Original research article

Assessment of Diagnostic Role of Adenosine Deaminase (ADA) Levels in Cases of Pleural Effusions

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Abstract

Background: The most economical pleural fluid marker is adenosine deaminase (ADA), which is frequently employed in high-prevalence situations whereas its usefulness is questioned in low-prevalence ones. It is well recognized that the lymphocyte percentage (LP) raises the specificity of ADA for this diagnosis. We evaluate ADA's diagnostic utility for tubercular pleural effusions in this study.

Methods: Cases of pleural effusion presented to our hospital were included in the study. The pleural fluid data (total and differential cell count, protein, lactate dehydrogenase-LDH, pH, glucose, ADA, cytology, aerobic and anaerobic culture, Lowenstein-Jensen, and MGIT-Bactec culture) was noted. we also kept track of demographic and clinical information (body temperature on admission, presence of cough, chest pain, and/or dyspnea), as well as serum glucose, protein, and LDH. In pleural biopsy specimens, the histopathological and microbiological results, the final diagnosis, the first and last days of treatment, and the combination of anti-tuberculosis medications administered were all documented.

Results: The difference between the mean adenosine deaminase values in the tuberculous pleural effusion group and the non-tuberculous group is statistically very significant (p 0.001). In n=2 of n=22 instances (two patients had bronchogenic carcinoma, and one patient each had lymphoma), the adenosine deaminase level of the non-tuberculous group exceeded the diagnostic cut-off (40 U/L) for TB. N=2 tuberculous individuals had adenosine deaminase levels below 40U/L.

Conclusion: The values of ADA found in this study is adequate to quickly (within 1-2 days) diagnose the majority of TPE cases. Additionally, it is the least expensive, comparable accurate, and least intrusive diagnostic treatment. The process of collecting samples for an ADA test is simple and does not require any particular planning.

Keywords: Adenosine deaminase (ADA), Tubercular Pleural Effusion (TPE), Extra Pulmonary Tuberculosis (EPTB)

Introduction

Tuberculosis (TB) is a disease from time immemorial. It is the main infectious disease cause of avoidable morbidity and mortality around the globe. Extra-pulmonary tuberculosis is a prevalent clinical issue, especially given the development of immunosuppressants in the current age of HIV infection. [1] Pulmonary tuberculosis is the most common manifestation of M. tuberculosis infection. Around 5% of TB patients develop pleural effusion. [2] 38% of all TB patients, according to Tanzanian research, had pleural involvement. [3] In India, EPTB

accounts for 15-20% of all cases of tuberculosis in immunocompetent individuals; 20% of these patients have a tubercular pleural effusion. [1]

Because typical non-invasive diagnostic methods have limited sensitivity and/or specificity, it can be challenging to make a definitive diagnosis of TPE. [4] Finding M. tuberculosis in pleural fluid and tissue is the gold standard for pleural TB diagnosis. Nevertheless, in practice, this identification is challenging since mycobacterium grows slowly in culture and has a poor bacillus identification rate (less than 30% in pleural fluid and around 50% in pleural tissue) (about 60 days). [5] Culture on Löwenstein-Jensen (L-J) medium has a positivity rate of little more than 40%, and direct investigation of pleural fluid for the identification of acid-fast bacilli (AFB) by Ziehl-Neelsen (Z-N) or a comparable approach is positive in fewer than 5% of cases. Just 10% of pleural fluid smears were reported to be positive by Z-N staining in another study. [6] Around 25% of pleural fluid cultures tested positive for mycobacteria. [7] Nevertheless, culture takes 4-6 weeks to produce M. tuberculosis growth, even with the radiometric mycobacterium culture system (BACTEC), which needs 18 days. In addition, cultures need between 10 and 100 viable bacilli. [2] On the other hand, pleural biopsy reveals granulomatous pleuritis in 50–80% of TPE patients and the diagnosis may be made in around 90% of cases when biopsy material is cultured and histologically examined. [7] Pleural biopsy histopathology is superior to all conventional tests and is the specimen of choice for the diagnosis, but the diagnostic process is challenging due to the potential for thoracentesis complications, the cost of patient care, the need for a doctor who is qualified to perform the procedure and has access to the necessary facilities, as well as the need for a pathological laboratory and a skilled pathologist who can interpret the results. [8] It doesn't always ensure that a representative sample will be taken. Moreover, it is an invasive blind treatment.

