

# Evaluation of ADA Activity in Pleural Fluid for Tuberculous Pleuritis

Talreja Khushbu<sup>1</sup>, Gupta Mohan Lal<sup>2</sup>

## ABSTRACT

**Introduction:** Tuberculous pleuritis occurs due to delayed hypersensitivity reaction with paucity of bacillary load in the fluid. Lymphocytic exudate alone is not able to confirm the diagnosis of tuberculosis. ADA has been found to be useful parameter to conclude the tubercular etiology.

**Material and Methods:** 250 patients suspected of tuberculous pleuritis were subjected to cytological and ADA evaluation. Tuberculosis was confirmed by ZN stain, AFB culture and other relevant investigations.

**Result:** Most of cases (60.8%) were in age group of 21 to 60 years. 73.3% patients were male and 26.7 % female. Lymphocytic exudate was seen in 68 patients (56.6 % cases). Malignant cells were found in 3.33 % patients. Tubercular diagnosis was confirmed by ZN stain, AFB culture and clinical features in 32 patients (28.6 %). Out of 32 patient 30 patients was ADA positive. 93.75% patients could be diagnosed by lymphocytic exudates and ADA value. Sensitivity of ADA was 93.75 %. Specificity was 93.18 %. Positive Predictive Value calculated was 83.33 % and Negative Predictive Value was 97.61 %.

**Conclusion:** ADA estimation with cytology increases the sensitivity, specificity and predictive value of ADA in the diagnosis of tuberculous pleuritis. Therefore ADA should be included as routine investigation for pleural fluid analysis.

**Keywords:** ADA, pleural fluid, predictive value, Sensitivity, specificity

## INTRODUCTION

Tuberculosis is leading cause of death from infectious disease worldwide.<sup>1</sup> Pleural tuberculosis is common manifestation of extra pulmonary TB, with or without pulmonary TB. Tuberculous pleurisy is present in around 4% of all TB cases.<sup>2</sup> Tuberculous Pleural effusion is diagnosed by demonstration of tubercular bacilli in pleural fluid or granuloma in pleural biopsy specimen.<sup>3</sup> Since pleural biopsy is more difficult than pleural aspiration, various parameters have been developed and evaluated as an alternative to pleural biopsy.<sup>4</sup> ADA is the most cost effective pleural tuberculosis marker and is routinely employed as a screening tool. The combination of ADA and pleural fluid lymphocyte proportion has come to be recognized as an excellent approach for increasing the specificity of ADA test.<sup>5</sup> Pleural TB occurs due to rupture of sub pleural focus followed by entry of TB Ag which evokes delayed hypersensitivity reaction. The activated macrophages enter the pleural space producing ADA during proliferating process. The increase in ADA in tubercular pleuritis has been studied and found to be higher in tuberculosis than other exudative fluid.<sup>6,7</sup> The pleural fluid of tubercular pleuritis is predominantly lymphocytic but in acute tubercular pleuritis there may be increase in neutrophils.<sup>8</sup> Other causes of lymphocytic pleural effusion include malignancies, collagen vascular disease, chylothorax and post coronary artery bypass graft (CABG).<sup>9</sup> Pleural effusion ADA levels in non tuberculous lymphocytic

pleural effusion seldom exceed the cut off for tuberculous effusion.<sup>10</sup>

The objective of present study was to find out the significance of ADA activity in pleural fluid to differentiate tuberculous lymphocytic pleural effusion and non tuberculous pleural effusion.

## MATERIAL AND METHODS

The study was done in Geetanjali Medical College and Hospital, Udaipur from Jan to June 2016. Two hundred and fifty cases suspected of tubercular pleural effusion fluid were sent for diagnostic evaluation. Biochemical examination (Protein and Glucose) cytological examination (cell count, cell type, malignant cells) and ADA measurement was done by Cobas by Diazyme kit. Microbiological demonstration of AFB by ZN stain and AFB culture was done by conventional LJ method. After all relevant investigation lymphocytic exudates were segregated with >50 % lymphocytic proportion of all nucleated cells.

