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Original Research Article

Diagnostic utility of adenosine deaminase in bronchoalveolar lavage (BAL) in pulmonary tuberculosis from a tertiary care hospital in North India

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ABSTRACT

Background: Adenosine Deaminase (ADA) has been acclaimed as a biochemical marker in numerous studies in the diagnosis of tuberculosis of pleura, peritoneum, meninges etc because of its high sensitivity and specificity. As a diagnostic test, ADA offers several advantages; it is rapid, simple, low cost, non-invasive and can be performed easily in most clinical laboratories.

Materials and Methods: The current retrospective study was carried out on a total of 91 specimens of bronchoalveolar lavage collected on bronchoscopy. The samples were collected and sediments were confirmed for presence of tubercular bacilli through Lowenstein Jensen(LJ) media (Gold standard), GeneXpert(CBNAAT).Simultaneously the ADA estimation was done from supernatant fluid obtained after centrifugation of sample.

Results: The Mean ADA level for Culture Positive samples in BAL was 5.899± 1.723 and from Culture Negative Samples the Mean ADA was1.217 ± 1.439. The ADA cut off levels of >4.0 IU/L in BAL when compared with LJ culture media (gold standard) showed a sensitivity of 90.0% and a specificity of 97.2%. Upon ROC analysis a high rate of accuracy was recorded in diagnosis of TB through ADA estimation with sensitivity and specificity reaching 100% and 97.2% at a ADA cut off 3.76 IU/L when compared with LJ culture media (gold standard). However, the sensitivity and specificity achieved was slightly lower when ADA results were compared with GeneXpert results.

Conclusion: The determination of ADA levels may help in early diagnosis, improve the prognosis and reduce the spread of disease and thereby the test should be included in routine investigations in patients suspected of tuberculosis.

1. Introduction

Tuberculosis (TB) is one of the oldest known diseases. The World Health Organization cites tuberculosis as the second most important fatal infection, after COVID-19. There are around 10 million new cases and 1.5 million deaths per year, 95% in developing countries.[1] It is a chronic granulomatous disease with variable manifestations, involving most commonly lungs and all other systems as well. It is a re-emergent killer, threatening to consume serious population all over the world. The reliable timely diagnosis of tuberculosis is important in preventing spread of disease in the community as well as in preventing morbidity and mortality. While the conventional mycobacterial culture and sensitivity testing is time-consuming, impetus has been on evolving rapid methods of reliable diagnosis.[2] Widespread use of newer imaging facilities such as ultrasonography (USG), computed tomography (CT) and magnetic resonance imaging (MRI) has revolutionized the diagnosis of pulmonary and extra-pulmonary TB. Alongside, with these new emerging techniques several biochemical markers like Adenosine deaminases (ADA),

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TNF-α and interferon-γ have been investigated for their potential role in diagnosis of tuberculosis because of being rapid, simple, low cost, non-invasive (especially in diagnosis of pleural tuberculosis) and can be performed easily in most clinical laboratories.[3] Different studies have been conducted to evaluate the diagnostic accuracy of ADA and have confirmed their high sensitivity (92%) and specificity (89%) in different body fluids including pleural, pericardial, ascitic fluid etc.[4] However, to the best of our knowledge no such study has been carried out in our population. In lieu of the above facts the present study was conducted with the objective to check the diagnostic accuracy of ADA levels in BAL in patients of tuberculosis while comparing with the gold standard i.e., conventional culture-based techniques, and GeneXpert/Cartridge-based nucleic acid amplification test (CBNAAT).

2. Materials and Methods

This cross-sectional study was conducted in the Post Graduate department of Microbiology Govt. Medical College, Srinagar for a period of eighteen months. The specimens of BAL were collected in sterile, leak-proof, disposable, and appropriately labelled containers without fixatives and placed in leak proof outer bags and were send to the laboratory immediately after collection. The specimens were stained following Ziehl-Neelson technique and the smears were examined for acid fast bacilli. However, the nonsterile specimens (BAL) were subjected to decontamination before concentration through Kubica's method. The sediments were then used for preparation of smears for inoculating on Lowenstein Jensen (LJ) media where each culture was inoculated with 0.2ml-0.4ml of centrifuged sediment and incubated at 35°C. The smears were further analysed with GeneXpert/MTBRIF. The supernatant was used for detecting ADA levels and analysed for its sensitivity and specificity in order to determine the accuracy for diagnosis of tuberculosis.

2.1. Ethical clearance

The study was approved by institutional ethics committee (IEC), Govt. Medical College Srinagar, J&K.

