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# Use of Adenosine Deaminase Activity in Pleural Fluid for the Diagnosis of Tubercular Pleural Effusion

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## ABSTRACT

***Background*** *Adenosine deaminase (ADA) activity is a quick non invasive method to diagnose of tubercular pleural effusion(TPE) compared to other methods which are less diagnostic and time consuming. A universal cut-off value for ADA in pleural fluid, is needed since results vary widely from country to country and even within a given region. The present study evaluated the effectiveness of the present cut-off point of (30 IU/L) in the diagnosis of TPE by determining the sensitivity and specificity and also evaluated the ability of the decreased cut off point 25 IU/L to improve the sensitivity and specificity and thereby the diagnostic accuracy.*

***Materials& methods****: ADA was estimated in pleural fluid specimens of 104 patients who were admitted for pleural effusion by the colorimetric procedure of Galanti and Guisti.*

*Based on the cytological and radiological examination the patients were divided into Tubercular group and Non tubercular group with 50 and 54 subjects respectively. The cut-off value for positive ADA result was 30 U/L according to clinical practice at this hospital.*

*Statistical analysis A Comparative correlation study of Adenosine deaminase levels in both groups was done based on Sensitivity/Specificity to diagnose the disease..*

***Results****: The mean ADA level in Tubercular and in Non-tubercular group were 39.64 ± 15.6IU/L and 13.17±11.40IU/L respectively with a P value <0.001** which is highly significant. The sensitivity, specificity was 74% and 90.74 respectively. Bringing down the cut-off value to 25 IU/L, the sensitivity improved from 74% to 84% which is significant and the specificity decreased to 89% from 90% which is not significant.*

***Conclusion****: The results of the present study it is suggested to bring down the cut-off value of Pleural Fluid ADA to 25 IU/L from 30IU/L to improve the efficiency of this test in diagnosing Tubercular pleural effusion.*

**KEYWORDS : ADA – Adenosine Deaminase ;TPE – Tubercular Pleural Effusion**

## INTRODUCTION:

Tuberculosis is an endemic disease in several regions, particularly in developing countries [2].Its incidence also rising in developed countries. .Pleural TB is one of the most common extra pulmonary manifestations of the disease and may represent up to 10% of all cases[4]. Tubercular pleural effusion (TPE) should be diagnosed at the earliest to avoid the occurrence of pulmonary or extra pulmonary tuberculosis. Acute onset of fever, cough and pleuritic chest pain are the main presenting symptoms of the patients.The histological examinations of caseous lesions, microbiological methods such as acid fast smears, and cultures take time for the conformation of the diagnosis of tubercular pleural effusion and also have a low yield when done in pleural fluid . Acid fast bacilli (AFB) by the Ziehl- Neilsen on pleural fluid is positive in less than 5% of cases, and the culture on Lowenstein-Jensen medium takes more than four weeks and positive in less than 40% of cases. [5]. Therefore a fast non-invasive test is required for the rapid diagnosis of TB.

Adenosine deaminase(ADA), which is produced from T- Lymphocytes and also plays an important role in the maturation of the lymphocyte is found to be a useful marker for the diagnosis of tubercular pleural effusion. The immune cellular response which occurs in the body against mycobacterium tuberculosis, suggests the possible role of ADA in the diagnosis of mycobacterium tuberculosis. [6]. Many studies supports the fact that an increased ADA activity is found in patients with TPE; Increased ADA activity in body fluids also present in bacterial infections, rheumatologic diseases and lymph proliferative disorders. ..The determination of adenosine deaminase (ADA) activity in fluids including serum, CSF, pleural, peritoneal and pericardial is found to be useful in the diagnosis of tuberculosis . A universal reference cut-off value for ADA is not established yet in pleural fluid because of the varied results obtained among different countries and also in different regions of the same country.[4]. The present study was undertaken to evaluate the efficiency of present cut-off point for Adenosine deaminase activity (30 IU/L) for the diagnosis of tubercular pleural effusion by determining the sensitivity and specificity of the same and also to study whether the decreased cut off point of 25 IU/L essentially improves the sensitivity and specificity and thereby the diagnostic accuracy.

