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Combined Use Of Pleural Fluid Adenosine Deaminase, Cytology, Pleural Biopsy And Pleural Fluid AFB Smears And CBNAAT In The Diagnosis Of Tubercular Pleural Effusion

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ABSTRACT
Introduction: *Tuberculosis is one of the leading cause of morbidity and mortality worldwide affecting more than 8 million persons annually with 2-3 million deaths. Tubercular pleural effusion is the 2nd most common extra pulmonary manifestation of tuberculosis.*
Materials and Methods: *Patients with signs and symptoms of pleural effusion were admitted. Routine investigations including chest X-ray, hemoglobin, total leukocyte count, differential leukocyte count, erythrocyte sedimentation rate, urea, sugar, etc were done. Specific investigations like sputum for acid fast bacilli, diagnostic thoracocentesis , pleural biopsy and CBNAAT were done. Pleural fluid was examined for its color, protein, sugar, cytology, total leukocyte count, differential leukocyte count, malignant cells, adenosine deaminase (ADA), lactate dehydrogenase and microorganisms. Pleural biopsy was attempted and biopsy specimen was sent for histopathology examination.*
Results and Discussion: *Study was conducted in 60 patients of pleural effusion. 42 cases had adenosine deaminase levels >40 U/L and 53 cases had lymphocyte predominance in pleural fluid cytology. Pleural biopsy by abrams needle done in 25 cases, 13 cases showed epithelioidcaseous granuloma and 22 cases had CBNAAT Positive,in 6 cases had pleural fluid smear AFB seen.*
Conclusion: *Lymphocyte predominant exudates with high ADA value ≥40 U/L and caseous granuloma on pleural biopsy are highly suggestive of tubercular pleurisy. Combined assessment of ADA, cytology, pleural biopsy and CBNAAT is more helpful in diagnosing tubercular pleural effusion rather than individual investigation*

Keywords*- Pleural effusion, tuberculosis, ADA, pleural biopsy, CBNAAT*

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I. INTRODUCTION

Tuberculosis (TB) is one of the deadliest infectious diseases caused by Mycobacterium tuberculosis. It is one of the top 10 causes of death worldwide. Approximately a third of the world's populations who are infected with Mycobacterium tuberculosis are at risk of developing TB disease. Pulmonary TB is the most common form of TB, with extrapulmonary tuberculosis (EPTB) in the form of pleural effusion, accounting for ~15% of cases. It may increases to 50% in high HIV prevalence settings. [1]

Mycobacteria infect the pleura and pleural space. This initiates the delayed hypersensitivity reaction resulting in the increase in fluid formation and decreases its removal.[2]There is increase in neutrophilic infiltrate initially followed by lymphocyte driven immune reaction and granuloma formation. This further increases the release of adenosine deaminase (ADA). Diagnosis of EPTB is a challenge due to the paucibacillary and non-uniform distribution of microorganisms and the variable clinical presentation. Clinical guidelines for diagnosis alone can lead to over-diagnosis and treatment which can increase the resistant strain and also the mortality and morbidity of the patient

The gold standard for diagnosis of tuberculous pleural effusion (TPE) depends on the demonstration of tubercle bacilli in pleural fluid either by culture or AFB positive and granuloma formation in pleural biopsy specimen and fluid. Each test has its own limitations. Due to paucibacillary nature of pleural fluid, direct lung involvement may not occur, and is found to be positive in less than 5% of cases.[3] Culture of pleural fluid also

has low sensitivity (24–58%) and time consuming as it takes approximate 2 to 8 weeks.[4,5]Thoracoscopic pleural biopsy is an invasive, time consuming procedure and is associated with risk, with a sensitivity ranging from 93 to 100%. [6-9] Rapid identification is essential for early treatment initiation and improved patient outcome. To overcome above limitations newer methods has been developed. One among many is GeneXpert MTB/RIF assay, a fully automated quantitative real-time hemi-nested PCR which can detect Mycobacterium tuberculosis complex directly from clinical samples and also rifampicin susceptibility in less than 2 hours. It is recently endorsed by the WHO as a rapid test for both smear-positive and smear-negative (paucibacillary) respiratory samples.[10,11] It is not prone to cross-contamination, requires minimal biosafety facilities, can be performed by technicians with little training; However, a recent meta-analysis reported the pooled sensitivity and specificity of GeneXpert in TPE as 46.4% and 99.1% respectively, compared with those of pleural fluid mycobacterial Culture.[12] However, there are limited data about the Xpert MTB/RIF assay using pleural fluid. The most widely used diagnostic marker for TPE is the pleural fluid adenosine deaminase (ADA) level. ADA testing also gives same day result. It is a relatively easy, inexpensive and rapid, with pooled sensitivity and specificity estimates of 92% and 90%, respectively.[13] Since it is biomarkers of the inflammatory process in the pleural space and thereby do not confirm the etiologic agent. So combinations of tests seem to perform better than any single test. Therefore, the present study was done to evaluate and compare the combined role of Adenosine deaminase, cytology of pleural fluid, pleural biopsy, pleural fluid AFB smears and CBNAAT in diagnosing tuberculous pleural effusion.

