Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2017; 5(7A):2555-2561 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2017.v05i07.014

Original Research Article

Predictive Role of ADA in Bronchoalveolar Lavage Sample in Sputum Negative Pulmonary Kochs

P. Eshwaramma*[1], Swetha. A[2], Mahaboob khan[3]
[1]Assistant Professor, Dept. of Pulmonary Medicine, Osmania Medical College Hyderabad, India Government General and chest hospital, Hyderabad, India.
[2]Post Graduate in Dept. of Pulmonary Medicine, Osmania Medical College Hyderabad, India, Government General and chest hospital, Hyderabad, India.
[3]Professor, Dept. of Pulmonary Medicine OMC. Hyderabad, India, Government General and chest hospital, Hyderabad, India.

***Corresponding author**
P. Eshwaramma
Email: eshwari.pampana@yahoo.com

Abstract: Diagnosis of sputum/smear-negative pulmonary tuberculosis patients can be both challenging and time consuming with many patients being put on empirical anti-tubercular treatment. Fiber optic bronchoscopy may provide a confirmative and early diagnosis in such patients and the role of BAL ADA in diagnosing tuberculosis has been studied in present study. The present study consisted of 60 cases suspected of having tuberculosis based on clinical or radiological features that are sputum for AFB negative. They were subjected to FOB and BAL sample was sent for CBNAAT and Adenosine deaminase (ADA) and results obtained and sent for statistical analysis. The cases constituted of 43 tuberculous cases that BALF CBNAAT had had MTB detected and 17 cases that had BALF CBNAAT negative for MTB. In the present study ADA cut off level in BAL fluid was 3.84U/L with CI of 95% with sensitivity of 93% and specificity of 58.8%, PPV of 85% NPV of 77% with overall accuracy of 83% with a significant p value (< 0.001).
Keywords: Bronchoalveolar lavage, Mycobacterium tuberculosis, Adenosine deaminase, Fiber optic bronchoscopy, sputum negative tuberculosis.

INTRODUCTION:

Pulmonary Tuberculosis is one among the most important health problems World wide[1]. The World Health Organization (WHO) recommends confirmation of pulmonary tuberculosis (PTB) by the detection of acid-fast bacilli (AFB) in respiratory Specimens [2]. Early diagnosis of pulmonary tuberculosis prevents progression of disease, morbidity, spread of disease and permanent damage by fibrosis. Culture of sputum for acid fast bacilli (AFB) takes long time and a reliable serological test is not yet available. In such a situation bronchoscopy has been tried for rapid diagnosis of tuberculosis in sputum smear negative cases. Fiber optic bronchoscopy with bronchial washing analysis for AFB either by smear stain or culture has significant role to establish the diagnosis when extensive search for AFB in expectorated sputum has repeatedly failed, when sputum expectoration is absent or sputum induction has failed.

Detecting patients with active pulmonary tuberculosis (PTB) disease is an important component of TB control as early appropriate treatment renders these patients noninfectious and interrupts the chain of transmission of TB. Under the programmatic conditions, such as those endorsed by the World Health Organization (WHO) [3] and implemented successfully in high burden countries including India's Revised National Tuberculosis Control Programme (RNTCP) of Government of India [4], the diagnosis of PTB is based on 3 sputum smear examination. Sputum microscopy is a highly specific test, a low-cost, appropriate technology and is an essential component of the directly observed treatment, short-course (DOTS) strategy of the WHO where a case detection rate of 70% or more is aimed at.

However, in patients with a compatible clinical picture, sputum smears do not reveal acid-fast bacilli (AFB) in all patients, smear negative – culture positive state has been observed in 22% to 61% of the cases [5-7]. Mycobacterial cultures take at least six to eight weeks time for confirming the diagnosis and thereby a valuable time is lost. Unlike the years prior to the DOTS era, where wide variations existed in the quality of sputum microscopy, the DOTS approach has provided access to standardized, quality assured microscopy [8]. However, sputum smear-negative pulmonary tuberculosis (SSN-PTB) still remains a common problem faced by the clinicians. This is particularly true in the case of children who are unable to produce an adequate sample of sputum, patients with immunosuppressed states such as those with HIV infection and the acquired immunodeficiency syndrome (AIDS) in whom SSN-PTB is quite common.