The purine salvage pathway enzyme adenosine deaminase (ADA) catalyzes the hydrolytic and irreversible deamination of deoxyadenosine and adenosine into deoxyinosine and inosine, respectively, with the release of ammonia. [9] While widespread in distribution, enzyme activity is present in all cells, with lymphocytes—primarily active T lymphocytes and monocytes—having the highest activity. [4] ADA has a role in the growth and maturation of lymphocytes, particularly T lymphocytes. [10] Increased ADA activity in TB patients may be a sign of disease-related T lymphocyte activation and cellular immune response. [11]

ADA levels in T lymphocytes are 10–12 times greater than in B lymphocytes. ADA activity changes according to the cell's maturity and proliferative state. [12] Because ADA measurement is a less costly, minimally invasive, quick, and easily accessible test, it has gained favor as a diagnostic test in the high-incidence area of TPE. The level of ADA is raised in TPE.

Material and methods

This cross-sectional study was conducted in the Departments of Pulmonology and Department of Microbiology and Immunology, Prathima Institute of Medical Sciences, Naganoor, Karimnagar. Institutional Ethical approval was obtained for the study. Written

consent was obtained for the study after explaining the nature of the study in the local language. In addition to pleural fluid data (total and differential cell count, protein, lactate dehydrogenase-LDH, pH, glucose, ADA, cytology, aerobic and anaerobic culture, Lowenstein-Jensen and MGIT-Bactec culture), we also kept track of demographic and clinical information (body temperature on admission, presence of cough, chest pain, and/or dyspnea), as well as serum glucose, protein, and LDH. In pleural biopsy specimens, the histopathological and microbiological results, the final diagnosis, the first and last days of treatment, and the combination of anti-tuberculosis medications administered were all documented. ADA levels in the pleural fluid were assessed using an automated UV kinetic assay (Roche Diagnostics, Mannheim, Germany). Where feasible, 200 cells were counted when doing differential cell counts in pleural fluid following Wright's staining on white blood cells in a Thoma chamber. By dividing the total number of white blood cells by the number of lymphocytes, the LP was computed.

***Statistical analysis*:**

SPSS 18.0 statistical software was used for the statistical analysis. The x^2 test was used to assess categorical variables, while the student's t-test was used to compare continuous variables. Moreover, Kruskal-Wallis one-way analysis of variance was employed to compare group means whenever parametric tests were not an option.

Results

A total of n=30 cases were included in the study out of which n=22(73.33%) were tuberculous pleural effusion and n=8(26.67%) were non-tubercular cases. Among the non-tubercular cases, 20% were malignancy and the rest cases were due to nephrotic syndrome and congestive cardiac failure details depicted in Table 1.

Table 1: Diagnosis of cases with pleural effusion included in the study

Diagnosis	*Frequency*	*Percentage*
Tuberculous pleural effusion	22	73.33
Nontuberculous pleural effusion	8	26.67
Malignancy	6	20.00
Nephrotic syndrome	1	03.33
Congestive cardiac failure	1	03.33
Total	30	100.0

Using 40 U/L as the cut-off value for ADA level, the results were positive in n=20 out of n=22 tuberculosis patients the sensitivity was 93% however, among the n=8 non-tuberculous cases n=2(25%) were positive and n=6(75%) were negative, and this decreased the test specificity to 90% depicted in table 2.

Table 2: ADA levels in pleural fluid of study group

ADA level in pleural fluid	*Tuberculous*	*Nontuberculous*	*Total*
Positive	20 (90.90)	2 (25.0)	22 (73.33)

Negative	02 (9.09))	6 (75.0)	08 (02.67)
Total	22 (100.0)	8 (100.0)	30 (100.0)

The difference between the mean adenosine deaminase values in the tuberculous pleural effusion group and the non-tuberculous group is statistically very significant (p 0.001). In n=2 of n=22 instances (two patients had bronchogenic carcinoma, and one patient each had lymphoma), the adenosine deaminase level of the non-tuberculous group exceeded the diagnostic cut-off (40 U/L) for TB. N=2 tuberculous individuals had adenosine deaminase levels below 40U/L. Table III showed the relationship and comparison between the ADA result and the results of the tuberculin test, histology of the pleural tissue, and bacterial analysis of the pleural fluid in instances with tubercular pleural effusion. Out of n=22 cases, n=20 cases were positive for ADA. Among these cases n=3 cases were positive for direct microscopy and n=6 cases were positive for culture n=11 cases were positive for pleural biopsy and n=14 cases were positive for MT. Among the ADA negative cases, 50% were positive for culture and 50% were diagnosed by the response to anti-tuberculous treatment who had a chronic inflammatory lesion on histopathology of pleural biopsy.

Table 3: Showing the ADA results with bacteriological, histopathology, and MT test results in pleural fluid among tuberculous pleural effusion cases.

