

Original Research Article

Diagnostic value of pleural fluid adenosine deaminase level in patients of tubercular pleural effusion

Amit Goyal*, Amanpreet Kaur, N. C. Kajal

Department of Chest and TB, GMC Amritsar, Punjab, India

Received: 27 October 2020

Revised: 03 December 2020

Accepted: 04 December 2020

*Correspondence:

Dr. Amit Goyal,

E-mail: goyal5757@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: The diagnosis of tuberculosis (TB) continues to be a challenge in clinical practice. Traditional diagnostic methods are very useful but don't provide enough sensitivity and specificity. Adenosine deaminase (ADA) has been developed and widely used for the diagnosis of TB. This article reviews the characteristics, metabolism and clinical uses of ADA for the diagnosis of TB in clinical practices.

Methods: This study was carried out in the department of chest and TB, GMC, Amritsar, Punjab, India. In this study total 50 who attended outpatient department (OPD) and indoor patients of adult age and either sex were taken. Patients with pleural effusion as determined by clinical and or radiological means, thoracentesis on who yield a minimum amount of fluid enough to carry out routine test were included in the study.

Results: Most of the patients were between the age group of 15-34 years, of those 72% were males and 28% female. Most of the patients of tuberculous effusion were from younger age group between 25-34 years. Most common symptom was breathlessness (90%) followed by fever (75%), cough (75%) then chest pain (72%). The diagnosis of TB was made in 40 patients (80%), while in 10 patients (20%) TB were excluded (malignancy and miscellaneous disease) based on history, clinical and laboratory findings. Sensitivity of ADA in diagnosing TB pleural effusion was 95% and specificity 80%.

Conclusions: ADA level of the pleural fluid is a non-invasive test. Pleural fluid ADA is useful in early diagnosing of tuberculosis pleural effusion. So the analysis of ADA levels can be done simply, quickly and cheaply.

Keywords: Adenosine deaminase, Pleural effusion, Tuberculosis

INTRODUCTION

Tuberculosis (TB) is a bacterial disease caused by the tubercle bacilli which includes Mycobacterium tuberculosis. TB remains one among the main health problems. Globally, the simplest estimate is that 10.0 million people (range, 9.0–11.1 million) developed TB disease in 2017: 5.8 million men, 3.2 million women and 1.0 million children. There were cases altogether countries and age groups, but overall 90% were adults (aged ≥ 15 years), 9% were people living with human-immunodeficiency virus (HIV) (72% in Africa) and two thirds were in eight countries: India (27%), China (9%),

Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). These and 22 other countries in the World Health Organization's (WHO) list of 30 high TB burden countries accounted for 87% of the world's cases.¹ The lung tissue is involved in pulmonary TB and therefore the tissue aside from lung tissue like pleural fluid, ascitic fluid etc. is involved in extrapulmonary TB (EPTB). Prompt diagnosis is important for effective TB control programme. Although we've many methods for the diagnosis of pulmonary TB, for instance Ziehl-Neelsen (ZN) staining, polymerase chain reaction (PCR) and culture, these methods don't provide enough sensitivity and specificity.

The sensitivities of ZN staining and culture are 10-40% and 8-49% respectively within the diagnosis of TB infection.² The definitive diagnosis of EPTB depends on the demonstration of tubercle bacillus within the specimens like pleural fluid, ascitic fluid, pericardial fluid, spinal fluid (CSF) or pleural biopsy specimen, and can also be established with reasonable certainty by demonstration of granuloma in the parietal pleura, peritoneum, pericardium etc.³

Although mycobacterial culture is the gold standard in diagnosing TB, *Mycobacterium* spp. grows very slowly and it can take up to six weeks to isolate it in culture. Determination of susceptibility to drugs can add another three to 6 weeks to the method. Meanwhile the disease may progress and be transmitted to others when appropriate treatment is delayed. There is a need of a simple, rapid and reliable test which can be easily carried out in the clinical laboratory. Thoracoscopy in diagnosing tubercular serositis offers a near 100% positive diagnostic yield on histology and 76% positive on culture.³

However, historically, since pleural biopsy is more invasive and unsafe than thoracentesis, alternative diagnostic approaches are extensively evaluated. Adenosine deaminase (ADA) has been developed and widely used for the diagnosis of TB thanks to its simplicity, low cost, and quickly available results. Many studies have confirmed the high sensitivity and specificity of ADA (sensitivity 92% and specificity 89%) for early diagnosis of EPTB, like tuberculous pleuritis, pericarditis, ascites and meningitis.⁴