**AIMS AND OBJECTIVE:** Evaluation of the present cut-off point for Adenosine deaminase activity (30 IU/L) in the diagnosis of tubercular pleural effusion by determining the sensitivity and specificity of the same. Also evaluation of the ability of decreased cut off point to 25 IU/L to essentially improve the sensitivity and specificity and thereby the diagnostic accuracy.

## MATERIALS& METHODS

104 patients who were admitted for pleural effusion in a tertiary hospital in Bangalore were used as subjects for this study. Pleural tap was performed in these patients and the pleural fluid specimens were sent for the estimation ADA to the Biochemistry Laboratory of the hospital. The samples were analyzed immediately or within 48 hours after receiving the sample.

Patients both males and females aged between 20-60 years included in the study.

The patients were divided into two groups: The tuberculosis group included 50 cases confirmed by cytological examination of pleural fluid, radiological examination..The Non- tubercular group included 54 subjects with (1) malignant pleural effusion confirmed by cytological examination of pleural fluid/histological examination of pleural biopsy; (2) Pleural effusion of other known etiology such as congestive cardiac failure or para-pneumonic effusion. The cut-off value for positive ADA result was 30 U/L according to clinical practice at this hospital.

ADA analysis was done by the colorimetric procedure of Guisti and Galanti [29] employing reagents optimized by Kaplan.[30] All chemicals employed as stabilizing agents were of reagent grade.

A Comparative correlation study with 54 non-tuberculosis and 50 Tuberculosis patients was undertaken to study the Adenosine deaminase levels and its correlation based on Sensitivity/Specificity to diagnose the disease.

Statistical analysis: Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance., Student t test ( two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis)

Diagnostic statistics viz. Sensitivity, Specificity, PPV, NPV and Accuracy PLR, NLR, DOR have been computed to find the correlation of ADA for diagnosis with tuberculosis patients. Student t test (Two tailed, independent), Sensitivity and Specificity using standard statistical softwarepackage.[44,45,46]

**RESULTS:**

Table1: Age distribution of patients studied

Table2: Gender distribution of patients studied.

Table 3&Fig1: Levels of ADA IU/L in two groups of patients;1.Tubercular group and 2.Non-

tubercular group.

Table 4&Fig2: Mean of ADA in two groups of patients

5&Fig3: Components of diagnostic accuracy with a cut off value of 30 IU/L

Table 6 Components of diagnostic accuracy with a cut off value of 25 IU/L

**DISCUSSION**:. ADA is required for converting Adenosine to Inosine in the Purine salvage pathway (16,21). Its role in immune response was first identified by Giblett in severe combined immunodeficiency disease(Absence of ADA) in1972.Increased levels were found in diseases such ash as Typhoid, Lymphoma, Leukaemia, and Tuberculosis which stimulates T-Lymphocyte response

**Tables 1-6**

| Age in years | NON-Tubercular group | | Tubercular group | |
|---|---|---|---|---|
| | No | % | No | % |
| 20-30 | 6 | 11.1 | 24 | 48.0 |
| 31-40 | 4 | 7.4 | 9 | 18.0 |
| 41-50 | 11 | 20.4 | 10 | 20.0 |
| 51-60 | 33 | 61.1 | 7 | 14.0 |
| Total | 54 | 100.0 | 50 | 100.0 |
| Mean ± SD | 50.83±11.19 | | 35.34±12.92 | |

**Table 1: Age distribution of patients studied**

| Gender | NON-Tuberculous group | | Tubercular group | |
|---|---|---|---|---|
| | No | % | No | % |
| Male | 43 | 79.6 | 42 | 84.0 |
| Female | 11 | 20.4 | 8 | 16.0 |
| Total | 54 | 100.0 | 50 | 100.0 |

| ADA IU/L | NON-Tubercular group | | Tubercular group | |
|---|---|---|---|---|
| | No | % | No | % |
| 1-10 | 25 | 46.3 | 2 | 4.0 |
| 11-20 | 19 | 35.2 | 4 | 8.0 |
| 21-30 | 5 | 9.3 | 7 | 14.0 |
| 31-40 | 3 | 5.6 | 14 | 28.0 |
| 41-50 | 1 | 1.9 | 8 | 16.0 |
| 51-60 | 1 | 1.9 | 11 | 22.0 |
| >60 | 0 | 0.0 | 4 | 8.0 |
| Total | 54 | 100.0 | 50 | 100.0 |

