ORIGINAL ARTICLE

UTILITY OF SPUTUM ADENOSINE DEAMINASE IN TUBERCULOSIS DIAGNOSIS: A COST-EFFECTIVE AND ACCESSIBLE TOOL

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ABSTRACT:
Introduction: Tuberculosis (TB) remains one of the deadliest communicable diseases globally, with nearly 9 million new cases reported annually. The limitations of conventional and molecular diagnostic methods pose significant challenges in timely diagnosis and management. Sputum Adenosine Deaminase (ADA) is a rapid and accessible diagnostic tool for pulmonary tuberculosis.
Objective: In this study, we aim to see the validity of sputum ADA levels in diagnosing pulmonary tuberculosis.
Methodology: 95 participants were included in the study, including 45 patients with culture-confirmed pulmonary tuberculosis and 50 individuals without tuberculosis (culture-negative controls). ADA levels were measured in all sputum samples. The diagnostic accuracy of sputum ADA was calculated by using sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).
Results: A statistically significant difference in sputum ADA levels was observed between tuberculosis and non-tuberculosis patients (p = 0.001). At a cutoff value of 150 U/L, the sensitivity, specificity, PPV, and NPV were 82%, 76%, 75%, and 83%, respectively.
Conclusion: The findings of this study support the utility of sputum ADA measurement as a rapid and reliable diagnostic tool for pulmonary tuberculosis. Its implementation could aid in the timely diagnosis and management of TB, particularly in regions with high disease prevalence.
Keywords: Tuberculosis, Diagnosis, Adenosine Deaminase, Sputum

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INTRODUCTION:

Tuberculosis (TB) remains the most lethal communicable disease worldwide, although effective treatments are available and live attenuated vaccines for its prevention. According to recent estimates, there are approximately 9 million new active TB cases, and about 2 million people die annually globally[1]. The burden of TB is disproportionately high in Asia and Africa, with South Asia accounting for 40% of total cases and Africa contributing 26%[2]. In Pakistan, TB is a significant public health challenge, necessitating improved diagnostic and management strategies.

The gold standard for TB diagnosis is mycobacterial culture, which, despite its high accuracy, is time-consuming, requiring up to six weeks for bacterial growth and an additional 3-6 weeks for drug sensitivity testing[3]. Alternative diagnostic methods, such as acid-fast staining and polymerase chain reaction (PCR), have limitations. Acid-fast staining, while highly specific in TB-endemic regions, exhibits variable sensitivity ranging from 20% to 80%[4,5]. PCR, though highly specific (78%-100%), has sensitivity issues due to factors such as primer type, genomic sequence, bacterial load, and the presence of inhibitors, resulting in a sensitivity range of 20%-90%[6]. These challenges underscore the urgent need for rapid, accurate, and non-invasive diagnostic tools.

Adenosine deaminase (ADA), an enzyme predominantly found in T-lymphocytes, has emerged as a promising biomarker for TB diagnosis. Meta-analyses have consistently demonstrated the high

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diagnostic accuracy of pleural fluid ADA, with a sensitivity of approximately 92%, specificity of 90%, and an area under the curve (AUC) of 0.95 for TB detection[7-10]. However, a systematic review concluded that ADA is not useful for diagnosing pulmonary TB, highlighting the need for further research[11]. Recent studies have explored the utility of ADA in other body fluids, such as serum and bronchoalveolar lavage fluid, for differentiating TB from conditions like pneumonia and lung cancer, with method-dependent cutoff values established[13].

Sputum, being a non-invasive and easily obtainable specimen, is an attractive medium for ADA measurement. Although limited studies have investigated sputum ADA levels, their findings have been inconsistent[14-16]. This study aims to evaluate the diagnostic validity of sputum ADA levels and establish cutoff values for diagnosing pulmonary TB in a high-incidence setting. By addressing the limitations of existing diagnostic methods, this research seeks to contribute to the development of a rapid, reliable, and accessible tool for TB diagnosis.