Characteristics, metabolism and assay of ADA

ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. It is also an essential enzyme of the purine catabolic pathway. There are 2 isoforms of ADA, ADA-1 and ADA-2. ADA-1 is found in many tissues including red blood cells. ADA-2 is found only in macrophages and monocytes.⁵ ADA acts in proliferation and differentiation of lymphocyte, especially T lymphocyte. It also acts in maturation of monocytes transforming them to macrophage. ADA may be a significant indicator of active cellular immunity. It increases in biological fluids in the course of infectious disease characterized by micro-organisms infecting the macrophages. For example, deficiency in ADA in humans manifests primarily as severe lymphopenia and immunodeficiency. Furthermore, ADA has been proposed to be a useful surrogate marker for TB because it can be detected in body fluids such as pleural, pericardial and peritoneal fluid. The levels of ADA increase in TB because of the stimulation of T cells by mycobacterial antigens.⁵

Aims and objectives

The goal of study is to evaluate the diagnostic role of pleural fluid ADA levels in patients with tubercular pleurisy.

METHODS

The cross sectional study was carried out on 50 patients suffering from pleural effusion who attended OPD or were admitted government medical college, Amritsar from May 2019 to March 2020 were enrolled. This study will be conducted after approval from the institutional ethics committee and informed written consent of each patient.

Detailed clinical history, physical examination and investigation e.g. acid-fast bacilli (AFB), cytological examination, biochemical examination and, wherever possible, biopsy and histopathological examination, ultrasonography (USG), X-ray chest, electrocardiogram (ECG), echocardiography (ECHO) and other appropriate investigations including pleural fluid ADA.

Exclusion criteria

Diagnosed cases of infectious mononucleosis, typhoid, leprosy, hepatitis, HIV, CA urinary bladder and haematopoietic malignancy.

ADA estimation was done by Galanti and Giusti's method. Pleural fluid ADA ≥ 39 U/l was taken as diagnostic cut off for TB effusion.

The data will be documented, tabulated and analysed by using Chi-square test wherever applicable. All statistical analyses were performed using statistical package for social sciences (SPSS) statistics version 16.0 software.

RESULTS

A total 50 patients were studied who were above the age of 12 year. Male were 36 (72%) and female were 14 (28%). Out of 50 patients tuberculosis was diagnosed in 40 cases (by history+sputum results+pleural fluid results). Pleural fluid ADA level was more than 39 IU/l in cases of tubercular pleural effusion. It ranged 39 to 245.4 IU/l. When 39 IU/l is taken as cut off point, sensitivity and specificity of ADA for TB is 95% and 80%. We found that when more than 90 IU/l was taken as cut of limit of ADA level, it was seen in tuberculosis only and in other pleural effusion cases 4 cases were diagnosed malignant in 1 cases pleural fluid is due to hypoproteinemia and 3 cases due to congestive cardiac failure.

Table 1: Incidence of TB pleural effusion among different age group.

Age group (year)	Male	Female
15-24	3	4
25-34	7	3
35-44	3	3
45-54	3	2
55-64	6	2
>65	3	1
Total (40)	25	15

Table 2: Clinical feature among patients diagnosed with TB pleural effusion.

Sign and symptoms	Variables	Percentage (%)
Prolonged cough	30	75
Cough with sputum	5	12.5
Haemoptysis	1	2.5
Breathlessness	36	90
Chest pain	28	70
Fever	30	75
Weight loss	23	57.5

Table 3: Pleural fluid ADA levels in various pleural effusions.

ADA (U/l) level	<39	40-55	>55
Tuberculous (40)	2	10	28
Malignant (4)	3	0	1
Parapneumonic (2)	1	0	1
Congestive cardiac failure (3)	3	0	0
Hypoproteinemia (1)	1	0	0
Total (50)	10	10	30

Table 4: No. of immunosuppressive condition among patient diagnosed with TB pleural effusion.

Condition	Tuberculous	Non-tuberculous
HIV	2	0
Diabetes mellitus	7	4
Cancer or patient receiving chemotherapy	0	0
Total	9	4

Table 5: Result of pleural fluid for CBNAAT in diagnosing TB pleural effusion.