**Table 2: Gender distribution of patients studied**
**Table 3: Levels of ADA IU/L in two groups of patients**

| ADA IU/L | NON-Tubercular group | Tubercular group |
|---|---|---|
| Min-Max | 1.00-53.50 | 6.50-71.10 |
| Mean ± SD | 13.17±11.40 | 39.64±15.56 |
| Significance | t=9.945; P<0.001** | |

**Table 4: Mean of ADA in two groups of patients**

| Components of diagnostic accuracy | Values |
|---|---|
| Specificity | 90.74% |
| NPV | 88.10% |
| PPV | 79.03% |
| Accuracy | 84.6% |
| PLR | 7.4 |
| NLR | 0.22 |
| Diagnostic odds ratio | 31.04 |

**Table 5 Components of diagnostic accuracy with a cut off value of 30 IU/L**

| Components of diagnostic accuracy | Values |
|---|---|
| Sensitivity | 82% |
| Specificity | 89% |
| NPV | 88.10% |
| PPV | 84% |
| Accuracy | 85.5% |
| PLR | 7.45 |
| NLR | 0.20 |
| Diagnostic odds ratio | 36.44 |

**Table 6 Components of diagnostic accuracy with a cut off value of 25 IU/L**

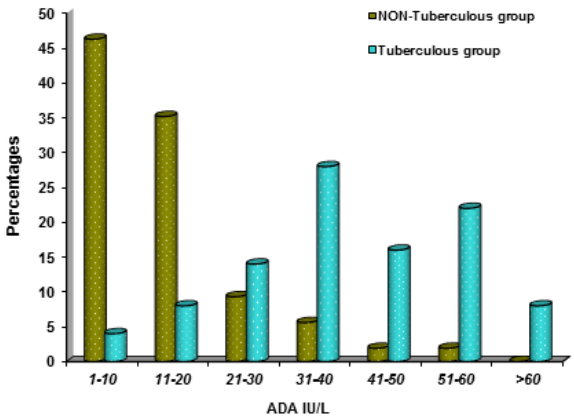


**Fig-1:Levels of ADA IU/L in two groups of patients**

( 24). In 1975, Piras was the first to demonstrate such of estimating Adenosine deaminase, in the diagnosis of Tuberculous pleural effusions achieving 100% sensitivity and specificity(25) .

The present study evaluated the usefulness of ADA with a cut-off value of 30 IU/L to diagnose Tubercular pleural effusion cases efficiently. Mean ADA level is significantly higher in tubercular group than in non tubercular group with 39.64+/- 14.56 IU/L in tubercular group and 13.17 IU/L +/- 11.40 IU/L in Non tubercular group respectively. This is in accordance with several other studies which showed ADA level in tuberculosis pleural effusion ranged from 6.50-71.0 IU/L with a mean of 39.64+/-15.56 IU/L while in Non tubercular group it ranged from 1.00-53.50 IU/L with a mean of13.17 IU/L