AIMS & OBJECTIVES OF THE STUDY: To evaluate the role of ADA in Broncho alveolar lavage sample in sputum negative pulmonary tuberculosis.

CAUSES OF SMEAR NEGATIVITY:

Diagnosing sputum negative tuberculosis is difficult and in spite of the widespread availability and accessibility to quality microscopy under the RNTCP of the Government of India, commercial laboratories with varying standards still provide sputum examination results. Occasionally in immunocompetent patients, poor quality of the sputum sample (e.g., submitting saliva as sputum), deficient preparation, staining, or examination of the sputum smear by inexperienced technicians can contribute to the negative results. Sputum smear negative should be used to refer the results obtained from a quality assured and periodically accredited laboratory. This is particularly relevant to developing countries like India, where [9] many patients with PTB who are co-infected with HIV and present during the late stages of HIV disease (CD4+ count less than or equal to 200 per mm3) and those who are severely immunosuppressed (non HIV) are more likely to be sputum smear-negative. However, in spite of best efforts at sputum collection, processing and examination, some patients with active PTB do not produce adequate sputum, while others who produce adequate sputum also remain smear-negative for reasons that are as yet unknown[10].

MATERIALS AND METHODS

STUDY DESIGN: A cross-sectional descriptive study was performed at the pulmonary medicine Department, Government General and Chest Hospital Hyderabad, Telangana between 2015 january and 2016 september.

SAMPLE SIZE: Sixty cases participated in the study who were suspected of having pulmonary tuberculosis and are sputum smear negative for AFB were included.

Inclusion criteria: Cases suspected of having pulmonary TB and were sputum negative for AFB with clinical suspicion and radiologically positive for tuberculosis were included in the study.

Exclusion criteria: Exclusion criteria were as follows:
(1) Arterial hypoxia,
(2) Uncooperative patients for bronchoscopy,
(3) Hemodynamic instability,
(4) Life-threatening cardiac arrhythmia,
(5) Known TB cases (sputum positive for AFB).

Sample collection procedure:

Informed written consent was obtained from all cases who participated in the present study for undergoing FOB. After over night fasting. Local anesthesia was given by 10% Xylocaine spray locally and 2% Xylocaine jelly was applied intra nasally. An Olympus FOB (type P20Dor 1T20D, Tokyo, Japan) was inserted trans nasally and BAL was performed on the pulmonary segment with maximal radiological involvement**.**

Cbnaat: The samples were sent to IRL (intermediate reference laboratory) Irrumnuma, Hyderabad, Telangana state.

ADA measurement:

To assess ADA activity, the samples were centrifuged and kept at −21°C. Then ADA levels in BAL fluid were measured by MTB-ADA kit. ADA activity was measured by Giusti's colorimetric method. In Giusti's, the first step is deamination of adenosine and release of ammonia. The second reaction is catalyzed by glutamate dehydrogenase accompanying an allosteric activator. Low light absorption at 340 nm has a direct relationship to ADA activity. By this method, ADA activity can be measured up to 150 IU/L.

Diagnosing of TB cases: Tuberculous case is diagnosed if the BAL sample sent for CBNAAT reported MTB detected.

OBSERVATIONS AND RESULTS

A total of 60 cases were included in the study during a period of one year and nine months (January 2015 to September 2016). The study was conducted in a

tertiary care teaching hospital to assess the predictive role of ADA in BAL in sputum negative pulmonary tuberculosis cases. The enrolled patients underwent bronchoscopy and BAL fluid was sent for ADA and CBNAAT and out of 60 cases 43 cases had pulmonary tuberculosis which was diagnosed by CBNAAT where the BAL fluid sent for CBNAAT reported MTB detected and 17 cases had CBNAAT negative for MTB.ADA levels were studied in these patients.

Age distribution among tuberculous cases (n =43): In the present study, the most common age group among tuberculous cases(n=43) was found to be 41-50 years followed by 31- 40 years. The mean age among the tuberculous cases was 40.55 years.