PF fluid for CBNAAT	Tubercular	Non tubercular
Positive	3	0
Negative	37	10
Total (50)	40	10

Table 6: Cytology picture in patients.

Cytology picture	Tuberculous	Non-tuberculous
Lymphocytic predominance	36	7
Neutrophilic predominance	4	3
Total (50)	40	10

Out of 40 diagnosed TB patients 36 patients pleural fluid cytology report show lymphocytic predominance picture that is more than 80% lymphocytes and 4 patient have neutrophilic predominance means PF lymphocytosis was sensitive (90%) for detecting TBE but with low specificity

and biochemistry report show that only 6 case have low sugar level less than 40 mg/dl and 22 patients have protein level more than 5 gm/dl that is exudative.

Table 7: Protein and glucose values in patients.

Values (gm/dl)	Tubercular	Non-tubercular
Protein		
>5	22	2
<5	18	8
Glucose		
>40	34	2
<40	6	8

Table 8: Gross appearance of pleural fluid.

Gross appearance of PF	Percentage (%)
Straw-coloured	84
Blood stained	10
Purulent	6
Chylous	0

DISCUSSION

ADA, a product of T lymphocytes, has been reviewed as a superb marker for the diagnosis of tuberculous pleural effusion. Tuberculosis may be a common explanation for pleural effusion especially in countries like India.^{6,7} If untreated TPE can become active tuberculosis so, it's important to form rapid and accurate diagnosis for TPE and initiation of treatment.⁸ Definitive diagnosis of tuberculosis may be a difficult task, as in additional than 50% of patients, pleura is that the only site of infection. Because bacterial load is less so pleural fluid culture for mycobacterium tuberculosis is also low (<20).⁹ Tuberculosis is a common cause of pleural effusion especially in countries like India.

In present study confirms that ADA level in tubercular pleural effusion is increased and in non-tubercular pleural effusion ADA level didn't exceed to 90 IU/l. TB pleural effusion is unilateral in more than 90% of cases. Although, lymphocytic predominant fluid is usually seen in tubercular pleural effusion but it is also seen in case of malignancy also. So there is a need to differentiate among various causes of pleural effusion. Pleural fluid ADA estimation is quick and relatively inexpensive. Present study was conducted to assess the diagnostic utility of ADA in case of undiagnosed exudative effusions. In present study we took ADA ≥39 U/l as in agreement with other studies. We found that 76% of TPE cases had ADA >39 U/l; whereas just one of these with malignancy had ADA >39. The sensitivity and specificity for diagnosing tubercular effusion was 95% and 80% with positive and negative predictive values of 95% and 80% respectively in present study (Table 9).

Tubercular PE predominates in men, with an overall male-to-female ratio of 2:1. In the present study also male to

female ratio is 5:3 (Table 1). Comparable with the studies of Hirsch (2.53:1).¹⁰ In this study TB pleural effusion were more common in age group 25-34 years of age group. So tubercular pleural effusion is more prevalent in younger age group.

Table 9: Sensitivity and specificity of ADA for tuberculous effusion.

ADA	Tuberculous	Non-tuberculous
Positive	38	2
Negative	2	8

Sensitivity=95%, specificity=80%, positive predictive value=95%, negative predictive value=80%

Most of the patients in present study had right sided pleural effusion (64%) which is comparable with the study of Ambethiya.¹¹ The foremost frequent symptoms of TPE are non-productive cough and pleuritic pain; if both cough and chest pain are present, the pain usually precedes the cough.¹²⁻¹⁴ In our study also the foremost common symptom among patients diagnosed with TB pleural effusion were (Table 2) breathlessness (90%) followed by fever (75%), cough (75%), chest pain (70%) and other rare symptoms were weight loss, loss of appetite, and haemoptysis. Non-productive cough (70%) and pain (75%) are the 2 commonest symptoms at presentation.

Table 3 shows that the majority of the tuberculous pleural effusions had elevated levels of pleural fluid ADA. So ADA is extremely sensitive in diagnosing tubercular pleural effusion. But a number of the malignant and parapneumonic pleural effusions also had raised pleural fluid ADA. So it is advisable to not rely only on this investigation for diagnosing tuberculous pleural effusion.