ADA level cut off of > 40IU/ L were considered as tuberculous exudates which were confirmed by AFB stain and AFB culture subsequently. ADA level cut off value < 40IU/L were studied for cytological examination for malignant cells and relevant investigation to confirm non tuberculous lesion.

## STATISTICAL ANALYSIS

Data were expressed as mean±SD. The Student *t* test was used for the comparison and the Mann-Whitney test was used if the distributions were not normal. The chi-squared analysis was used for comparison of proportions. The results of the diagnostic tests were expressed as sensitivity, specificity, predictive values (positive and negative) and accuracy, with 95% confidence intervals (95% CI).

## RESULT

In our study most of the cases (60.8%) were in the age group of 10 to 60 years. 73.3% patients were male and 26.7% were female (Table 1). Cytological examination revealed lymphocytic exudates in (68) 56.6% cases (Table 2). Malignant cells were found in 3.33% patients. In our study ZN stain was positive in 8.33% (10 patients) while AFB culture was positive in 16.6% patients (20 patients). On the basis of clinical feature, X-ray, cytology, 12 cases were considered to be tubercular by excluding other etiologies. Total numbers of tubercular cases were 32 (26.6% cases). Out of 32 tubercular patients 30 patients

<sup>1</sup>Junior Resident, <sup>2</sup>Associate Professor, Department of Pathology, Geetanjali Medical college and Hospital, Udaipur, Rajasthan, India

**Corresponding author:** Dr Khushbu Talreja, 63 B, Shakti Nagar, Opposite Community Hall, Udaipur, Rajasthan (313001), India

**How to cite this article:** Talreja Khushbu, Gupta Mohan Lal. Evaluation of ADA activity in pleural fluid for tuberculous pleuritis. International Journal of Contemporary Medical Research 2016;3(10):3083-3085.

showed ADA > 40 IU/L ( 93.75%) and 2 patients (6.25%) were negative for ADA. 68 patients (56.6%) showed lymphocytic exudates (>50% lymphocytic) and 52 patients (43.4%) patients showed neutrophilic exudates or transudes. 36 patients (30.0%) were positive for ADA, while 84 patients (70%) were negative for ADA. 32 patients (47%) for lymphocytic exudates were positive for ADA and 36(53%) were negative for ADA (Table 2). Majority of patients of non lymphocytic exudates 48 (92.3%) were negative for ADA. Correlating tuberculosis confirmed patients and ADA value; it was found that 93.75% patients could be diagnosed by lymphocytic exudates and ADA value (Table 3). Majority of tubercular patients (88.8%) showed lymphocytic exudates.

Sensitivity of ADA =  $30 / 30 + 2 \times 100 = 93.75\%$

Specificity of ADA =  $82 / 82 + 6 \times 100 = 93.18\%$

Positive Predictive Value =  $30 / 36 \times 100 = 83.33\%$

Negative Predictive Value =  $82 / 84 \times 100 = 97.61\%$

**DISCUSSION**

The diagnosis of tubercular pleural effusion is challenge in clinical practice. The commonest cause of pleural effusion has been found to be tuberculosis (60.2%) followed by malignancy (29.1%) and pneumonitis (7.7%).<sup>11-14</sup> The age group and sex

Age Groups (years)	Number of patients	Male	Female	Percentage
0-10	2	2	0	1.6%
11-20	5	3	2	4.16%
21-30	18	12	6	15.0%
31-40	22	16	6	18.33%
41-50	10	7	3	8.33%
51-60	23	18	5	19.16%
61-70	17	13	4	14.16%
>70	23	17	6	19.16%
Total	120	88	32	
Percentage		73.3%	26.7%	

**Table-1:** Age and Sex distribution of patients of pleural effusion

	ADA > 40 IU/L	ADA < 40 IU/L	Total
>50% Lymphocytic Exudate	32	36	68
<50% Lymphocytic Exudate	4	48	52
Total	36	84	120

