



## Original Research Article

## Role of ADA and CBNAAT (Cartridge Based Nucleic Acid Amplification Test) in diagnosis of Tuberculosis in straw coloured exudative pleural effusion in patients attending Government General Hospital, KAKINADA

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## ARTICLE INFO

## Article history:

Received 20-01-2020

Accepted 18-06-2020

Available online 16-09-2020

## Keywords:

Pleural effusion

ADA

CBNAAT

## ABSTRACT

**Introduction:** India is presently one of the high burden countries for tuberculosis and accounts for 23% of global TB burden with 2.2 million patients. Of these reported cases in India around 20% are of Extra Pulmonary Tuberculosis (EPTB). Pleural effusion due to TB is currently the second common location for EPTB next to TB lymphadenitis. The bacteriological confirmation to diagnose EPTB is more difficult due to its paucibacillary nature. The present study was done to determine the role of ADA and CBNAAT both being rapid and non-invasive diagnostic methods for early detection of tuberculous pleuritis, which is essential for treatment initiation, improved patient outcome and for more effective public health intervention.

**Materials and Methods:** An observational study was done on 100 patients presenting with clinico-radiological picture suggestive of pleural effusion, either admitted or attending OPD of Department of Pulmonary Medicine from Nov 2017 to Nov 2019 at GGH, Kakinada.

**Results:** Out of 100 exudative pleural effusions in study, male predominance with 80% of males and 20% females. Lymphocyte predominance is 74%, sputum AFB was positive in 6 cases. With ADA cut off as 40IU/L, 70 cases had an ADA > 40IU/L out of which 64 were tubercular, and 6 were malignant effusions. ADA has a sensitivity of 94% and specificity of 60%. Pleural fluid CBNAAT was positive in 30 cases, out of which 2 cases had an ADA < 40 IU/L. All are rifampicin sensitive. The sensitivity of CBNAAT is 40% and specificity 82%. There is a positive correlation between the lymphocyte predominance with ADA and CBNAAT with  $P < 0.05$ .

**Conclusion:** Estimation of ADA in pleural fluid is a simple, rapid, and less expensive laboratory investigation where the diagnosis is uncertain. The sensitivity of ADA, when combined with lymphocyte-predominant exudates, helps to diagnose tubercular effusions. The role of CBNAAT in diagnosing pleural TB is limited due to its poor sensitivity.

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### 1. Introduction

An excessive collection of fluid in the pleural space is defined as Pleural effusion; it indicates an imbalance between pleural fluid formation and absorption.<sup>1</sup> According to the global TB report of the World Health Organization, a total of 10.0 million people were infected with the disease in 2018. India being in the high TB burden, accounts for 27% of the world's TB cases with an estimated 27.5 lakhs

patients as per Global TB report 2018.<sup>2</sup> TB can involve any organ in the body, with pulmonary TB being the most common. Pleural effusion due to TB is currently the most common location for Extra Pulmonary Tuberculosis (EPTB) next to lymph node TB. Major hindrances for diagnosing EPTB are due to variable clinical presentation and lack of standardized laboratory methods. Early detection of TB and drug resistance is important in the management of TB. The diagnosis is compromised due to the paucibacillary nature of disease in extra pulmonary specimens.

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The definitive diagnosis of tuberculous pleuritis is - demonstration of tubercle bacilli either in the pleural fluid, sputum or by demonstrating granulomas in the pleura or pleural biopsy specimen. AFB in a pleural fluid smear is positive in less than 10% of reported instances, while the culture of mycobacteria from the pleural fluid is seen in 10-70% cases in various studies.<sup>3</sup> Pleural fluid culture for AFB is time-consuming, taking 6-8weeks & pleural biopsy is invasive procedure with its complications. In view of the above observations, alternative diagnostic approaches have been extensively evaluated.

We considered to study the role of ADA and CBNAAT both being rapid and non-invasive diagnostic methods for early detection of tuberculous pleuritis, which is essential for treatment initiation.

### 1.1. Adenosine Deaminase

Levels of Adenosine deaminase (ADA) are particularly useful in areas where the prevalence of TB is high. An ADA level greater than 40 U/L has a sensitivity of more than 90% and a specificity of about 85% for the presence of tuberculosis.<sup>4,5</sup>

In lymphocyte-predominant effusions, the specificity of ADA for tuberculosis increases to more than 95%. Elevated ADA also occurs with malignant neoplasm, empyema, and rheumatoid arthritis.<sup>6</sup> (64). The ADA is an enzyme involved in purine catabolism. It catalyzes the deamination of adenosine to inosine and of deoxy adenosine to deoxyinosine. Adenosine deaminase is involved in the proliferation and differentiation of lymphocytes, specifically the T-lymphocytes. The T-cells release ADA during the process of activation in the presence of live intracellular pathogens. Thus ADA has been looked upon as a marker of cell-mediated immune response and specifically T-cell activation.