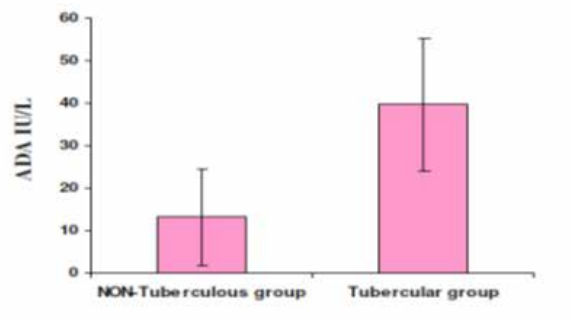


**Figure - 2 Mean of ADA in two groups of patients**

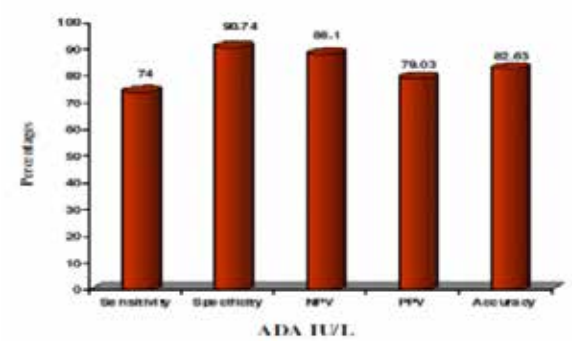


**Figure - 3 Components of diagnostic accuracy with a cut off value of 30 IU/L**

(p<0.001)**which is highly significant,. Statistically analyzed data showed significance of ADA in diagnosis of TPE with a sensitivity , specificity of 74% and 90.74%and , and a Positive Predictive and Negative predictive values of 79.03% and 88.10% respectively. Also it was observed that the Positive likelihood ratio(PLR)is 7.4 and negative likelihood ratio is 0.288(NLR) with an odds ratio is 31.04 and Accuracy of 0.846 respectively. The results correlated well with other studies, apart from showing low sensitivity. Several other studies have suggested that an elevated pleural fluid ADA level predicts Tubercular pleuritis with a sensitivity of 90-100% and specificity of 89-100% utilizing GALANTI AND GIUSTI method (16). Gupta, D.K (47)found in 53 cases of pleural effusion 36 were of tubercular etiology, concluded the mean ADA level in tubercular cases as 50.75 IU/L while in cases of Non tubercular group comprising other cuses as 14.47 IU/L and 28.65 IU/L respectively. His study showed the significance of ADA in diagnosis of TPE with sensitivity and specificity of 100% and 94.1% respectively for diagnosing tuberculosis. Burgess L.J (48) showed ADA activity in tubercular effusion was higher at a level of 50 IU/L with the sensitivity and specificity being 90% and 89% respectively. The most important finding of our study was the high negative predictive value for a cut off of 30 U/L; this allowed us to exclude TB in our community where there is a relatively high prevalence of TB pleuritis. Positive likelihood ratio of 7.4 showed moderate increase in the likelihood of disease and negative likelihood ratio of 0.288 showed moderate decrease in the likelihood of disease and with Diagnostic odds ratio ( DOR)of 31.04 indicates greater discriminatory ability of the test to diagnose the disease. DOR is a global measure that is easy to interpret and does not depend on the prevalence of the disease, Accuracy 0.84 shows it is a good test to diagnose the disease. Bringing down the cut-off value of ADA from 30 IU/L to 25 IU/L, gives a sensitivity , specificity of 82% and 89% respectively, with a Positive Predictive and Negative predictive values of 87% and 84% respectively. The sensitivity and the Positive Predictive value of the present study improved from 74% to 82% and from79% to 84% which is significant. But the specificity and negative predictive value of the study decreased from 90% to 89% and from 89% to 88% which is not significant. There is also significant increase in positive likelihood ratio from 7.4 to 7.45 and decrease in negative likelihood ratio from 0.22 to 0.20, increase in diagnostic odds ratio from 31.66 to 36.44 and increase in accuracy from 84.6%to 85.5%hows that decreasing the cut off value to 25 IU/l increases the diagnostic accuracy of the test. So from the results of the present study it is suggested to bring down the cut-off value of Pleural Fluid ADA to 25 IU/L from 30IU/L to improve the efficiency of this test in diagnosing Tubercular pleural effusion.

