Original Article

Role of Adenosine Deaminase in Diagnosis of ExtrapulmonaryTuberculosis: Study from a Tertiary Care Hospital of North India

Rukhsana Taj, Unairah Naqash, Shazia Mushtaq

Abstract:

Background: Tuberculosis has been reported worldwide. Mycobacterium tuberculosis (MTB) primarily affect the lungs but can eventually spread to any other organ resulting in extra pulmonary tuberculosis (EPTB). The high morbidity associated with EPTB necessitates rapid development of sensitive tests. Adenosine Deaminase (ADA)is a proven biochemical marker with several advantages over conventional and molecular methods having high sensitivity and specificity for diagnosing TB.

Aims and Objective: To evaluate the sensitivity and specificity of Adenosine Deaminase (ADA) in diagnosis of extrapulmonary tuberculosis from different body fluids.

Methods: The current study was carried out on 190 specimens collected from different body fluids in orderto rule out extrapulmonary tuberculosis. The smearswere confirmed for presence of tubercular bacilli through ZN staining, LJ Media (Gold standard) andGeneXpert (CBNAAT). Simultaneously the ADA estimation was done from supernatant and readings were taken and expressed in IU/L.

Results: Out of the total 190 samples taken31 (16.3%) samples tested positive for TB on culture media, 30 (15.8%) through GeneXpert and 2 (1.1%) on ZN staining.Through ROC analyisADA estimation achieved the sensitivity of 100% in pleural, ascetic and synovial fluid. In CSF only 94.1% of sensitivity was achieved through ROC analysis. The specificity achieved was 100% for synovial fluid; however it reached to a maximum of 93.2% for pleural fluid, 95% for CSF and 96.4% for ascitic fluid.

Conclusion: Although conventional culture methods and GeneXpert are established diagnostic tools for identification of TB;the ADA levels estimation showed high sensitivity and specificity for diagnosis of tuberculosis in our study.

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Introduction:

Tuberculosis has been found to infect all age groups and its occurrence has been reported worldwide. The causative organism Mycobacterium tuberculosis (MTB) is the 13th leading cause of death world-wide.[1] The bacteria primarily affect the lungs but can eventually spread to any other organ directly or via lymphatics or hematogenous routes, resulting in extra pulmonary tuberculosis (EPTB).[2] In India 15%-20% of TB cases were reported to have TB involving extrapulmonary sites. 50% cases were seen in HIV positive individuals. [3] Extrapulmonary TB was seen mostly in adults above 44 years and children below 14 years of age.[4] Clinical presentation in EPTB is variable, depending on the organ involved, host immune response and the degree of tissue damage that has occurred. [5] Common disease manifestations include meningitis, lymphadenitis, pleuritis, pericarditis, peritonitis cutaneous, musculoskeletal, abdominal, genitourinary and miliary forms of tuberculosis.[6] The most frequent extrapulmonary sites of disease seen in India were extra thoracic lymph nodes (35%), pleural tuberculosis (20%), abdominal (14%) bone and joint tuberculosis (10%), genitourinary (5%), central nervous system (4.5%).[7]

The high morbidity associated with extra pulmonary tuberculosis (EPTB) necessitates rapid development of sensitive tests, the preliminary aim being to identify the presence of mycobacteria tuberculosis. Although a variety of tests including traditional,

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Keywords
ADA, Diagnostic Accuracy, Extrapulmonary Tuberculosis

Conventional culture-based techniques, Molecular Methods and Adenosine deaminase assay are available but the current study was limited to determine the diagnostic accuracy of ADA levels and a comparison to that of conventional and molecular methods for diagnosis of tuberculosis.

ADA is a proven biochemical marker that offers several advantages of being simple, rapid, low cost and easy to perform in most clinical laboratories.[8] The sensitivity and specificity of ADA in the different body fluids including those of pleural, pericardial, ascitic fluid etc has been ascertained in many studies.[9] In lieu of above facts the current study was undertaken to investigate the accuracy of ADA levels over other diagnostic approaches, wherein the specimens were collected from those of pleural fluids, ascitic fluids, synovial fluids, cerebrospinal and pericardial fluids for the diagnosis of EPTB. To evaluate the sensitivity and specificity of Adenosine Deaminase (ADA) in diagnosis of extrapulmonary tuberculosis from different body fluids.