### 1.2. CBNAAT- cartridge based nucleic acid amplification test

The rapid, fully automated NAAT also known as Xpert MTB/RIF assay –has been described as a major breakthrough in TB control and program. The GeneXpert is currently the only one of its kind using a cartridge containing lyophilized reagents, buffers, and washes. The Xpert MTB/RIF assay is based on hemi-nested real-time PCR amplifying the rpoB gene target.

The original WHO policy guidance on Xpert MTB/RIF(2010) advises its use as the diagnostic test in individuals suspected of having multidrug-resistant TB or HIV associated TB. In 2013 updated policy, it recommended Xpert MTB/RIF for the diagnosis of EPTB, for suspected cases of pulmonary TB (conditional recommendations) and TB in children.

CBNAAT Testing involves three manual steps:

1. Addition of sample treatment reagent to liquefy and inactivate the bacteria in the sputum.
2. Transfer of 2ml of liquefied sputum into the cartridge.
3. Loading of the cartridge into the device for the assay . All further steps are automated.

The role of CBNAAT for early diagnosis of tubercular effusion has been evaluated as an alternative diagnostic tool with an added advantage to detect rifampicin resistance.

## 2. Materials and Methods

An observational study was done on 100 patients presenting with symptoms, medical history, radiological picture suggestive of pleural effusion, either admitted or attending OPD of Department of Department of pulmonary medicine from Nov 2017 to Nov 2019 at Government General Hospital, Kakinada.

### 2.1. Inclusion criteria

1. Patients of age >18yrs with a medical history suggestive of pleural effusion.
2. Patients with pleural effusion were identified by- Clinical examination, chest x-ray, ultrasonography, and diagnostic thoracentesis for fluid analysis showing exudative nature.

### 2.2. Exclusion criteria

1. Patients age <18 yrs.
2. Patients not giving consent for thoracentesis
3. Transudative pleural effusions.
4. Hemothorax (hemorrhagic pleural effusion).

### 2.3. Methodology

After obtaining informed consent, 100 cases of pleural effusion were subjected for following investigations- Medical history taking, full clinical examination, Sputum for AFB smear, diagnostic thoracentesis under aseptic conditions. Aspirated pleural fluid was sent to biochemical tests sugar, protein, LDH, microbiological tests –AFB stain, total cytological count and differential count. Simultaneously Serum protein and serum LDH were also measured. All patients fulfilling Light's criteria for exudative pleural effusion were further subjected to ADA and CBNAAT.

In the study, presence of first or more than one of the following criteria was adopted to label a case as tuberculous

1. Bacteriological confirmation of the presence of Mycobacterium tuberculosis in pleural fluid or sputum by z-n stain, Fluorescent stain, CBNAAT
2. Clinical presentation consistent with TB with the exclusion of other clinical considerations;

3. Exudative (according to Light's criteria), lymphocytic pleural effusion with ADA > 40 IU/L.
4. Definite clinical and radiological improvement in 2 months of administration of exclusive ATT. All cases of malignancy diagnosed on cytology by pleural fluid, showing the malignant cells.

ADA activity was measured by the standard method, as suggested by Guisti.<sup>7</sup> 2 mL of pleural fluid was collected in a sterile container and was either immediately analyzed or refrigerated at 4°C and analyzed within two days. An ADA value > 40 IU/L was taken as a cut off value for diagnosing tubercular effusion<sup>8,9</sup>.

### 3. Results

Out of 100 patients 80 are male and 20 are female. There is a male predominance with a male: female ratio of 4:1.

- Tubercular effusions were seen predominantly in the age group of 18- 30 yrs, accounting for 36%.
- The mean age of tubercular effusion in the study is 39.88±14.43 years.
- The minimum age affected by TB in study is 19 years and maximum age is 73 years. The mean age among males is 39.84, and among females is 36.35.
- The most common presenting symptom in study group is cough (85%), followed by chest pain (78%), fever (68%), breathlessness (65%), loss of weight (34%).
- Right-sided effusions are predominant in the study, accounting for 65%.
- 54% are indoor workers, 64% are smokers and with a low literacy rate, which is important confounding factor for TB.

Sputum AFB was positive in 6% of patients. Lymphocyte predominance was seen in 74% of effusions.

With an ADA cut off of 40 IU/L, 70 cases had ADA > 40 out of which 64 were tubercular, and 6 were malignant effusions.

- ADA has a sensitivity of 94% specificity of 60%.
- Pleural fluid CBNAAT was positive in 30 cases, out of which 2 cases had an ADA < 40 IU/L. All rifampicin sensitive. The sensitivity of CBNAAT was 40%, and specificity was 82%.
- There is a positive correlation between the lymphocyte predominance and ADA with P < 0.05%. A positive correlation was also seen between lymphocyte predominance and CBNAAT.