Table-1: Age distribution among tuberculous cases

Age groups	No. of cases(n=43)	percentage of cases
21 -30	9	20.9%
31- 40	13	30.2%
41-50	15	34.9%
51-60	6	14%

Gender distribution among tuberculous cases(n= 43): Present study there were 28 males and 15 females among tuberculous cases with male to female ratio of 1.9 :1.

Table-2: showing Gender distribution among tuberculous cases (n= 43)

Gender	Count	Percentage %
Female	15	34.9%
Male	28	65.1%
Total	43	100%

Table-3: showing gender distribution among CBNAAT negative cases.

Gender	BALF CBNAAT MTB not detected (n =17)	
	Count	%
Female	6	35.3%
Male	11	64.7%
Total	17	100.0%

Table-4: Symptoms among tuberculous cases(n=43):

Symptom	No.of cases	Percentage(%)
Cough with sputum	38	88.3%
Breathlessness	22	51%
Hemoptysis	12	28%
Fever	25	58%
Anorexia	14	32.5%

The most common presenting symptom among tuberculous cases was cough with sputum which was present in 38 cases (88.3%) followed by fever which was present in 25 (58%) cases.

Symptoms among CBNAAT negative cases (n= 17): Among the 17 CBNAAT negative cases the most common symptom was cough with sputum which was present in 82.4% cases followed by fever which was present in 52.9% cases, breathlessness in 41.2% cases, hemoptysis was present in 29.4%,anorexia was present in 23.5 % cases.

Bronchoscopy findings among tuberculosis cases (n=43): The most common bronchoscopy finding among the 43 tuberculous cases was mucosal edema which was present in 44.2% cases followed by

erythematous mucosa which was present in 30.2% cases.

ADA Level in BAL fluid: BAL Fluid ADA levels among tuberculous cases and those with CBNAAT for MTB negative cases:

Table-5: ADA Level in BAL fluid

Variable	Cases	N	Min	Max	Mean	SD	P-value
ADA	**CBNAAT negative cases**	17	0.5	6.0	3.06	1.55	<0.0001
	Tuberculous cases	43	2.0	18.0	8.08	3.86	

Receiver operator curve for ADA: ADA cut off was obtained by using receiver operator curve

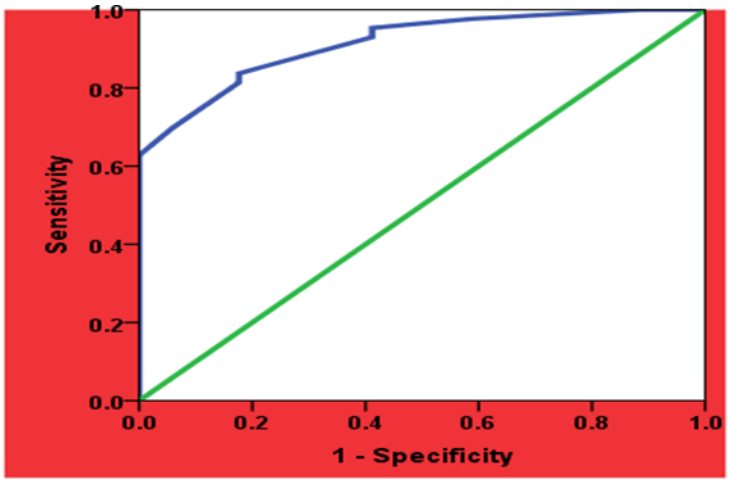

Fig-1: Receiver operator curve for ADA

Statistical Analysis: Descriptive statistics, Fisher exact test, Mann-whitney U test, Kappa-statistics, Logistic Regression, Sensitivity, Specificity, ROC curves were applied. SPSS V22 software was used for data analysis.

ADA cut off value after drawing ROC curve:

ADA cut off of greater than 3.84 U/L was obtained using a ROC curve with a sensitivity of 93 % specificity of 58.8% PPV and NPV of 85% and 77% respectively.