**Table-2:** Distribution of ADA and Lymphocytic Exudate

	ADA Positive	ADA Negative	Total
AFB Culture Positive	30	2	32
AFB Culture Negative	2	34	36
Total	32	36	68

**Table-3:** AFB culture and ADA levels among the study subjects

	Tubercular Pleuritis	Non Tubercular	Total	Predictive value
ADA Positive	30	6	36	(Positive) 83.33%
ADA Negative	2	82	84	(Negative) 97.61%
Total	32	88	120	
Sensitivity/Specificity	93.75% (Sensitivity)	93.18% (Specificity)		

**Table-4:** Evaluation of ADA test for Tubercular and Non tubercular Pleuritis

incidence in our study is similar to observed in other studies.<sup>15,16</sup> Although lymphocytic predominance is usually seen in tubercular pleural effusion but many fluid may be malignant as well. So there is need to differentiate among various causes of pleural effusion. It has been observed that determination of ADA is more sensitive than histopathological examination of pleural tissue.<sup>17</sup> The combination of effusion and sputum culture may give a good diagnostic clue but tuberculous pleurisy is a hypersensitivity reaction, therefore an alternate approach to diagnose tubercular pleurisy is ADA determination. The Mc Neuar test demonstrated that from a statistical view point ADA determination was more sensitive than pleural histopathological examination.<sup>18</sup> Cut off value of ADA varies in various studies from 30 IU/L to 40 IU/L. We have used cut off 40 IU/L to increase specificity, because in malignancy cut off may rise up to 67 IU/L.<sup>19,20</sup>

Overall sensitivity of ADA in our study in the diagnosis of tuberculosis is 93.75% (Table 4). It is close to the value reported in previous studies performed.<sup>21,22</sup> Using cut off range from 30 to 55.8 IU/L. Chen et al<sup>22</sup> has reported sensitivity ranging from 76.5-94.3% and specificity ranging from 86.2-95.7%. Specificity in our study of ADA for the diagnosis of tuberculosis is 93.18% very close to other study.<sup>21,22</sup> This study emphasizes the usefulness of ADA measurement in lymphocytic effusion. Depending only on lymphocytic exudates may lead to false positive diagnosis, thus with high ADA level the predictive value is increased. In our study negative predictive value of ADA is high (Table 4). Therefore the measurement of pleural fluid ADA is an excellent tool to rule out a tubercular etiology of lymphocytic exudates so that in the patient of pleural effusion with unknown diagnosis with negative ADA level, further workup can be done for diagnosis.

**CONCLUSION**

ADA estimation with cytology increases the sensitivity and specificity and predictive value for the diagnosis of tuberculosis. Although varying cut off levels have been used in various studies. A cut off 40 IU/L is considered to be adequate to exclude tuberculosis. All case of lymphocytic pleural effusion should be screened for ADA to exclude tuberculosis. Further studies may be required to incorporate use of ADA isoenzyme for the diagnosis of tuberculosis.

**REFERENCES**

1. Global Tuberculosis Control: Surveillance, Planning, Financing. WHO Report 2003, Geneva, Switzerland, WHO/CDS/TB/2003.316, www.who.int/gtb/publications/globrep
2. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian J Med Res. 2004;120:316-53.
3. Light RW. Tuberculous pleural effusions. In: Light RW, editor. Pleural Diseases. 5th Edition. Baltimore: Lippincott, Williams and Wilkins. 2001:182-195.