Materials and Methods

This cross-sectional study was conducted at Postgraduate Department of Microbiology Govt. Medical College, Srinagar for a period of 18 months. A total of 190 specimens were collected from different body fluids like pleural fluid, ascitic fluid, cerebrospinal fluid (CSF), synovial fluid and pericardial fluid under all proper aseptic precautions from patients suspected of extra pulmonary tuberculosis. Specimens were collected in sterile, leak-proof, disposable, and appropriately labelled containers without fixatives and were transported to laboratory immediately. By keeping in consideration, the RNTCP guidelines for grading of ZN-stained smears, the differential staining technique (Ziehl - Neelson) was done for proper visualization and separation of acid-fast bacilli through microscope. Further, the specimens were concentrated by sedimentation in a refrigerated centrifuge at 3000 g for 30 minutes. The sediments were used for preparation of smears and were inoculated with 2-4 drops (0.2ml - 0.4ml) of centrifuged sediment on Lowenstein Jensen (LJ) media and were incubated at 35°C in the dark atmosphere and high humidity. Culture media was incubated in slanted position with screw caps and most isolates appeared between 3 and 6 weeks of incubation. After 8 weeks of incubation, negative cultures (those showing no growth) were reported, and the culture bottles were discarded. The sediments were further analysed through GeneXpert /MTBRIF which is a rapid, nested real-time PCR for diagnosis of tuberculosis and drug resistance. It is a cartridge based nucleic acid amplification test (CBNAAT) which simultaneously detects DNA of Mycobacterium tuberculosis complex (MTBC) and resistance to rifampicin (RIF) in less than 2 hours. The supernatant was used for detecting adenosine deaminase (ADA) levels at the Department of Biochemistry through calorimetric method using the ADA assay kit in analyzer. Reading of ADA levels in different body fluids was done and ADA level was expressed in IU/L. The whole procedure was carried out in the biological safety cabinet.

Data Collection

The detailed history including chief complaints, co morbidities, history of contact with patients of tuberculosis and diagnostic resultswere entered on a self devisedproforma.The data was entered in excel and analysed using SPSS version 20.0.

Ethical clearance

The ethical clearance for the study was granted by Ethical Clearance committee of Government Medical College, Srinagar.

Results:

The current study was conducted to rule out the efficiency of elevated Mean ADA levels in suspected cases along with a comparison to different multimodalities for the diagnosis of extrapulmonary tuberculosis. **(Table:1)** below shows the summarized report of different body fluids wherein a total of 190 samples were investigated for EPTB, out of which 64 (33.7%) samples were of pleural fluid, 57 (30.0%) of CSF, 33 (17.4%) of ascitic fluid, 32 (16.8%) of synovial fluid and 4 (2.1%) of pericardial fluid.

Outcome of culture on LJ media is shown in **Table 1**. In pleural fluid 64 samples were inoculated on LJ media and growth was seen in 5 (7.8%) samples and the same samples also tested positive on Genexpert. In 57 CSF samples growth was seen on 17 (29.8%) samples and same tested positive on GeneXpert. After inoculating 33 samples of ascitic fluid on LJ media 5 (15.2%) samples were positive and same tested also positive on GeneXpert.Four samples of synovial fluid tested positive on LJ

Table:1 Exudate types and their outcome on different diagnostic modalities.

Type of fluid	**Total Samples**	**Culture Positive**	**GeneXpert Positive**	**ZN Staining Positive**
Pleural Fluid	64	5 (7.8%)	5 (7.8%)	0 (0.0%)
CSF	57	17 (29.8%)	17 (29.8%)	1 (1.8%)
Ascitic Fluid	33	5 (15.2%)	5 (15.2%)	0 (0.0%)
Synovial Fluid	32	4 (12.5%)	3 (9.4%)	1 (3.1%)
Pericardial Fluid	4	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total	**190**	**31 (16.3%)**	**30 (15.8%)**	**2 (1.1%)**

Table 2: Comparison of Mean ADA levels according to culture results in different body fluids

Type of sample & ADA Cutoff	No. of Samples	Overall Mean ADA	Std. Deviation	Mean ± SD ADA levels among Culture Positives	Mean ± SD ADA levels among Culture Negatives	P-value
Pleural Fluid	64	35.2077	46.57159	100.40 ±22.490	29.68 ±20.858	<0.001
CSF	57	18.6335	28.81398	51.00 ±34.808	4.877 ±1.660	<0.001
Ascitic Fluid	33	35.6430	56.71333	129.00±103.114	18.97 ±8.293	<0.001
Synovial Fluid	32	22.2941	20.77581	73.25±19.653	15.01 ±10.632	<0.001
Pericardial Fluid	4	17.0750	10.49170	-	17.07 ±10.491	-

media and 3 of them were positive on GeneXpert also.Only 1 sample each from CSF and synovial fluid tested positive for TB on ZN staining. All the 4 pericardial fluid samples were negative on all testing modalities.