3. Results

The current study was conducted to rule out the role of elevated ADA levels in diagnosing Tuberculosis. In present study a total of 91 BAL samples were processed on (LJ) culture media, out of which 20 (21.9%) were found to be positive and the remaining 71 (78.1%) were negative. Similarly, when subjected to GeneXpert analysis a total of 17 (18.7%) samples for BAL were found positive and the remaining 74 (81.3%) were found negative (Figure 1).

The mean levels of ADA among tuberculosis positive and negative patients were compared. The mean ADA level among culture positive and negative samples of BAL (91) was 5.89± 1.723 and 1.217±1.439 respectively with a statistically significant P value (<0.0001). Similarly, Mean ADA Levels upon GeneXpert analysis for positive smear was 5.703 ± 1.571 and for negative smear was 1.452 ± 1.866 with a significant P-value (<0.0001) Table 1.

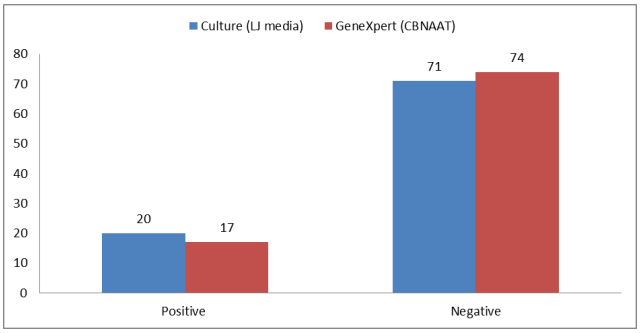

Fig. 1: Diagnosis of tuberculosis from BAL on culture and GeneXpert

Table 1: Comparison of Mean ADA levels in BAL according to culture and GeneXpertresults

Diagnostic Test	Result	Mean ± SD ADA levels in BAL	P-value
Culture	Positive (20)	5.899 ± 1.723	<0.0001
	Negative (71)	1.217 ± 1.439	
GeneXpert	Positive (17)	5.703 ± 1.571	<0.0001
	Negative (74)	1.452 ± 1.866	

Performance characteristics of ADA in BAL with culture on LJ media taken as gold standard is shown in Table 2. In BAL samples (91), 18 samples were positive in both ADA and culture media while as 69 samples were negative in both. Sensitivity of ADA for the diagnosis of tuberculosis was found 90.0% and specificity was 97.2%. The area under curve achieved was 0.984 (95% CI =0.957-1000) through receiver operating characteristic (ROC) analysis, and it was also observed that at a cut off of 3.76 IU/L the sensitivity and specificity attained was 100% & 97.2%, respectively (Figure 2).

Out of the total 91 samples of BAL, 15 samples were positive in both ADA and GeneXpert while as 69 samples were negative. Sensitivity of ADA for the diagnosis of tuberculosis was found 88.2% and specificity was 93.2% (Table 2). The area under curve achieved was 0.955 (95% CI =0.910-0.999) through receiver operating characteristic (ROC) analysis, and it was also observed that at a cut off of 3.76 IU/L the sensitivity and specificity attained was 100% & 93.2%, respectively (Figure 3).

Table 2: Performance characteristics of ADA Levels in BAL as compared to culture on LJ media and GeneXpert

Standard Test		ADA Levels (Cut off 4U/L) Positive	Negative	Sensitivity	Specificity	PPV	NPV
Culture on LJ media	Positive	18	2	90.0%	97.2%	90.0%	97.2%
	Negative	2	69				
GeneXpert	Positive	15	2	88.2%	93.2%	75.0%	97.2%
	Negative	5	69				

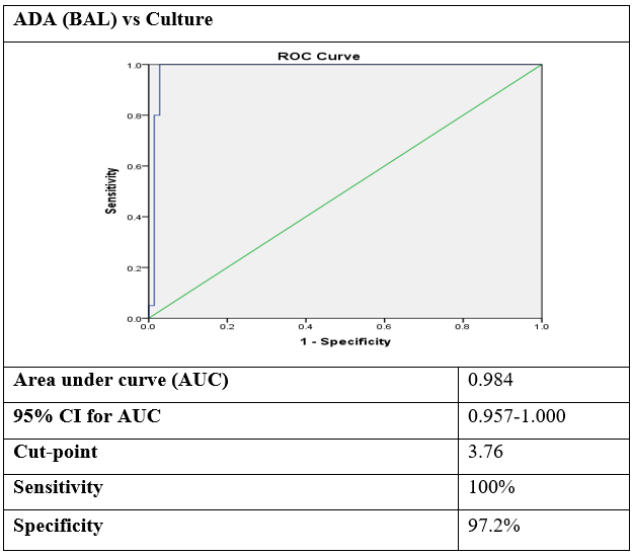

Fig. 2: ROC analysis of ADA in BAL when compared with culture (gold standard) for TB diagnosis