Table-6: ADA cut off value after drawing ROC curve

ADA	No. of cases n = 60		Total
	Tuberculous cases	CBNAAT negative cases	
>3.84	40	7	47
	93.0%	41.2%	78.3%
≤ 3.84	3	10	13
	7.0%	58.8%	21.7%
Total	43	17	60
	100.0%	100.0%	100.0%

Mean ADA among tuberculous cases and CBNAAT negative cases: Mean ADA among tuberculous cases was 8.08±3.86U/L and among CBNAAT negative cases was 3.06±1.55 U/L.

Table-7: Showing sensitivity, specificity, PPV, NPV, Overall accuracy and p value, kappa value :

Sensitivity	93.0%
Specificity	58.8%
PPV	85%
NPV	77%
Overall Accuracy	83%
Kappa	0.56
P-value	<0.001
AUC	0.92
95% CI	0.85-0.98

Data Analysis: The observed results were statistically analyzed. The sensitivities, specificities, negative predictive values (NPV) and positive predictive values (PPV) of BAL ADA was determined. Their 95% confidence intervals were also determined and the kappa statistics (with its p-value) was used to determine the level of agreement between the two methods.

DISCUSSION:

As India has the highest TB burden in the world, early detection of tuberculosis and prompt treatment is essential to decrease the spread, morbidity and mortality of disease. In the present diagnostic algorithm of RNTCP, sputum smear is the sheet anchor of diagnosis but its main drawback is its low sensitivity, while culture, which is the gold standard for the diagnosis of tuberculosis, gives delayed results. Other diagnostic modalities which require less time should be considered for early detection and diagnosis of sputum negative tuberculosis. Activity of ADA in pleural fluid, peritoneal fluid and pericardial fluid has been widely studied but very few studies have been done to determine the role of Adenosine deaminase level in BAL fluid. So this study was aimed at determining the role of ADA in BAL fluid, which is cost effective in comparison to CBNAAT, and gives earlier results when compared to the gold standard Culture methods.

A substantive number of pulmonary tuberculosis patients remain undiagnosed by conventional sputum microscopy. These cases also play an important role in the disease transmission. Moreover, on the basis of chest radiography alone as the diagnostic tool, many patients are wrongly started on Anti-Tubercular Treatment (ATT). In the above two situations, Cartridge Based Nuclear Acid Amplification Test (CBNAAT) of BAL (Broncho alveolar Lavage) appears convincing as a good diagnostic method for the purpose of diagnosing or ruling out pulmonary tuberculosis. One of the drawbacks of CBNAAT is its cost. By detecting active pulmonary TB early, an appropriate treatment can be initiated, lung damage can be prevented and disease transmission can be prevented.

Fiber optic bronchoscope is considered a good option for these cases that pose a diagnostic challenge; [11] although smear microscopy exhibits low sensitivity on fiber optic bronchoscope samples with sensitivities of 5-35% on bronchial aspirates (BA) and 10-30% on bronchi alveolar ravages (BAL)[12,13].

ADA activity has been studied as a valuable marker for differentiating tuberculous pleural effusion from other causes of exudative pleural effusions [22, 23], as well as a marker of tuberculosis in various body fluids. However, there are not many studies to depict the role of ADA in BAL fluid in differentiating tuberculous cases and non-tuberculous cases.

Some studies have reported that ADA activity in BALF of patients with pulmonary TB is higher than in other non-tuberculous pulmonary disorders [14-16].

In present study the minimum age among the tuberculous cases was 26 years and maximum age was 56 years and mean age of tuberculous cases was 40.55±9.98 years. The above data suggests that tuberculosis is more common in younger population.

Adenosine deaminase in BAL fluid:

In present study 60 cases were enrolled to determine the diagnostic value of ADA in BALfluid in sputum negative pulmonary tuberculosis cases who are highly suspicious of having tuberculosis. Among 60 cases 43 cases were diagnosed as pulmonary tuberculosis. The study group was selected based on clinical and radiological features suggestive of tuberculosis who were sputum negative. After obtaining the results 43 cases were diagnosed as tuberculous cases

based on BALF CBNAAT which was positive for MTB.

The mean ADA in pulmonary Tuberculosis cases was 8.08±3.86 U/L and among CBNAAT negative cases was 3.06±1.55 U/L($p< 0.0001$).