Table 2 shows comparison of mean levels of ADA among tuberculosis positive and negative patients in different body fluids. In pleural fluid the Mean ADA level among culture positive samples was 100.4±22.490 and among culture negative samples was 29.68±20.858. The mean ADA activity among culture positive CSF samples was 51.00±34.808 and among the culture negative samples it was 4.877±1.660. In ascitic fluid mean ADA level was 129.00 ± 80.114 among culture positive subjects and among culture negative mean ADA was 18.97±8.293. In synovial fluid the mean ADA level among culture positive samples was 73.25±19.653 and in culture negative samples it was 15.01±10.632. The mean difference was statistically significant among positive and negative samples of pleural fluid, CSF, ascetic fluid and synovial fluid [p value (<0.001)].

Table 3: Performance characteristics of ADA Levels in different types of fluids as compared to culture on LJ media in diagnosis of EPTB

Type of fluid			Culture on LJ media		Sensitivity	Specificity	PPV	NPV
			Positive	Negative				
Pleural Fluid	ADA Levels (Cut off 40U/L)	Positive	5	10	100.0%	81.4%	31.2%	100.0%
		Negative	0	49				
CSF	ADA Levels (Cut off 10U/L)	Positive	16	2	94.1%	95.0%	88.9%	97.4%
		Negative	1	38				
Ascitic Fluid	ADA Levels (Cut off 40U/L)	Positive	5	2	100.0%	92.9%	71.4%	100.0%
		Negative	0	26				
Synovial Fluid	ADA Levels (Cut off 40U/L)	Positive	4	0	100.0%	100.0%	100.0%	100.0%
		Negative	0	28				
Pericardial Fluid	ADA Levels (Cut off 40U/L)	Positive	0	0	-	100.0%	-	100.0%
		Negative	0	4				
Total	**ADA Levels**	**Positive**	**48**	**17**	**94.1%**	**92.6%**	**73.8%**	**98.6%**
		Negative	**3**	**213**				

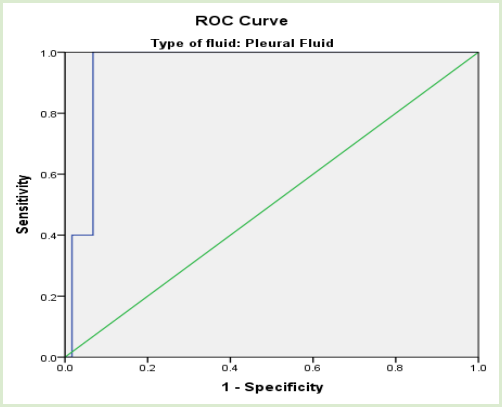
Performance characteristics of ADA in different body fluids (at conventional cut off) when culture results were taken as gold standard is shown in **Table 3**. The sensitivity of ADA for the diagnosis of tuberculosis on pleural fluid was found to be 100% and specificity was 81.4%.In CSF the sensitivity and specificity of ADA levels in the diagnosis of tuberculosis was found 94.1% and 95.0% respectively. In case of ascitic fluid the sensitivity of ADA for the diagnosis of TB in ascitic fluid is 100% and specificity was92.9%. Synovial fluid showed complete correspondence between ADA levels and culture positive growths, hence the sensitivity and specificity were calculated at 100% for both.

Table 4shows performance characteristics of ADA in different body fluids with geneXpert as gold standard. The sensitivity of ADA for the diagnosis of tuberculosis in pleural fluid was found to be 100% and specificity was 81.4%.In CSF the sensitivity and specificity of ADA levels in the diagnosis of tuberculosis was found 94.1% and 95.0% respectively. In case of ascitic fluid the sensitivity of ADA for the diagnosis of TB in ascitic fluid is 100% and specificity was 92.9%. The ADA levels in synovial fluid showed sensitivity and specificity 100% & 96.6%, respectively.

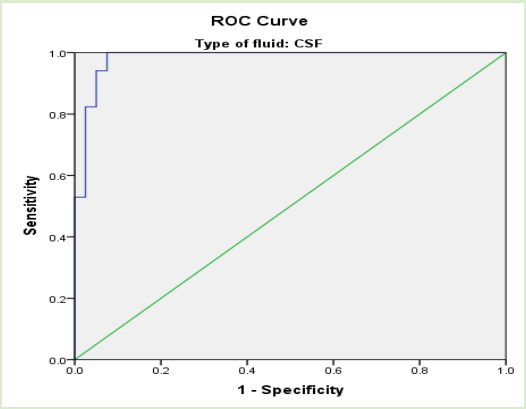
Table 4: Performance characteristics of ADA Levels in different types of fluids as compared to GeneXpert in our study population