METHODOLOGY:

Study Design: This was a cross-sectional analytical study conducted to evaluate the diagnostic validity of sputum Adenosine Deaminase (ADA) in detecting pulmonary tuberculosis.

Study Setting: The study was conducted at the Pathology Department of Sahiwal Medical College, Sahiwal, in collaboration with the Pulmonology Department. The research was carried out from June 2023 to July 2024.

Study Population: The study included patients suspected of having pulmonary tuberculosis (TB) who had not received any anti-tuberculosis treatment before sample collection. Sample Size and Sampling Technique: A total of 95 sputum samples were analyzed, including 45 culture-positive tuberculosis patients and 50 culture-negative controls. Patients were selected using a purposive sampling technique based on predefined criteria.

Inclusion Criteria:

Patients were included in the study if they:

- Had clinical suspicion of pulmonary tuberculosis.
- Had not received anti-TB treatment before sample collection.
- Provided a sputum sample suitable for analysis.

Exclusion Criteria:

Patients were excluded if they:

- Had a history of prior TB treatment.
- Had extrapulmonary tuberculosis.
- Had coexisting lung conditions that could influence ADA levels (e.g., chronic obstructive pulmonary disease, lung cancer).
- Provided insufficient or compromised sputum samples.

Data Collection Procedure: The study was approved by the Institutional Review Board, and all participants provided written informed permission before sample collection. Sputum samples were expectorated spontaneously and transported to the Pathology Laboratory in a controlled environment. Mycobacterial growth was achieved using the Löwenstein-Jensen solid medium, which was incubated at 37°C with 5-10% CO_2. Cultures were checked weekly for six weeks to ensure the presence of Mycobacterium tuberculosis.

Sputum samples were homogenized for ADA measurement in a 70 mmol phosphate buffer (pH 6.0) with 0.5 mol NaCl. After centrifugation of the processed samples for 30 minutes at 5000 rpm, the supernatant ADA activity was determined using the

Table 1: Comparison between two groups regarding demographic data

Characteristics		Study	Control
Gender	Female	19	22
	Male	26	28
Age (years)	< 30	10	16
	31-45	19	11
	46-60	11	10
	>60	05	13

Diazyme enzymatic technique of the Cobas c303 fully automated chemistry analyzer.

Statistical Analysis: The data was analyzed with SPSS version 24 (SPSS Inc., Chicago, IL, USA). The mean, standard deviation (SD), and other descriptive statistics were computed. A student's t-test was used to compare the mean ADA levels in the TB and non-TB groups; a p-value less than 0.05 is considered statistically significant. To assess the diagnostic efficacy of sputum ADA, we calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

Ethical Considerations: All patient data remained confidential, and no additional financial burden was imposed on participants for diagnostic procedures.

Table 2: Comparison of sputum ADA between groups

Characteristics	Mean	SD	p-value
Study group (TB) IU/L	167	±19.7	<0.0001
Control group (Non-TB) IU/L	85	±27.1	

Table 3: Diagnostic characteristics of sputum ADA at cut-off 150 IU/L

Sensitivity	82%
Specificity	76%
Positive Predictive Value (PPV)	75%
Negative Predictive Value (NPV)	83%
Likelihood Ratio	3.4

The study adhered to the ethical principles outlined in the Declaration of Helsinki to ensure participant rights and safety.

RESULTS:

The study enrolled a total of 95 patients, comprising 45 individuals with culture-confirmed pulmonary tuberculosis (TB) and 50 patients with non-tuberculosis pulmonary diseases (culture-negative). Participants were stratified into four age groups: ≤30 years (26 patients), 31-45 years (30 patients), 46-60 years (21 patients), and >60 years (18 patients). The cohort included 41 females (43%) and 54 males (57%). A statistically significant difference was observed between TB and non-TB patients

Table 4: Categorical Comparison of ADA and TB Results

	TB+	TB-	Total
ADA+	37	12	49
ADA-	8	38	46
Total	45	50	95

(p=0.001). Using an ADA cutoff value of 150 U/L, the diagnostic performance of sputum ADA was evaluated, and sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated accordingly.