In present study BAL ADA was higher in cases with pulmonary Tuberculosis cases (Mean ADA =8.08±3.86 U/L) when compared to those who had CBNAAT negative for MTB (Mean ADA =3.06± 1.55 U/l) with a p value of< 0.0001(significant).

ADA activity has been used as a valuable marker for differentiating tuberculous pleural effusion from other causes of exudative pleural effusions [24,25].Similarly ADA activity has been studied in various body fluids like Ascitic fluid, cerebrospinal fluid but very few studies have been done to know the activity of ADA in BAL fluid in tuberculous cases.

In the present study ADA cut off level in BAL fluid was 3.84U/L with CI of 95% with sensitivity of 93% and specificity of 58.8%, PPV of 85% NPV of 77% with overall accuracy of 83% with a significant p value (< 0.001).

Some studies have reported that ADA activity in BALF of patients with pulmonary TB is higher than in comparators [17, 18, 19].

ADA is an enzyme found in peripheral blood and tissue lymphocytes and is increased in BAL fluid in diseases with lymphocytic reactions like sarcoidosis and hypersensitivity pneumonitis [20, 21].

LIMITATIONS OF THE STUDY

- BAL is an invasive procedure and cannot be performed in all cases.
- Contraindications for Bronchoscopy is one of the limitations.
- ADA 2 is more specific marker than total ADA in the present study total ADA was done.
- As the sample size is small the present study and the derived ADA cut off value is not applicable to general population.

CONCLUSION

BAL fluid ADA can be used as one of diagnostic modality for early diagnosis of tuberculosis but no clear guidelines are there which show its diagnostic efficacy. BAL ADA can be used as a screening test but not as a diagnostic modality. This study has to be substantiated by further studies.

REFERENCES:

1. Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Global burden of tuberculosis: Estimated incidence, prevalence, and mortality by country. ITS Global Surveillance and Monitoring Project. JAMA 1999;282:677-86
2. World Health Organization: Treatment of Tuberculosis: Guidelines for National Programmes, 3rd edn. Switzerland: WHO, 2003.
3. World Health Organization, Stop TB Department. Treatment of Tuberculosis: Guidelines for National Programmes; 3rd edn. Geneva: World Health Organization; 2003. WHO/CDS/TB/ 2003.313.
4. TB India 2007. RNTCP Status Report. New Delhi: Central TB Division, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India; 2007
5. Hong Kong Chest Service / Tuberculosis Research Center Madras/ British Medical Research Council. Sputum smear negative tuberculosis: controlled clinical trial of 3-month and 2-month regimen of chemotherapy (first report). Lancet 1979; 1:1361-3.
6. Narain R, Subbarao MS, Chandrasekhar P, Pyarelal. Microscopy positive and microscopy negative cases of pulmonary tuberculosis. Am Rev Respir Dis 1971; 103: 761-3.
7. Kim TC, Blackman RS, Heatwole KM, Rochester DF. Acid fast bacilli in sputum smears of patients with pulmonary tuberculosis: prevalence and significance of negative smears pretreatment and positive smears post treatment. Am Rev Respir Dis 1984; 29: 264-8.
8. Davis JL, Cattamanchi A, Cuevas LE, Hopewell PC, Steingart KR. Diagnostic accuracy of same-day microscopy versus standard microscopy for pulmonary tuberculosis: a systematic review and meta-analysis. The Lancet infectious diseases. 2013 Feb 28; 13(2):147-54.
9. Toman K. How many bacilli are present in a sputum specimen found positive by smear microscopy? In: FriedenT, editor Toman's Tuberculosis Case Detection, Treatment and Monitoring: Questions and Answers; 2nd edn. Geneva: World Health Oraganization; 2004: pp 11-13.
10. Hillemann D, Richter E, Rusch-Gerdes S. Use of the BACTEC Mycobacteria Growth Indicator Tube 960 automated system for recovery of Mycobacteria from 9,558 extrapulmonary specimens, including urine samples. Journal of clinical microbiology. 2006 Nov 1;44(11):4014-7