4. Krenke R, Korczyński P Use of pleural fluid levels of adenosine deaminase and interferon gamma in the diagnosis of tuberculous pleuritis. *Curr Opin Pulm Med.* 2010;16:367-75.
5. Burgess LJ, Maritz FJ, Le Roux I, Taljaard JJ. Combined use of pleural adenosine deaminase with lymphocyte/neutrophil ratio. Increased specificity for the diagnosis of tuberculous pleuritis. *Chest* 109:414-419. *Chest.* 1996; 109:414-9.
6. Shibagaki T, Hasegawa Y, Sarito H, Yamori S, Shimokata K. Adenosine deaminase isoenzymes in tuberculous pleural effusion. *J Lab Clin Med.* 1996;127:348-52.
7. Pérez Walton IJ, Sanchez Hernández JJ, Pallarés E, Rubi J, Jimenez Castro D, Diaz Nuevo G. ADA1/ADAp ratio in pleural tuberculosis: an excellent diagnostic parameter in pleural fluid. *Respir Med.* 1999;93:816-21.
8. V B Antony, S A Sahn, A C Antony, and J E Repine. Bacillus Calmette-Guérin-stimulated neutrophils release chemotaxins for monocytes in rabbit pleural spaces and in vitro. *J Clin Invest.* 1985;76:1514–1521.
9. Blake J, Berman P. Useful of adenosine deaminase determination for the diagnosis of tuberculosis. *S Afr Med.* 1982;62:782-786.29.
10. Lee YC, Rogers JT, Rodriguez RM, Miller KD, Light RW. Adenosine deaminase levels in nontuberculous lymphocytic pleural effusion *Chest.* 2001;120:356-61.
11. L. Valdés, D. Alvarez, E. San José, J. R. Juanatey, A. Pose, J. M. Valle, M. Salgueiro, and J. R. Suárez. Value of adenosine deaminase in the diagnosis of tuberculous pleural effusions in young patients in a region of high prevalence of tuberculosis. *Thorax.* 1995;50:600–603.
12. Bañales JL, Pineda PR, Fitzgerald JM, Rubio H, Selman M, Salazar-Lezama M. Adenosine deaminase in the diagnosis of tuberculous pleural effusions. A report of 218 patients and review of the literature. *Chest.* 1991;99:355-7.
13. Mostafa MG, Khatun A, Islam K, Zaman AMI. Diagnostic significance of pleural fluid adenosine deaminase activity in tuberculous pleurisy *Chest and Heart Bulletin.* 1996;10:47-52.
14. Haque ME, Ahmad MM, Hiron MM. Aetiological diagnosis of pleural effusion. *Chest and Heart Journal.* 2000;24:1-4.
15. Lima DM, Colares JK, da Fonseca BAL. 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;124:909-14.
16. Morrys Casagrande Kaiseemann; Afrânio Lineu Kritski; Maria de Fátima C Pereira; Anete Trajman. Pleural fluid adenosine deaminase detection for the diagnosis of pleural tuberculosis. *J bras pneumol.* 2004;30:549-546.
17. S.K. Verma, A.L. Dubey, P.A. Singh, S.L. Tewerson, Davashish Sharma. Adenosine Deaminase (ADA) Level in Tubercular Pleural Effusion. *Lung India.* 2008; 25:109–110.
18. Blake J, Berman P. The use of adenosine deaminase assays in the diagnosis of tuberculosis. *S Afr Med J.* 1982;62:19-21.
19. Valdés L, San José E, Alvarez D, Sarandeses A, Pose A, Chomón B. Value of adenosine deaminase in the diagnosis of tuberculosis of pleural effusion in young patients in a region with high prevalence of tuberculosis. *Thorax.* 1995;50:600–603.
20. Porcel JM, Esquerda A, Bielsa S. Diagnostic performance of adenosine deaminase activity in pleural fluid: a single-center experience with over 2100 consecutive patients. *Eur J Intern Med.* 2010;21:419-23.
21. Diacon HA, Van de wal, Wyser C, Smedema JP et al. Diagnostic tools in tuberculous pleurisy: a direct comparative study. *Eur Respir J.* 2003;22:589-591.
22. Chen ML, Yu WC, Lam CW, Au KM, Kong FY, Chan AY. Diagnostic value of pleural fluid Adenosine deaminase activity in tuberculous pleuritis. *Clin Chim Acta.* 2004;341:101-7.

**Source of Support:** Nil; **Conflict of Interest:** None

**Submitted:** 16-09-2016; **Published online:** 30-10-2016