Out of 100 effusions, 66% are tb effusion, 21% are parapneumonic, 10% were malignant, 3% are empyema.

Data analysis was done with the help of computer using MS-Excel, SPSS 22.0 (Trail version). Using this software, frequencies, percentage, range, mean, standard deviation. Student t test and p values were calculated.

**Table 1:** Age wise distribution of study population

Age group	Males	Females	%
18-30	28	8	36%
31-40	20	7	27%
41-50	13	2	15%
51-60	8	1	09%
>60	11	2	13%
Total	80	20	100%

**Table 2:** Clinical features in study population

Symptoms	No. of cases	Per cent
Cough	85	85%
Fever	68	68%
SOB	65	65%
Chest pain	78	78%
Weight loss	34	34%
Loss of appetite	56	56%

**Table 3:** ADA level in pleural fluid

Pleural fluid ADA (IU/L)	No. of cases	Percent
≤ 40	30	30%
> 40	70	70%
Total	100	100%

### 4. Discussion

Pleural effusions are traditionally divided into transudates and exudates based on Light's criteria. Exudative pleural effusion is manifested by various diseases. In the Indian context, where Tuberculosis is endemic, an exudative pleural effusion is considered as tuberculous in origin until proven otherwise.

In this study of 100 patients, 80 (80%) are males and 20 (20%) females with sex ratio 4:1 (M: F). The male predominance seen in our study (80%) is similar to studies done by Modi et al.<sup>1</sup> (73.33%), Anushree Chakraborty et al.<sup>8</sup> (76%), Shubham Kumar Sharma et al.<sup>11</sup> (78%) and Kate et al.<sup>9</sup> (72%). Male predominance is nearer to the study done by Sharma et al. The incidence of tuberculosis is more in males due to exposure to outdoor pollution, the presence of confounding factors like smoking, and migration to high prevalent areas. The lower number of females attending to the hospital due to social stigma may also be a cause for less incidence of females in our study.

The predominant age group affected is 18-30 years in the study (36%) similar to Modi et al. (40%). These studies indicate tubercular effusion more in productive age group.

The mean age of the present study group is 39.88±14.43. Modi et al study has mean age 43.19±1, and Mohammed Zainul et al.<sup>10</sup> has mean age of 45.17±14.69. As stated in literature and by WHO, TB commonly affects productive age groups and vulnerable populations. From all these study findings, including the present study, it can be emphasized that TB forms an unavoidable differential diagnosis among

**Table 4:** ADA and CBNAAT in study

	No. of cases	Cbnaat MTB detected	Cbnaat MTB not detected
ADA>40 IU/L	70	28	42
ADA<40 iu/l	30	2	28
Total	100	30	70

**Table 5:** Distribution of patients according to etiologic diagnosis

ETIOLOGICAL	No. of patients	Percent
Lymphocytic effusion suggestive of TB	66	66%
Malignant effusion	10	10%
Empyema	3	3%
Parapneumonic Effusion	21	21%
total	100	100%

**Table 6:** Pleural fluid lymphocyte wise ada and cbnaat in study population

Lymphocytes	Cases	ADA		CBNAAT	
		>40IU/L	<40IU/L	MTB detected	MTB not detected
60-69	6	4	2	3	3
70-79	8	6	2	3	5
80-89	16	15	1	4	12
90-100	44	39	5	19	25

There was significant positive correlation seen with ADA detected patients and lymphocyte count  $r = 0.71$ ,  $P$ -value = 0.003 HS\* ( $p < 0.05$ ). Significant positive correlation seen with CBNAAT detected patients and lymphocyte count  $r = 0.95$ ,  $P$ -value = 0.0015 HS\* ( $p < 0.05$ ).

younger patients with pleural effusion.

In developing countries, TB frequently affects young productive age groups who did not acquire natural immunity and reflects the high prevalence rate of the disease in the community.

The most common presenting symptom is cough (85%) in the study, similar to that of Anushree Chakraborty et al. (89%), Lokeswara Reddy et al.<sup>11</sup> (73.3%) whereas the most common presenting symptom is a fever in Shukla et al.<sup>12</sup> and Sachin kate et al. The second most common symptom in our study is chest pain (78%), similar to Sharma et al. study (71.5%). In the present study, fever accounts for (68%), which is comparable to Lokeswarareddy et al. (70%). The other symptoms, like loss of weight and loss of appetite are similar to Anushree Chakraborty et al., indicating that the disease is a chronic process.

The right-sided predominance (65%) of effusion is similar to Modi et al. (56.2%), Shukla et al. (68%), Chakraborty et al. (58.6%), and Lokeswarareddy et al. (59%).

The study has 54% indoor workers similar to Zainul et al study with 52.4% indoor working group. Indoor workplace with overcrowding and poor ventilation are well-known risk factors for the increase of germ in air & facilitates tubercular infection.