In immunosuppressive people (Table 4) TB are more prevalent than non-immunosuppressive person. 26% cases were immunosuppressive and out of this 18% cases were diagnosed with tubercular pleural effusion. In regions, like Zimbabwe, with a high prevalence rate of pleural TB, 85% of patients with TB pleuritis patients are human immunodeficiency virus (HIV)-positive.¹⁵ So it means immunocompromised patients are more likely to develop TB than non-immunocompromised patients. Table 5 shows that pleural fluid for Cbnaat in diagnosing tuberculosis is extremely less sensitive but high specificity. The Xpert MTB/Rif test indentified 3 (7.5%) of the 40 confirmed pleural TB cases. The sensitivity were 7.5% and specificity 100% compare with a recent study which also show the sensitivity and specificity of Xpert MTB/Rif on pleural fluid for pleura TB diagnosis was 15% and 100% respectively.¹⁶ The majority of patients has greater than 50% of small lymphocytes in their pleural fluid, and some have more than 90%.^{17,18} However, a patient who presents with symptoms for less than 2 weeks in duration is more likely to have predominantly neutrophils in his pleural fluid.¹⁹ Present study also (Table 6) shows that pleural fluid lymphocytosis is very sensitive for diagnosing TB pleural effusion. In a model of TB

pleurisy, when BCG-sensitized rabbits are given injections with BCG into the pleural space, the resulting pleural effusion contains predominantly neutrophils within the first 24 hours, followed by macrophages peaking at 96 hours, then lymphocytes.²⁰ In this study 90% tubercular patient show lymphocytosis and 10% show neutrophilic predominance which suggests pleural fluid lymphocytosis is very sensitive for diagnosing TB pleural effusion. Pleural fluid with tuberculosis is invariably an exudate. Usually, the pleural fluid protein level exceeds 5 g/dl, which suggests TB pleuritis.²¹ In this study (Table 7) 55% person diagnosed with tubercular pleural effusion had protein level more than 5 g/dl. The pleural fluid protein level exceeds 5 g/dl. Biochemical analysis of the pleural fluid is otherwise of limited value. Although within the past it had been observed that pleural fluid glucose was reduced in most patients, but newer studies show that majority of patients have a pleural fluid glucose level more than 60 mg/dl. In this study 50% cases have sugar level more than 60 mg/dl. In this study majority of the patient (87%) had straw colored fluid followed by blood stained (10%) (Table 8).

Burgess showed ADA activity in tuberculous effusion was higher than in any other diagnostic group.²² At a level of 50 U/l the sensitivity and specificity for the identification of tuberculosis was 90% and 89% respectively.

Value of ADA activity in pleural effusion was studied by Shibagaki et al.²³ He concluded that tuberculous pleural effusion had a much higher ADA activity than cancer effusion and total ADA activity in tuberculous pleural effusion decreases after anti tubercular therapy.

In another retrospective study, Chen et al evaluated 63 TPE and 147 non-tuberculous pleurisy cases and reported higher diagnostic values like the sensitivity of 87.3%, specificity 91.8%, PPV 82.1% and NPV 94.4% for the cut off value similar to ours (55.8 U/l) in the TPE diagnosis. They concluded total ADA value of the pleural fluid is an appropriate and fast diagnostic tool for the diagnosis of tuberculosis.²⁴

Out of the tuberculosis, high levels of ADA in the lymphocytic pleural effusion have been also reported in the fungal infections such as coccidioidomycosis and histoplasmosis. Among the noninfectious cases, high levels of the ADA are seen in the malignancies and collagen vascular diseases (e.g. rheumatoid arthritis and systemic lupus erythematosus).²⁵

So it has been clearly shown that ADA levels are significantly high in tuberculosis as against non tuberculous causes.

Limitations

This study has limitations that number of patients studied is small. So definitive criteria can't be established on this sample size. A large number of patients are required to

confirm our findings further and establish the definitive criteria.