ADA levels in pleural fluid	*M/E for AFB Positive*	*Culture for M TB positive*	*Biopsy for granuloma Positive*	*Tuberculin test positive*
ADA Positive	3 (15.0)	6 (30.0)	11 (55.0)	13 (65.0)
ADA Negative	0 (00.0)	1 (50.0)	1 (50.0)	0 (00.00)
Total	3 (59.09)	7 (3.18)	12 (54.54)	13 (59.09)

ADA estimation in pleural fluid of the non-tuberculous pleural effusion cases has been depicted in Table 4. Among these cases, n=2 of cases were ADA positive which was due to malignancy, and one case each of nephrotic syndrome and congestive cardiac failure was found to be ADA negative.

Table 4: ADA positive and negative in pleural fluid among non-tubercular effusions

Nontuberculous cases	*Frequency*	*ADA Positive*	*ADA Negative*
Malignant	6	2 (33.33)	4 (66.67)
Nephrotic syndrome	1	0 (00.00)	1 (100.0)
Congestive cardiac failure	1	0 (00.00)	1 (100.0)
Total	8	2 (25.00)	6 (75.00)

Figure 1 shows the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of the ADA assay. The specificity of ADA was found to the lower at the cut-off value of 35.0 U/L however, the sensitivity remains the same. At a higher cutoff value of 40 U/L we found the sensitivity to be 90.91%, (70.84% – 98.88%) at 95% CI. Similarly, the specificity was 75% with (34.91% - 96.81%) at 95% CI. The positive predictive value was 90.91% (90.84 – 98.88%) at a 95% CI negative predictive value of 75% (34.91% - 96.81%). The positive likelihood ratio in this study was 3.64 and the negative likelihood ratio was 0.12.

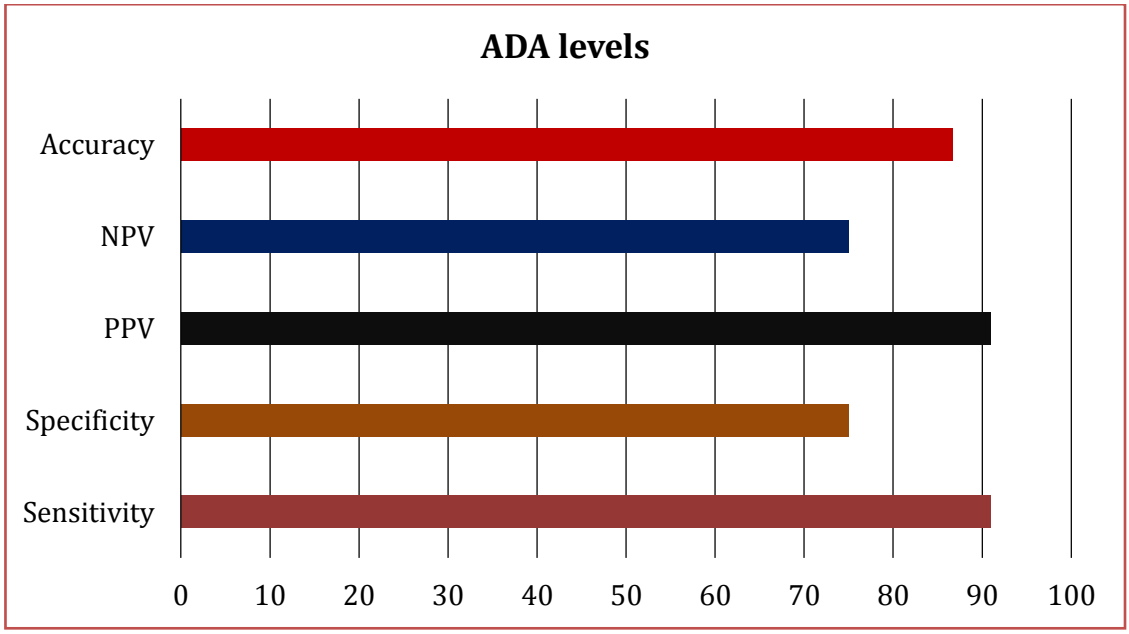

Figure 1: Sensitivity, specificity, Positive predictive value (PPV) Negative Predictive Value (NPV), and accuracy of ADA essay.