Type of fluid			GeneXpert		Sensitivity	Specificity	PPV	NPV
			Positive	Negative				
Pleural Fluid	ADA Levels (Cut off 40U/L)	Positive	5	10	100.0%	81.4%	31.2%	100.0%
		Negative	0	49				
CSF	ADA Levels (Cut off 10U/L)	Positive	16	2	94.1%	95.0%	88.9%	97.4%
		Negative	1	38				
Ascitic Fluid	ADA Levels (Cut off 40U/L)	Positive	5	2	100.0%	92.9%	71.4%	100.0%
		Negative	0	26				
Synovial Fluid	ADA Levels (Cut off 40U/L)	Positive	3	1	100.0%	96.6%	75.0%	100.0%
		Negative	0	28				
Pericardial Fluid	ADA Levels (Cut off 40U/L)	Positive	0	0	-	100.0%	-	100.0%
		Negative	0	4				

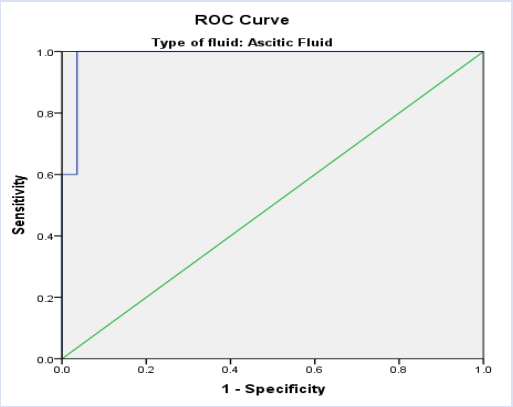
Fig. 1: ROC analysis of ADA in different body fluids when compared to culture (gold standard) for EPTB diagnosis.



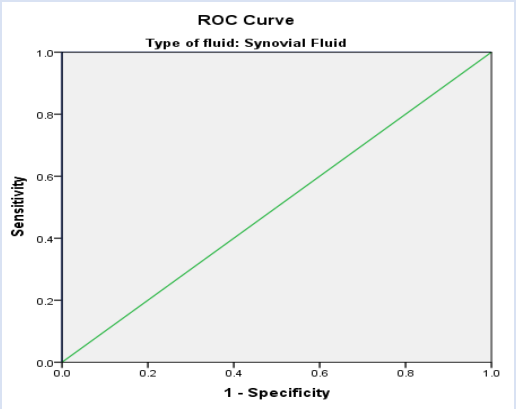
Area under curve (AUC) = 0.953
95% CI for AUC = 0.900-1.000
Cut-point = 74.0
Sensitivity = 100.0%
Specificity = 93.2%



Area under curve (AUC) = 0.982
95% CI for AUC = 0.955-1.000
Cut-point = 10.75
Sensitivity = 94.1%
Specificity = 95.0%



Area under curve (AUC) = 0.986
95% CI for AUC = 0.951-1.000



Area under curve (AUC) = 1.000
95% CI for AUC = 1.000-1.000

Cut-point = 53.5 Sensitivity = 100% Specificity = 96.4%	Cut-point = 45.5 Sensitivity = 100.0% Specificity = 100.0%

Figure 1shows receiver operating characteristic (ROC) of ADA in different body fluids. The area under curve achieved was 0.953 (0.900-1000) for ADA in pleural fluid. At a cutoff of 74.0 IU/L for ADA in pleural fluid the sensitivity and specificity achieved was 100% & 93.2%, respectively. In CSF the area under curve for ADA achieved was 0.982 (0.955-1000). At a cutoff of 10.75 IU/L for ADA in CSF the sensitivity and specificity achieved was 94.1% & 95.0%, respectively. In ROC analysis(ADA in ascitic fluid) the area under curve achieved was 0.986 (0.951-1000). At a cutoff of 53.5 IU/L for ADA in ascitic fluid the sensitivity and specificity achieved was 100% & 96.4%, respectively. **Figure 1** also shows receiver operating characteristic (ROC) of ADA in synovial fluid. The area under curve achieved was 1.000 (1.000-1000). At a cutoff of 45.5 IU/L for ADA in synovial fluid the sensitivity and specificity achieved was 100%.