## REFERENCES


1. Burtis C A, E R, Ashwood, Bruns.E, tietz text book of clinical chemistry and molecular diagnostics 4th ed. Clinical enzymology; 597.
2. Aston N O. Abdominal tuberculosis. World J Surg. 1997; 21: 492- 499
3. Richard W. Light: Update on tuberculous pleural effusion,Official journal of the Asian pacific society of Respirology, Volume 15 Issue 3, Pages 451 – 458.
4. Morrys Casagrande Kaisemann, Afrânio Lineu Kritski et'al: Pleural fluid adenosine deaminase detection for the diagnosis of pleural tuberculosis J Bras Pneumol 2004; 30(6).
5. Light RW. Useful tests on the pleural fluid in the management of patients with pleural effusions. Curr Opin Pulm Med 1999; 5: 245-52
6. Segura R M, Pascal C, ocana I, et al. Adenosine deaminase in body fluids: a useful diagnostic tool in tuberculosis. Clin Biochem. 1989; 22: 141- 148.
7. MPS Menon: Pulmonary tuberculosis.
8. Kasper, Braunwald, Harrison's :Principles of Internal medicine. Volume 1, 16th Edition. Pg.953-961.
9. Anthony Harries, Dermot Maher et'al: TB/HIV a clinical Manual WHO, 2nd Edition.
10. Schlossberg: Tuberculosis and Non-Tuberculous Mycobacterial infections 4th Edition. Pg.143-153.
11. Richard W. Light, M.D.,et'al: Pleural Effusions: The Diagnostic Separation of Transudates and Exudates. October 1972 Annals of Internal Medicine, Volume 77, Number 4.
12. Arun Gopi, Sethu M. Madhavan: Diagnosis and treatment of Tuberculous pleural effusion in 2006. Chest 2007;131;880-889.
13. A.H. Diacon, B.W. Van de Wal et'al: Diagnostic tools in tuberculous pleurisy: A direct comparative study. European Respiratory journal2003; 22: 589–591.
14. P.C. Mathur, K.K Tiwari et'al: Diagnostic utility of Adenosine deaminase (ADA) activity in Tubercular serositis.Indian journal of Tuberculosis 2006;53:92-95.
15. Mostafa Ghanei. M.D, Jafar Aslani,M.D, et'al: Simple Method for Rapid Diagnosis of Tuberculosis Pleuritis: A Statistical Approach. Asian Cardiovasc Thorac Ann 2004;12:23-29.
16. A. Merrikhi M.D: Diagnostic Value of Adenosine deaminase Activity and Its isoenzymes In Tuberculous Effusions. Shiraz E-Medical Journal.
17. Koneman: Colour atlas and textbook of diagnostic microbiology 5th edition.
18. José M. Porcel, M.D., Richard W. Light, M.D: Diagnostic Approach to Pleural Effusion in Adults Am Fam Physician 2006;73:1211–20.
19. A Trajman, M. Pai, et'al: Novel tests for diagnosing tuberculous pleural effusion: what