Discussion

In this study we found even after significant investigation, tubercular pleural effusion frequently poses a diagnostic challenge. In the current investigation, TB was shown to be the most frequent cause of pleural effusion, followed by cancer (26.67%) and pneumonia (3.3%). These results were to those seen in earlier studies. [13] This result, however, contrasts with those of certain other studies that identified malignancy as the most prevalent reason for pleural effusion. [14] In the current investigation, there were only a few cases of pleural effusion caused by nephrotic syndrome and congestive heart failure. AFB is seldom seen in individuals with tubercular effusions when the microscopic investigation and sputum culture are performed. [15] Mycobacterium tuberculosis culture results from individuals with pleural TB are often regarded as having minimal diagnostic value28. Only 9% (2/22) of the patients in the Berger et al., [16] study, in whom no indication of a parenchymal lesion could be seen on the chest radiograph, had a positive sputum culture. In their study Yew et al., [17] reported a 12.2% sputum microscopy positive rate for AFB. In a Brazilian study, 16.7% of patients with sputum cultures for Mycobacterium TB were found to be positive. [18] In 50% to 80% of patients with tuberculous pleural effusion, a biopsy of the parietal pleura reveals a characteristic epitheloid granuloma, which is widely employed as a diagnostic criterion for pleural tuberculosis. In this study, 55% of the cases with tuberculous pleural effusion were correctly diagnosed. Prabhudesai et al., [19] also reported a low positivity rate in the histological analysis of pleural biopsy tissue, finding positive biopsy in only 38.4% of cases. The lack of repeat pleural biopsy in those studies may account for the low rate of positive. Furthermore, because epithelioid granulomas are not equally distributed throughout the pleural tissue, a biopsy needs more skill and is vulnerable to sampling error. [18] In the current study, 65% of patients had positive tuberculin test results. Similar to this, other studies reported greater tuberculin test positive results. [18, 19] Not all ADA-positive

instances were tuberculin-test positive, but all tuberculin-test-positive cases were ADA-positive. A negative PPD test does not rule out the diagnosis, and the cutaneous reaction to PPD may be negative in one-third of patients. [20] The prevalent incidence of viral illness and poor nutritional state may be contributing factors that inhibit the tuberculin response. Ages (newborn, elderly); vaccinations (polio, mumps, measles); medications (corticosteroids, anti-TB); bacterial (typhoid), viral (measles); severe illnesses; stress; are some factors that may impact tuberculin sensitivity. However, the presence of adherent suppressor cells in the peripheral blood but not in the pleural fluid of tuberculous patients has been suggested as an explanation for the occurrence of false negative tuberculin skin tests in tuberculous pleural effusion. [21] Since Piras et al.'s [22] initial suggestion, several investigations have supported the usefulness of ADA for the diagnosis of tuberculous pleural effusion. Measurements of ADA activity in this study also contributed to accurate TPE diagnosis. In other contexts where ADA has been assessed in the differential diagnosis of TPE, the cutoff value used in this investigation, 40U/L, is comparable to that determined elsewhere. [23] Since the literature provides a wide range of these levels, ranging from 30 to 50 U/L31, the highest cut-off values (70 U/L) have been documented by Banales et al., [21] the ADA cut off value suggestive of TB is up for controversy. It is challenging to establish a standard cut-off point for ADA activity. The ROC curve indicates that the 40U/L ADA level in the pleural fluid of tuberculous patients is the best cutoff, with extremely high sensitivity (94%) and specificity (88%). The sensitivity and specificity of high levels of ADA in TPE vary from 81 to 100% and 83 to 100%, respectively, according to almost all study workers. [24] In the current study, we found the mean ADA levels of TPE was 72.5 ± 12.5 U/L while in the non-tuberculous cases, the value was 28.5 ± 10.5 U/L the differences were found to be significant ($p < 0.05$) other studies have also reported such findings [18, 19]. This study showed a sensitivity of 90.91% specificity of 75%, PPV of 90.91%, and NPV of 75% The positive likelihood ratio in this study was 3.64, and the negative likelihood ratio was 0.12. the results of this study showed that greater severity of the disease is likely to result in greater sensitivity. The lower negative likelihood ratio indicated that the patients who were negative for TPE had fewer chances of showing positive ADA values.

Conclusion

The present study supports the case for using ADA measurement as a diagnostic tool in the treatment of pleural effusion. The values of ADA found in this study are adequate to quickly (within 1-2 days) diagnose the majority of TPE cases. Additionally, it is the least expensive, comparable accurate, and least intrusive diagnostic treatment. The process of collecting samples for an ADA test is simple and does not require any particular planning. Errors in the laboratory are also less likely. Pleural fluid culture is another crucial diagnostic procedure, but it delays diagnosis since it takes at least six weeks to provide findings. Pleural tissue biopsy and histopathology are having a high probability of complications as compared to ADA measurement.