Discussion:

The present study was conducted on 5 different types of body fluids including those of Pleural fluid, CSF, Ascitic fluid, synovial fluid and pericardial fluid in order to find out the utility of ADA levels indiagnostic accuracy of EPTB. Out of the total 64 samples taken, 5 samples were positive in both ADA and culture media while 49 were being found negative. The Sensitivity of ADA for the diagnosis of extrapulmonary tuberculosis was found to be 100% while the specificity attained was 81.4% on both culture and GeneXpert. Thisanalysis showed a close resemblance with the studies of **San José et al. [10]**wherein they analysed the use of serum, pleural ADA and lysozyme in tuberculous pleurisy in Spain, and found that pleural ADA had a sensitivity of 100% and a specificity of 93% for the diagnosis of TB.**Muranishi et al. [11]** while working on measurement of ADA activity and tuberculostearic acid (TSA) in pleural effusions attained a sensitivity of 56% and specificity of 76% for the diagnosis of tuberculous pleuritis. However, the observed results were relatively low when compared with our data. Our results further showed a concordance with the studies of **De Oliveira et al. [12]** who concluded that the use of these tests in combination was a highly efficient diagnostic strategy of low cost that merits wider use. On ROC analysis of pleural ADA, at a cut off of 74.0 IU/L, 100% sensitivity and 93.2% specificity were achieved. The predictive value (cut-off) as reported by **Villena et al.[13]** in their study was 33 IU/L for ADA whereby a sensitivity of 90% and specificity of 85% was achieved for tuberculous effusion, however, this value was much lower than our study.

The mean ADA activity among culture positive samples in CSF was 51.00±34.808 and among culture negative samples was 4.877±1.660 with a significant p value (<0.001).**Gambhir et al**.[14] reported mean ADA of 9.6 ± 4.1 U/L in 36 patients with tubercular meningitis, which was lower than the mean ADA in our study (18.6335 ±28.813) however, the fact may be attributed to the inclusion of more adults in our study. Upon both culture media and GeneXpert, the sensitivity and specificity of ADA levels in the diagnosis of EPTB came out to be 94.1% and 95.0% respectively. The results went in a complete harmony with the studies of **Choi et al.** [15] who observed ADA activity in CSF of 36 TBM patients and reported that at a cut-off of 7 IU/L, the sensitivity of the test for TBM group as compared to aseptic meningitis group was 83% and the specificity was 95%. Same sensitivity and specificity like that on culture media and GeneXpertwas achieved upon ROC analysis, at a cut-off of 10.75 IU/L for ADA in CSF. However, **Corral et al. [16]** used ROC curve analysis and suggested a cut off value of 8.5 U/L for the diagnosis of TBM with 57% sensitivity and 87% specificity.

For ascitic fluid the mean ADA level observed among culture positive subjects was 129.00 ± 80.114 and among culture negative subjects was 18.97±8.293. The sensitivity of ADA for the diagnosis of EPTB in ascitic fluid when compared with LJ media (Gold Standard) and GeneXpert was 100% and the specificity was 92.9%. UponROC analysis area under the curve achieved was 0.986 (0.951-1000) and at a cut-off of 53.5 IU/L for ADA in ascitic fluid the sensitivity and specificity achieved was 100% & 96.4%, respectively for diagnosis of peritoneal tuberculosis.However,**Hillenbrand DJ et al. [17]** obtained a sensitivity of only 30% in their study and concluded that the ADA is inferior in cirrhosis as a diagnostic method for peritoneal TB. Furthermore, they inferred that this could be because of a positive correlation between ADA and ascitic fluid total protein as cirrhotic patients generally have low ascitic fluid total protein. In a study conducted by **Dahale AS et al. [18]** the ADA had very high sensitivity (93%) and specificity (94%) at a cut-off of 41.1 IU/L. They also showed the presence of elevated ADA levels in the cirrhotic peritoneal TB group than that of cirrhotic non-peritoneal group and all other types of ascites patients in the control group.

Similarly, 4 samples of synovial fluid out of 32 samples taken were found positive on Culture media and there was a complete correspondence between ADA levels and culture growth, hence the sensitivity and specificity calculated was 100% for both. Upon ROC analysis thearea under curve achieved was 1.000

(1.000-1000) and at a cut-off of 45.5 IU/L, the sensitivity and specificity achieved was 100%.**Gupta VK et al. [19]** while studying Musculo-skeletal disease observed a positive predictive value of 85.71% and a negative predictive value of 66.67%, however, the predictive values being lower than that found in our study.

Conclusion:

Although culture and GeneXpert are good diagnostic tools for identification of TB yetestimation of ADA levels in patients suspected of TB especially EPTB is inexpensive, rapid and efficient with high sensitivity and specificity. It is also efficient in differentiating tubercular pulmonary infections from non-tubercular pulmonary infections thereby,suggesting that the test should be included in routine investigations in patients suspected of tuberculosis.

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