DISCUSSION:

The present study demonstrated that sputum adenosine deaminase (ADA) levels were significantly higher in patients with culture-confirmed pulmonary tuberculosis (TB) compared to non-TB controls. A statistically significant difference was observed between both groups ($p = 0.001$), with a mean ADA level of 167 ± 19.7 IU/L in the TB group and 85 ± 27.1 IU/L in the control group. At a cutoff of 150 IU/L, sputum ADA exhibited a sensitivity of 82% and specificity of 76%, indicating its potential as a rapid diagnostic biomarker for pulmonary TB. Positive and negative predictive values (PPV and NPV) were 75% and 83%, respectively, reinforcing its clinical utility in TB-endemic regions.

These findings align with previous studies that have evaluated the role of ADA in TB diagnosis. Ahmed et al., reported that serum ADA levels were significantly elevated in TB patients compared to non-TB individuals, highlighting its diagnostic relevance in pulmonary and extrapulmonary TB cases[14]. Similarly, a study by Fenhua et al. emphasized the value of ADA in both pulmonary and extrapulmonary TB, further corroborating the present study's findings[15]. The ability of ADA to differentiate TB from other pulmonary conditions has been previously established, with research demonstrating a higher diagnostic yield when ADA levels are assessed alongside other TB-specific markers[16].

However, conflicting results have also been reported. For instance, Han et al. found that pleural ADA results were unreliable in some pediatric TB cases, suggesting that additional markers may be required to improve diagnostic accuracy in specific patient subgroups[17]. Furthermore, Mandal et al. highlighted that although ADA is useful for TB diagnosis, its interpretation should be cautiously considered in conjunction with clinical and microbiological findings[18].

In contrast to these concerns, Gao et al. reported that ADA-based assays remain highly effective for differentiating TB from other pleural effusions, particularly when used in high-incidence settings[19]. The discrepancies among studies may be attributed to variations in ADA measurement techniques, study populations, and differing TB prevalence rates in various regions. Srinidhi and Kumar emphasized the importance of using ADA alongside molecular diagnostic techniques such as CBNAAT (Cartridge-Based Nucleic Acid Amplification Test) to enhance diagnostic sensitivity[20]. This suggests that ADA should not be considered a standalone diagnostic tool but rather a supplementary marker in TB detection.

The findings of the present study also highlight the importance of establishing standardized cutoff values for sputum ADA. Kumar and Anusha demonstrated that combining ADA measurements with CBNAAT significantly improves TB diagnosis in pleural effusions, reinforcing the need for multimodal diagnostic approaches[21]. Similarly, Balisan et al. reported that nucleic acid amplification tests (NAAT) complement ADA assays by enhancing specificity and reducing false positives, particularly in smear-negative TB cases[22]. These observations suggest that integrating ADA with molecular and microbiological tests could optimize TB diagnostic protocols.

However, this study has some limitations. Firstly, ADA isoenzymes were not analyzed, which could provide additional insights into its diagnostic performance. Secondly, the small sample size limits broader applicability, necessitating further large-scale validation studies. Lastly, sputum ADA measurement remains technically challenging due to the presence of inflammatory cells and mucus, which may interfere with assay accuracy.

study was conducted in a TB-endemic region, enhancing the generalizability of its findings to similar high-prevalence settings. The relatively high sensitivity and specificity of sputum ADA reinforce its potential as a rapid and cost-effective TB diagnostic tool.

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CONCLUSION:

In conclusion, the present study underscores the diagnostic utility of sputum ADA in pulmonary TB and highlights its potential role in resource-limited settings where conventional diagnostic methods are inaccessible. Future research should focus on standardizing ADA cutoff values and integrating ADA measurements with advanced molecular techniques to enhance TB diagnosis.

DECLARATION OF INTEREST: The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS:

M.R: Conceptualization, literature review, write up and references

M.W: Data collection, data analysis, proofreading and final editing of manuscript.

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