works and what does not?
20. Dr VM Katoch: Advances in Molecular Diagnosis of Tuberculosis. MJAFI, Vol. 59, No. 3, 2003.
21. Madhab Lamsal, Narayan Gautum et'al: Diagnostic utility of Adenosine deaminase (ADA) in pleural fluid and serum of Tuberculous and Non-Tuberculous respiratory disease patients. Southeast Asian journal of Tropical Medicine Public health, Volume 38, No.2, March 2007.
22. Thomas Dolezal : Adenosine deaminase, Review of physiological roles 2001.
23. Jhamaria. J P, R K. Jenaw, S. K. Luhada, D. K. Mather, H. C Parithan and S.K Shama. Serum adenosine deaminase in differential diagnosis of pulmonary tuberculosis and common nontubercular respiratory diseases. Ind. J. tub 1988, 35,25.
24. S.K. Teo, LF Chio: Adenosine deaminase in pleural fluid. An enzymatic test for tuberculous pleural effusion. Singapore medical journal.
25. Burgess L.J: Use of Adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax 1995 June 50 (6):672-674.
26. Nune A.Andreasyn, Hripsime L. Hairapetian et'al: Activity of Adenosine deaminase and its isoforms in pleural fluid in tuberculous pleuritis.
27. Carl A. Burtis, Edward R. Ashwood: Tietz textbook of clinical chemistry, 3rd edition.
28. Miller, KD, Barnette, R, Light, RW Stability Of Adenosine Deaminase During Transportation. Chest 2004;126,1933-1937
29. Guisti, G, Galanti, B Adenosine deaminase. Bergmeyer, HU eds. Methods of enzymatic analysis 1974,1092-1096 Academic Press. New York, NY
30. Kaplan, A The determination of urea, ammonia, and urease. Methods Biochem Anal 1969;17,311-324
31. Piras, MA, Gakis, C, Badroni, M, et al Adenosine deaminase activity in pleural effusion: an aid to differential diagnosis. BMJ 1978;4,1751-1752.
32. Ocana, I, Vazquez, JM, Sequra, RM, et al Adenosine deaminase in pleural fluids: test for diagnosis of tuberculous pleural effusion. Chest 1983;84,51-53.
33. Valdes, L, San Jose, E, Alvarez, D, et al Diagnosis of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme, and interferon-γ. Chest 1993;103,458-465.
34. De Oliveira, HG, Rossatto, ER, Prolla, JC Pleural fluid adenosine deaminase and lymphocyte proportion: clinical usefulness in the diagnosis of tuberculosis. Cytopathology 1994;5,27-32.
35. Burger, LJ, Maritz, FJ, Le Roux, I, et al Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax 1995;50,672-674.
36. Valdes, L, San Jose, E, Alvarez, D, et al Adenosine deaminase (ADA) isoenzyme analysis in pleural effusions: diagnostic role and relevance to the origin of increased ADA in tuberculous pleurisy. Eur Respir J 1996;9,747-751.
37. Villena, V, Navarro-Gonzalvez, JA, Garcia-Benayas, C, et al Rapid automated determination of adenosine deaminase and lysozyme for differentiating tuberculous and nontuberculous pleural effusions. Clin Chem 1996;42,218-221.
38. Villegas, MV, Labrada, LA, Saravia, NG Evaluation of polymerase chain reaction, adenosine deaminase, and interferon-γ in pleural fluid for the differential diagnosis of pleural tuberculosis. Chest 2000;118,1355-1364.
39. Perez-Rodriguez, E, Perez Walton, IJ, Sanchez Hernandez, JJ, et al ADA1/ADAp ratio in pleural tuberculosis: an excellent diagnostic parameter in pleural fluid. Respir Med 1999;9,816-821.
40. Sharma, SK, Suresh, V, Mohan, A, et al A prospective study of sensitivity and specificity of adenosine deaminase estimation in the diagnosis of tuberculosis pleural effusion. Indian J Chest Dis Allied Sci 2001;43,149-155.
41. Reechaipichitkul, W, Kawamatawong, T, Teerajetgul, Y, et al Diagnostic role of pleural fluid adenosine deaminase in tuberculous pleural effusion. Southeast Asian J Trop Med Public Health 2001;32,383-389.
42. Lima, DM, Colares, JK, da Fonseca, BA Combined use of the polymerase chain reaction and detection of adenosine deaminase activity on pleural fluid improves the rate of diagnosis of pleural tuberculosis. Chest 2003;124,909-914.
43. Tahhan, M, Ugurman, F, Gozu, A, et al Tumour necrosis factor-α in comparison to adenosine deaminase in tuberculous pleuritis. Respiration 2003;70,270-274.
44. Bernard Rosner (2000), Fundamentals of Biostatistics, 5th Edition, Duxbury, page 80-240.
45. M. Venkataswamy Reddy (2002), Statistics for Mental Health Care Research, NIMHANS publication, INDIA, page 108-144.
46. Sunder Rao P S S , Richard J(2006) : An Introduction to Biostatistics, A manual for students in health sciences , New Delhi: Prentice hall of India. 86-160.
47. Gupta DK, Suri JC et'al: Efficiency of ADA in diagnosis of pleural effusions. Ind J chest Dis 1990;32(4):205-208.
48. Burgess L.J Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax 1995 June:50(6): 672-674.