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## **AFB culture and adenosine deaminase levels in subjects with tubercular pleural effusion**

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**Abstract**---Background: Tuberculosis is the communicable infectious disease usually caused by Mycobacterium Tuberculosis Bacteria (MTB). Tuberculosis generally affects lungs, but can also affect other parts of the body. ADA has been found to be useful parameter to conclude the tubercular etiology. Tuberculous Pleural effusion is diagnosed by demonstration of tubercular bacilli in pleural fluid or granuloma in pleural biopsy specimen. Objectives of this study: The objectives of this study were to estimate ADA levels and to study AFB culture in subjects with and without tubercular pleural effusion. Materials and Methods: Biochemical analysis for Protein and Glucose were performed on Automated Biochemistry Analyser and ADA was estimated by ADA-MTB kit method. Cytological examination and ADA Microbiological demonstration of AFB by ZN stain and AFB culture was done by conventional LJ method. Statistical Analysis: Data were expressed as mean  $\pm$  SD. The Student t test was used for the comparison. Statistical analysis was done using Microsoft Excel spreadsheet, and statistical package for the social sciences (SPSS) version 20.0 software. Results: It is evident from our study that 126 subjects had ADA levels less <40 U/L and 74 subjects had ADA levels >40 U/L, similarly out of 74 subject's AFB culture was found to be positive in 68 subjects and was negative in 6 subjects, and out of 126 subjects 6 subjects had AFB culture positive and 120 subjects had AFB culture negative. Discussion & Conclusion: ADA estimation with AFB culture increases the sensitivity and specificity and predictive value for the diagnosis of tuberculosis. A cut off 40 IU/L considered to be adequate to exclude tuberculosis.

**Keywords**---adenosine deaminase, acid fast bacilli culture, tuberculosis, pleural effusion, non-tubercular pleural effusion, pleural fluid.

## Introduction

Pleural effusion is a common chest problem, yet it is difficult to establish the aetiological diagnosis in as many as 20% cases despite good history, thorough clinical, radiological, full examination of aspirated fluid and pleural biopsy. So, there is a need of simple, rapid and reliable diagnostic test to establish the aetiology of pleural effusion. Considering this a prospective hospital based study was designed to estimate pleural fluid adenosine deaminase level and pleural AFB culture in establishing the diagnosis of tubercular pleural effusion. On a global scale, tuberculosis (TB) remains one of the most frequent causes of pleural effusions. Our understanding of the pathogenesis of the disease has evolved and what was once thought to be an effusion as a result of a pure delayed hypersensitivity reaction is now believed to be the consequence of direct infection of the pleural space with a cascade of events including an immunological response. Pulmonary involvement is more common than previously believed and induced sputum, which is grossly underutilised, can be diagnostic in approximately 50%. The gold standard for the diagnosis of tuberculous pleuritis remains the detection of *Mycobacterium tuberculosis* in pleural fluid, or pleural biopsy specimens, either by microscopy and/or culture, or the histological demonstration of caseating granulomas in the pleura along with acid fast bacilli (AFB).

The pleural effusion is likely a manifestation of paucibacillary mycobacterial infection within the pleural space, which is acquired from initial parenchymal lesions and results in an immunological response that both increases pleural fluid formation and decreases pleural fluid removal. Initially, there is a rapid neutrophilic inflammatory response within the pleura which is symptomatic. This is followed by a protracted lymphocyte driven immune reaction which is accompanied by pleural granuloma formation and release of adenosine deaminase (ADA). It is therefore plausible that the likelihood of a positive pleural fluid culture decreases with time, as the effusion becomes lymphocyte predominant, and viable mycobacteria are contained. ADA levels are most useful when there is a moderate to high suspicion of TB in patients with negative pleural fluid or biopsy cultures, and non-diagnostic histology. There is a wide range of cut-off values used by authors but in most studies the most accurate threshold was found to range between 40 and 60 U/L.

## Objectives of the study

The objectives of this study were to estimate ADA levels and to study AFB culture in subjects with and without tubercular pleural effusion.

## Materials and Methods

### Study site

This study was conducted at the Department of Microbiology S Shri Balaji Institute of Medical Sciences, Mowa Raipur

### Study population

Subjects admitted in the department of Respiratory Medicine and General Medicine with Pleural Effusion.

### Study design

A Hospital Based Prospective Cross-Sectional Study was conducted on “AFB culture and adenosine deaminase levels in subjects with tubercular pleural effusion” was conducted at our hospital.

### Sample size

included 200 subjects admitted in Respiratory Medicine and General Medicine.

### Time frame to address the study

12 months from January 2021 to December 2021.

### Inclusion Criteria

We included a total 200 subjects with pleural effusion admitted in the department of Respiratory Medicine and General Medicine, in whom AFB culture and ADA levels were estimated.