References

1. Sharma SK and Mohan A. Extrapulmonary tuberculosis. India J Med Res 2004; 120: 316-353.

2. Gopi A, Madhavan SM, Sharma SK and Sahn SA. Diagnosis and treatment of tuberculous pleural effusion in 2006. Chest 2007; 131: 880-889.
3. Mlika-Cabanne N, Brauner M, Muguisi F. Radiographic abnormalities in tuberculosis and risk of coexisting human immunodeficiency virus infection: results from Dares Salaam, Tanzania, and scoring system. Am Rev Respir Crit Care Med 1995; 152: 786-793.
4. Laniado-Laborin R. Adenosine deaminase in the diagnosis of tuberculous pleural effusion: Is it really an ideal test? A word of caution. Chest 2005; 127: 417-418.
5. Antonangelo L, Vargas FS, Seiscento M, Bombarda S, Teixera L, de Sales RKB. Clinical and laboratory parameters in the differential diagnosis of pleural effusion secondary to tuberculosis or cancer. Clinics 2007; 5: 85-90.
6. Khatami K. Pleural tuberculosis. Shiraz E-Medical Journal 2001; 3: 1-15.
7. Laniado-Laborin R. Adenosine deaminase in the diagnosis of tuberculous pleural effusion: Is it really an ideal test? A word of caution. Chest 2005; 127: 417-418.
8. Conde MB, Loivos CA, Rezende VM, Soares SLM, Mello FCQ, Reingold AL, Daley CL Kritski AL. The yield of sputum induction in the diagnosis of pleural tuberculosis. Am J Respir Crit Care Med 2003; 167: 723-725.
9. Ungerer JPJ, Oosthuizen Bissbort SH, Vermaak WJH. Serum adenosine deaminase: Isoenzymes and diagnostic application. Clin Chem 1992; 38: 1322-6.
10. Canbolat O, Ulusdoyuran S, Ozgun G, Ceyhan I, Gumuslu F, Akbay A. The comparison of adenosine deaminase activity value with polymerase chain reaction results in patients with tuberculosis. Journal of Clinical Laboratory Analysis 1999; 13: 209-212.
11. Riquelme A, Calvo M, Saleh F, Valderrama S, Pattiollo A, Arellano M, Arrese M, Soja A. Viviani P, Letelier LM, Value of adenosine deaminase (ADA) in ascitic fluid for the diagnosis of tuberculous peritonitis. J Clin Gastroenterol 2006; 8: 705-710.
12. Carstens ME, Burgess LJ, Maritz FJ, Taljaard JJF. Isoenxymes of adenosine deaminase in pleural effusion: a diagnostic tool? Int J Tuberc Lung Dis 1998; 10: 831-835.
13. Lima DM, Colares JKB and de 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: 909-914.
14. Reechaipichitkul W, Kawamatawong T, Teerajetgul Y, Patjanasoontorn B. Diagnostic role of pleural fluid adenosine deaminase in tuberculous pleural effusion. Southeast Asian J Trop Med Public Health 2001; 32: 383-389.
15. Kumar D, Watson JM. Charlett A. Tuberculosis in England and Wales in 1993: results of a national survey. Thorax 1997; 52: 1060-1067.
16. Berger HW and Mejia E. Tuberculous pleurisy. Chest 1973; 63: 88-93.
17. Yew Ws, Chan CY, Kwan SY, Cheung SW and GL French. Diagnosis of tuberculous pleural effusion by the detection of tuberculo stearic acid in pleural aspirates. Chest 1991; 100: 1261-1263.
18. Valdes L, Alvarez D, Jose ES, Juanatey JRG, Pose A, Valle JM, Salgueiro M, Suarez JRR. 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.
19. Prabhudesai PP, Mahashur AA, Mehta N, Ajay R. Exudative pleural effusions in patients over forty years age- an analysis of seventy-six patients. J Postgrad Med 1993; 39: 190.
20. Rossi GA, Balbi B, Manca F. Tuberculous pleural effusions. Evidence for selective presence of PPD-specific T-lymphocytes at the site of inflammation in the early phase of the infection. Am Rev Respir Dis 1987; 136: 575-9.
21. Banales JL, Pineda PR, Fitzgerald JM, Rubio H, Selman M, and 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: 355-357.

22. Burgess LJ, Maritz FJ, Roux IL, Taljaard JJF. Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax 1995; 50: 672-674.
23. Castro JD, Nuevo GD, Perez-Rodriguez E, Light RW. Diagnostic value of adenosine deaminase in nontuberculous lymphocytic pleural effusions. Eur Respir J 2003; 21: 220-224.
24. Gorguner M, Cerci M and Gorguner I. Determination of adenosine deaminase activity and its isoenzymes for diagnosis of pleural effusions. Respirology 2000; 5: 321-324.