11. Chen NH, Liu YC, Tsao T, Wu TL, Hsieh MJ, Chuang ML, Huang CC, Kuo AJ, Chen MC, Yang CT. Combined bronchoalveolar lavage and polymerase chain reaction in the diagnosis of pulmonary tuberculosis in smear-negative patients. The International Journal of Tuberculosis and Lung Disease. 2002 Apr 1; 6(4):350-5.
12. Mahat R, Rizvi N, Saifullah N, Tawfik M, Kumar A. Comparison of GeneXpert MTB/RIF Assay in Broncho Alveolar Lavage and Gastric Lavage Samples. Advances in Infectious Diseases. 2016 Aug 2; 6(03):107.
13. Ioannidis, P., Papaventsis, D., Karabela, S., Nikolaou, S., Panagi, M., Raftopoulou, E., ... & Kanavaki, S. (2011). Cepheid GeneXpert MTB/RIF assay for Mycobacterium tuberculosis detection and rifampin resistance identification in patients with substantial clinical indications of tuberculosis and smear-negative microscopy results. *Journal of clinical microbiology*, *49*(8), 3068-3070.
14. Kayacan O, Karnak D, Delibalta M, Beder S, Karaca L,Tutkak H. Adenosine deaminase activity in bronchoalveolar lavage in Turkish patients with smear negative pulmonary tuberculosis. Respir Med 2002; 96 (7): 536- 41.
15. Boonsarngsuk, V., Suwannaphong, S. and Laohavich, C. Combination of adenosine deaminase activity and polymerase chain reaction in bronchoalveolar lavage fluid in the diagnosis of smear-negative active pulmonary tuberculosis. International Journal of Infectious Diseases, 2012; 16(9), pp.e663-e668.
16. Halvani A, Binesh F. Adenosine deaminase activity in bronchoalveolar lavage fluid in patients with smear-negative pulmonary tuberculosis. Tanaffos 2008; 7:45–9.
17. Garcia-Vila LM, Pereira S, and Rodriguez-Martinez D. Ascites adenosine deaminase activity is decreased in tuberculous ascites with low protein content. Am J Gastroenterol 1991; 86 (10): 1500-3.
18. Hillebrand DJ, Runyon BA, Yasmineh WG, Rynders GP. Ascitic fluid adenosine deaminase insensitivity in detecting tuberculous peritonitis in the United States. Hepatology 1996; 24 (6): 1408-12
19. Orphanidou D, Gaga M, Rasidakis A, Dimakou K, Toumbis M, Latsi P, Pandalos J, Christacopoulou J, Jordanoglou J. Tumour necrosis factor, interleukin-1 and adenosine deaminase in tuberculous pleural effusion. Respiratory medicine. 1996 Feb 1; 90(2):95-8.
20. Kubota M, Katagiri M, Yanase N, Soma K, Tomita T. Measurement of adenosine deaminase activity in bronchoalveolar lavage fluids as a tool for diagnosing miliary tuberculosis. Nippon Kyobu Shikkan Gakkai Zasshi 1996; 34: 139–144.
21. Albera C, Mabritto I, Ghio P, Solidoro P, Marchetti L, Pozzi E. Adenosine deaminase activity and fibronectin levels in bronchoalveolar lavage fluid in sarcoidosis and tuberculosis. Sarcoidosis 1993; 10 (1): 18- 25.
22. Dwivedi M, Misra SP, Misra V, Kumar R. Value ofadenosine deaminase estimation in the diagnosis oftuberculous ascites. Am J Gastroenterol 1990; 85 (9): 1123-5.
23. Fernandez-Rodriguez CM, Perez-Arguelles BS, Ledo L, Garcia-Vila LM, Pereira S, Rodriguez-Martinez D. Ascites adenosine deaminase activity is decreased in tuberculous ascites with low protein content. Am J Gastroenterol 1991; 86 (10): 15003.
24. Goto M, Noguchi Y, Koyama H, Hira K, Shimbo T, Fukui T. Diagnostic value of adenosine deaminase in tuberculous pleural effusion: a meta-analysis. Ann Clin Biochem 2003; 40:374–81.
25. 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: 909–14.