### Technique and Tools and Data collection

Biochemical analysis for Protein and Glucose were performed on Automated Biochemistry Analyser and ADA was estimated by ADA-MTB kit method. Cytological examination and ADA Microbiological demonstration of AFB by ZN stain and AFB culture was done by conventional LJ method.

### Statistical Analysis

Data were expressed as mean  $\pm$  SD. The Student *t* test was used for the comparison. Statistical analysis was done using Microsoft Excel spreadsheet, and statistical package for the social sciences (SPSS) version 20.0 software.

### Results

Table 1 Shows Age and Gender wise distribution of the subjects with Pleural Effusion				
Age Groups (years)	Number of Subjects	Males	Females	
11-20	16	10	6	
21-30	32	16	16	
31-40	36	20	16	
41-50	40	22	18	
51-60	40	26	14	

61-70	36	32	4
Total	100	126	74

Table 2 Distribution of Subjects Depending on ADA levels <40 and >40 IU/L	
ADA <40 IU/L	126
ADA >40 IU/L	74

Table 3 Distribution of Subjects Depending on AFB culture and ADA levels		
	ADA <40 IU/L	ADA >40 IU/L
AFB Culture Positive	6	68
AFB Culture Negative	120	6
Total	126	74

It is evident from the above table 2 & 3 that 126 subjects had ADA levels less <40 U/L and 74 subjects had ADA levels >40 U/L, similarly out of 74 subject's AFB culture was found to be positive in 68 subjects and was negative in 6 subjects, and out of 126 subjects 6 subjects had AFB culture positive and 120 subjects had AFB culture negative.

## Discussion

Pleural effusion is a common clinical entity: approximately 4% of all attendances at chest clinics. The initial step in diagnosis is to distinguish between transudates and exudates. This is indicative of the underlying pathophysiological process involved. Such a distinction allows appropriate investigations to be instigated, enabling better patient management. The commonest cause of exudative pleural effusion has been found to be tuberculosis (60.2%) followed by malignancy (29.1%) and pneumonitis (7.7%). Although lymphocytic predominance is usually seen in tubercular pleural effusion but needs to be differentiated from malignancies.

Hence, 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. 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. 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. Hence, in our study we used the combination of ADA levels and AFB culture. We found that that 126 subjects had ADA levels less <40 U/L and 74 subjects had ADA levels >40 U/L, similarly out of 74 subject's AFB culture was found to be positive

in 68 subjects and was negative in 6 subjects, and out of 126 subjects 6 subjects had AFB culture positive and 120 subjects had AFB culture negative.

Present study confirms that ADA level in tubercular pleural effusion is increased as compared to non-tubercular pleural effusion at cut-off value of 40 IU/L. Tuberculosis is a common cause of pleural effusion<sup>4</sup> especially in countries like India. More over incidence of tuberculosis is increasing world-wide. Although tubercular pleural effusion can resolve spontaneously but up to 65% untreated tubercular pleural effusion can develop active tuberculosis. So, rapid and accurate diagnosis and prompt treatment is necessary for tubercular pleural effusion. Whenever a patient of pleural effusion presents we usually investigate online of gross, microscopic and biochemical parameters. Although lymphocytic predominant fluid is usually seen in tubercular pleural effusion but all lymphocytic predominant fluid can't be tubercular, it could be malignant. So, there is a need to differentiate among various causes of pleural effusion. Definitive diagnosis of tubercular is often difficult as in more than 50% of patients, pleura is the only site of infection. Tuberculin test is non-specific and finding can be negative<sup>8</sup>. Because bacterial load is less so pleural fluid culture for mycobacterium tuberculosis is also low (< 20). Pleural fluid ADA estimation is quick and relatively inexpensive.

In present study ADA level in tuberculosis cases was more than 40 IU/L in agreement with Jindal et al(1993)> 40U/L, different studies have found the cut-off values of 38 and 37 IU/L as with Niwa et al. (1985) >38IU/L; Rodriguez (1962)>37 U/L. In case of malignant pleural effusion our findings co- relate with most of the authors. ADA level in malignancy was up to 87.6 IU/L. ADA level more than 100 IU/L observed only in cases of tubercular pleural effusion so from the study we concluded that if ADA level of more than 100 IU/L is taken as cut off point it is exclusively seen in cases of tubercular pleural effusion. So we can say that estimation of ADA level in pleural fluid is extremely helpful in establishing the aetiology of tubercular pleural effusion and to rule out other diagnosis especially of other diseases in which lymphocyte predominance of pleural effusion is seen such as malignancy and collagen vascular diseases (i.e. rheumatoid arthritis and systemic erythematosus).

## Conclusion

ADA estimation with AFB culture increases the sensitivity and specificity and predictive value for the diagnosis of tuberculosis. A cut off 40 IU/L considered to be adequate to exclude tuberculosis.

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