The study has more no. of cases with the primary school as educational qualification (35%). High school qualification is more in Zainul et al. study. In Sharma et al. study, 37% patients were illiterates. Low literacy rate

similar to Zainul et al study. Illiteracy is the main constraint for any health program as patients with low literacy fail to use available health information properly and fail to follow health instructions.

The present study comprised 45% malnourished with BMI < 18.5, while it is 57% in Sharma et al. and 66.7% in Zainul et al. study. The percentage of underweight in the present study is near to Sharma et al. study. The high prevalence of malnourishment may be due to low socioeconomic status or the underlying disease itself, causing reduced BMI. Underweight patients are of the high risk of developing EPTB.

Lymphocyte predominance in the study (74%) is similar to study by Modi et al. (67.87%), Kate et al. (90%), and chakraborty et al. (97%). The lymphocyte percentage in our study is closer to Modi et al. study. High lymphocyte predominance in exudative effusions favors tubercular etiology, which is further confirmed by ADA levels.

The sputum positivity was 6% in the present study, while 11.6% in Lokeswarareddy et al., 14% in Sharma et al.

The present study has 70% of cases with ADA > 40 IU/L, similar to Modi et al., with 72.38%. Kate et al. showed a high value of 93.33%.

In the present study ADA > 40 IU/L seen in 64 out of 66 TPE accounting to 96.9%. In A. Lokeswara Reddy et al study 88.3%, Modi et al (81.93%). No elevated ADA among parapneumonic effusions similar to A. Lokeswara Reddy et al' study while Modi et al., study has 4 PPE with elevated ADA.

Among malignant effusions 6 out of 10 had ADA > 40 in the current study and 4 out of 17 cases in Modi et al. study. A. LokeswaraReddy.et.al study, there were no ADA elevation among malignant effusions.

The high positivity for ADA suggesting tubercular effusion in our study can be due to the high prevalence of TB in India and Southeast Asian countries than in the West. In India, tubercular effusion is the commonest cause of all exudative effusions. This observation is different from the Western studies, where the incidence of PPE effusion and malignant effusion are much higher compared to tubercular effusion.

In our study TB is common among straw coloured exudative pleural effusion accounting to 66%, which is similar to A.LokeswaraReddy.et.al 60%, while its 79% in Modi et al. study.

24% are parapneumonic in the study similar to Lokeswara Reddy et al., 21.9% in Modi et al. study. 10% are malignant effusions, 7% in Lokeswara Reddy et al., 16.19% in Modi et al.

Sensitivity of ADA in the study is 94%, and specificity is 60%.Modi et al. study ADA have a sensitivity of 89.47% and specificity of 48.28%. In Kate et al. study, ADA has sensitivity and specificity of 93.3% and 90%, respectively. Pleural fluid ADA is valuable biochemical marker having high sensitivity for TB diagnosis.

CBNAAT positivity detected in our study is 30%, Chakraborty et al (32%), Ghosh et al (45.3%) Sharma et al (18.5%), Zainul et al(31%),Shukla et al (20.58%).

Sensitivity of CBNAAT in our study is 40% similar to Zainul et al study (40.7%). Low sensitivity of CBNAAT in pleural effusions may be due to paucibacillary nature of specimen. So, in patients with negative CBNAAT test, tuberculous etiology of pleural effusion cannot be ruled out with the degree of certainty. The specificity of CBNAAT was 82% in our study, in Zainul et al study it is 86.6%.

## 5. Conclusion

1. Pleural effusions are the most commonly encountered disease in medical practice posing a diagnostic difficulty. In developing countries like India, TB is the most common cause of straw-colored exudative lymphocyte-predominant effusions.
2. Estimation of ADA in pleural fluid is a simple, rapid, and less expensive laboratory investigation where the diagnosis is uncertain by other investigations .The sensitivity of ADA, when combined with lymphocyte-predominant exudates, helps to diagnose tubercular effusions.
3. The role of CBNAAT in diagnosing pleural TB is limited due to its poor sensitivity. In CBNAAT detected tubercular effusions there is added advantage of detecting Rifampicin resistance.
4. No single test available at present is able to diagnose tubercular effusions . Thus combining

clinical, radiological, pleural fluid analysis along with ADA levels itself can diagnose most of the tubercular effusions.

## 6. Source of Funding

None.

## 7. Conflict of Interest

None.

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**Cite this article:** Srinidhi R, Mathangi K, Kumar KR. **Role of ADA and CBNAAT(Cartridge Based Nucleic Acid Amplification Test) in diagnosis of Tuberculosis in straw coloured exudative pleural effusion in patients attending Government General Hospital, KAKINADA.** *IP Indian J Immunol Respir Med* 2020;5(3):152-157.