CONCLUSION

The definitive diagnosis of tubercular pleural effusion depends on the demonstration of tubercle bacillus within the sputum, pleural fluid, or pleural biopsy specimens. ADA level of the pleural fluid is a non-invasive, inexpensive and repeatable test that gives the results quickly. It takes only 2 hours to give the result. This study shows that an easy, inexpensive, sensitive and specific test like ADA estimation should be used routinely to differentiate between tubercular and non-tubercular etiology in patients of pleural effusion.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

- World Health Organization. World Health Organization Global tuberculosis report. Geneva: World Health Organization. 2013.
- Jay SJ. Diagnostic procedures for pleural disease. *Clin Chest Med.* 1985;6(1):33.
- Porcel JM. Tuberculous pleural effusion. *Lung.* 2009;187(5):263.
- Greco S, Girardi E, Masciangelo R, Capocchetta GB, Saltini C. Adenosine deaminase and interferon gamma measurements for the diagnosis of tuberculous pleurisy: a meta-analysis. *Int J Tuberculosis Lung Dis.* 2003;7(8):777-86.
- Gakis C. Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2: diagnostic and biological role. *Eur Respir J.* 1996;9(4):632-33.
- World Health Organization. Global tuberculosis report 2013. World Health Organization. 2013.
- Krenke R, Korczynski P. Use of pleural fluid levels of adenosine deaminase and interferon gamma in the diagnosis of tuberculous pleuritis. *Curr Opin Pulmonary Med.* 2010;16(4):367-75.
- Light RW. Pleural diseases. Baltimore: Lippincott, Williams and Wilkins. 2001;151-81.
- Bothamley GH. Tuberculous pleurisy and adenosine deaminase. *Thorax.* 1995;50(6):593.
- Hirsch AP, Ruffie P, Nebut M, Bignon J, Chretien J. Pleural effusion: laboratory tests in 300 cases. *Thorax.* 1979;34(1):106-12.
- Kataria YP, Khurshid I. Adenosine deaminase in the diagnosis of tuberculous pleural effusion. *Chest.* 2001;120(2):334-6.
- Berger HW, Mejia E. Tuberculous pleurisy. *Chest.* 1973;63(1):88-92.
- Seibert AF, Haynes J, Middleton R, Bass JB. Tuberculous pleural effusion: twenty-year experience. *Chest.* 1991;99(4):883-6.
- Wang Z, Xu LL, Wu YB, Wang XJ, Yang Y, Zhang J, Tong ZH, Shi HZ. Diagnostic value and safety of medical thoracoscopy in tuberculous pleural effusion. *Respir Med.* 2015;109(9):1188-92.
- Aljohaney A, Amjadi K, Alvarez GG. A systematic review of the epidemiology, immunopathogenesis, diagnosis, and treatment of pleural TB in HIV-infected patients. *Clin Develop Immunol.* 2012.
- Porcel JM, Palma R, Valdes L, Bielsa S, San-Jose E, Esquerda A. Xpert® MTB/RIF in pleural fluid for the diagnosis of tuberculosis. *Int J Tuberculosis Lung Dis.* 2013;17(9):1217-9.
- Pefura Yone EW, Kengne AP, Moifo B, Kuaban C. Prevalence and determinants of extrapulmonary involvement in patients with pulmonary tuberculosis in a Sub-Saharan African country: a cross-sectional study. *Scand J Infectious Dis.* 2013;45(2):104-11.
- Valdés L, Alvarez D, San José E, Penela P, Valle JM, García-Pazos JM, Suárez J, Pose A. Tuberculous pleurisy: a study of 254 patients. *Arch Int Med.* 1998;158(18):2017-21.
- Levine H, Szanto PB, Cugell DW. Tuberculous pleurisy: an acute illness. *Arch Int Med.* 1968;122(4):329-32.
- Antony VB, Repine JE, Harada RN. Inflammatory responses in experimental tuberculosis pleurisy. *Acta Cytol.* 1985;27:355.
- Light RW. Pleural diseases. Lippincott Williams & Wilkins. 2007.
- Burgess LJ, Maritz FJ, Le Roux I, Taljaard JJ. Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. *Thorax.* 1995;50(6):672-4.
- Shibagaki T, Hasegawa Y, Saito H, Yamori S, Shimokata KJ. Adenosine deaminase isozymes in tuberculous pleural effusion. *J Lab Clin Med.* 1996;127(4):348-52.
- Chen ML, Yu WC, Lam CW, Au KM, Kong FY, Chan AY. Diagnostic value of pleural fluid adenosine deaminase activity in tuberculous pleurisy. *Clinica Chimica Acta.* 2004;341(1-2):101-7.
- 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(2):417-9.

Cite this article as: Goyal A, Kaur A, Kajal NC. Diagnostic value of pleural fluid adenosine deaminase level in patients of tubercular pleural effusion. *Int J Adv Med* 2021;8:93-7.