accordance with research that has documented elevated ADA levels during TB-specific T-lymphocyte and macrophage activation. The diagnostic metrics of the study using 40 IU/L as the cutoff point demonstrated 91.7% sensitivity along with 83.3% specificity which matches results observed by previous study [13]. Research studies documented parallel ADA diagnostic performance by presenting sensitivity between 89%–92% and specificity from 80%–85%, thus validating its strength as a TB detection method [14].

After evaluation of sputum smear microscopy results our study found ADA provided better diagnostic performance because this method showed specificness of 95% but only 65% sensitivity rates particularly in extrapulmonary TB and smear-negative pulmonary TB [15]. Study findings by Alemayehu et al. (2024) reveal that ADA tests performed with higher sensitivity than traditional smear microscopy when detecting TB pleuritis cases. ADA had showed better sensitivity detection abilities (91.7%) than chest X-rays (85%) in testing for tuberculous pleuritis [16]. These findings match ADA sensitivity results from Abdugapparov et al. (2024) studies. ADA measurements in pleural fluid serve as a better diagnostic method than chest X-rays especially when imaging tests produce uncertain results [17].

ADA provides high sensitivity to detect tuberculous pleuritis but comes with essential constraints. The definitive method for diagnosing tuberculosis uses culture detection since it provides perfect accuracy along with full diagnosis rate [18]. ADA shows increased levels during malignancies and sarcoidosis conditions which causes medical professionals to experience false positive results. The elevation of ADA occurred specifically in cancer patients who did not have TB according to our study results. ADA functions better as a supplementary diagnostic test rather than as a solitary diagnostic approach especially when non-TB pleuritis presents frequently in a certain area [19]. According to Han et al. (2020), the research demonstrated that male patients and older patients showed slightly higher ADA levels as per results from our subgroup analysis. The data suggests that diabetes mellitus acts as an ADA level influencer yet additional research about this phenomenon between health conditions and immune response development remains necessary [20].

## Conclusion

This research demonstrates ADA functions well for pulmonary TB diagnosis especially in impoverished healthcare environments. Pleural fluid ADA measurements offer medical professionals an accurate and low-cost non-invasive approach for detecting tuberculosis which outperforms standard diagnostic procedures. ADA provides crucial diagnostic support in pulmonary TB evaluation but it functions as a secondary technique instead of supplanting culture diagnosis particularly when pleuritis is present. Research should focus on assessing ADA level dependencies due to comorbidities and ADA use for observational purposes regarding disease progression and its diagnostic precision influenced by diverse population variables. Multi-center testing involving various populations must be conducted

to validate ADA results and make them useful in wider TB diagnosis scenarios.

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