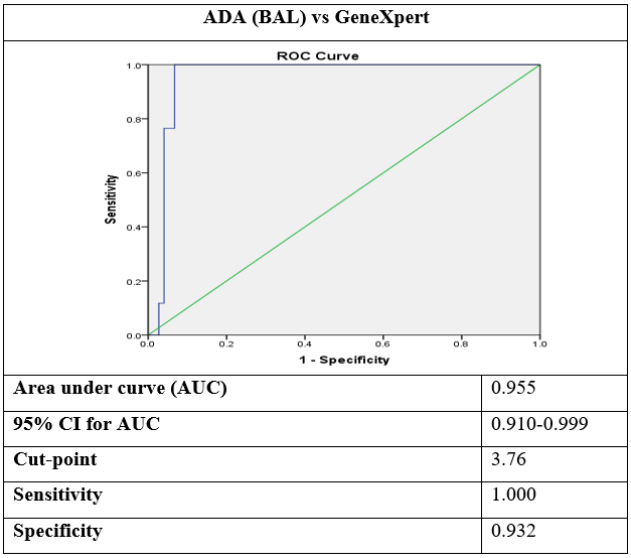

Fig. 3: ROC analysis of ADA in BAL when compared with GeneXpert for TB diagnosis

4. Discussion

The study was conducted on bronchoalveolar lavage (BAL) collected by bronchoscopy taken from the patients suspected for tuberculosis and were subjected to the different diagnostic modalities simultaneously for the confirmation of tuberculosis. The samples were processed through LJ culture media, GeneXpert and for ADA estimation. The mean fluid ADA levels estimated on LJ culture media for 20 BAL positive subjects was 5.89±1.723 while as,for 71 BAL negative subjects it was 1.74 ±1.436. Similarly, Mean ADA Levels estimated upon GeneXpert analysis for 17 positive subjects was 5.703 ± 1.571 and for 74 negative subjects was 1.452 ± 1.866. The results obtained were in concordance with the studies of Binesh F et al.[5] who measured and compared mean ADA levels in BAL fluids in pulmonary TB, pulmonary diseases other than TB, and in normal bronchoscopy wherein the levels reported were 4.13±2.55, 2.42±1.06 and 1.93±0.88, respectively. This observed rate was significantly higher in the pulmonary TB group compared to the other two groups (P = 0.001). However, the sensitivity and specificity obtained was much lower than our study in diagnosis of TB. Kayacan O et al.[6] in their study also observed that local ADA was highest in sputum smear-negative subjects highly suggestive for pulmonary TB when compared with non-tuberculous pulmonary diseases subjects and control group (P < 0.001). During the current study the ADA levels of >4.0 IU/L in BAL showed the sensitivity and specificity of 90.0% & 97.2%, respectively when compared to solid culture (gold standard) and at the same cutoff of ADA the sensitivity and specificity remained 88.2% & 93.2%, respectively upon comparing with GeneXpert. However, the results obtained were pretty higher when compared with the studies of Boonsarngsuk V et al.[7] whereby the sensitivity and specificity achieved for BAL fluid at a cut-off >3U/L was (90.0%) and (97.2%) respectively.

ROC analysis for the calculation of a cut-off point for ADA when compared with LJ media in BAL smears was being performed, it was observed that at a cut off of 3.76 IU/L, the test showed 100% sensitivity and 97.2% specificity in diagnosing pulmonary tuberculosis. Similarly, ROC analysis when compared with GeneXpert analysis in BAL was performed, it was observed that at the same cut-off point, the test showed 100% sensitivity and 93.2% specificity. Similar results were conferred by Hassanein K

et al.[8] who predicted an 80% accuracy value of ADA level in BAL fluid for diagnosing tuberculosis. Our results also showed an agreement with the studies of Perez et al.[9] who reported an average sensitivity for ADA as 93.3% and an average specificity as 91.3%. The reports also showed a close conformity with the studies of Chen et al.[10] who reported an average sensitivity and specificity of 88.6% and 80.5-96% respectively.

5. Conclusion

The determination of ADA levels in the patients suspected of pulmonary tuberculosis is inexpensive, rapid and efficient diagnostic tool with sensitivity and specificity of more than 90% and helpful in differentiating tubercular from non-tubercular etiology both in pulmonary and extra-pulmonary disease. For this reason, this test may help in early diagnosis, improve the prognosis and reduce spread of disease and its sequlae. Thus, we are of a suggestion that the test should be included in routine investigations in patients suspected of tuberculosis.

6. Source of Funding

None.

7. Conflict of Interest

None.

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