II. Material and Methods

The study population consisted of 60 suspected patients of tuberculous pleural effusion admitted in Chest & TB ward of NATIONAL INSTITUTE OF MEDICAL SCIENCES AND RESEARCH, JAIPUR from July 2022 to December 2022. Pleural fluid samples were collected for routine microbiology, biochemical and cytological analysis and pleural biopsy was also done. The samples were processed as follow:

Adenosine deaminase activity in pleural fluid was determined by colorimetric technique . Pleural fluid ADA levels greater than 40 U/L, were reported as suggestive of pleural TB.

CBNAAT - 1 ml aliquot of raw pleural fluid and a 1 ml aliquot of concentrated pleural fluid (Prepared by centrifugation of 10-20 ml pleural fluid at 3000×g for 15 min, with the supernatant discarded and the pellet made up to 1 ml with phosphate buffer solution) from each patient was diluted with 2 ml of the Xpert MTB/RIF sample buffer. It was mixed vigorously and incubated at room temperature for 15 min, finally run on the GeneXpert machine.

For Cytology the slides were made from centrifuged deposits and stained with Giemsa. The total count was done by using modified neaubaur chamber and differential count was done on giemsa stained slides. Acid fast bacilli staining results were also documented.

PLEURAL BIOPSY-The skin is cleaned and local anesthetic is administered . A small incision is made over the area to insert the Abrams biopsy needle and the stylet is placed in the inner cannula, which in turn is placed in the outer trocar. The needle is pushed into the pleural space in closed position and the nob inferiorly by exerting firm pressure on the stylet. Once the tip of needle is felt to be in the pleural space, remove the stylet and with the inner cannula in the closed position, a 20 ml syringe is attached to the connection. The inner cannula is rotated counterclockwise to open the distal notch and by making the nob on outer trocar looking inferior. The biopsy needle is then slowly withdrawn with constant aspiration until it hooks onto the pleura. Then the outer trocar is held firmly with one hand while, the inner cannula is rotated into the closed position with the other hand to cut off a small piece of parietal pleura. Once the initial biopsy specimen obtained, the needle is withdrawn in a closed position. The pleural biopsy specimen is collected from tip of needle. At least three bits of pleural tissue should be obtained from the inferior and lateral aspects. The samples are then sent for histo-pathological examination in 10% formalin. Then small adhesive bandage should be placed over the biopsy incision in a crosswise fashion.

Inclusion criteria

1. Patients of all age and sex with suspected tubercular pleural effusion (i.e. sign and symptoms an acute febrile illness characterised by cough and Pleuriticchest pain, night sweats, chills, weakness, dyspnea, haemoptysis and weight loss)
2. Chest X ray findings of pleural effusion.

Exclusion criteria

1. Patients on anti-tubercular therapy.
2.Diagnosed case of carcinoma of any site.
3.Transudative pleural effusion

III. Objectives

1. To determine the role of CBNAAT in the diagnosis of tubercular pleural effusion
2. To study the association between pleural fluid CBNAAT, ADA and lymphocytes percentage in cytology and biopsy.

IV. Results

There were 38 male (63.3%) and 22 (36.6%) female patients, with a mean age of 40 years. Cough and low grade fever was the most common symptoms followed by weight loss and loss of appetite. CBNAAT was positive in 22 patients (36.6%). ADA was increased (>40IU) in 42 patients (70%) with a mean value of 65IU. 20 cases who were CBNAAT positive, had increased ADA value. All CBNAAT positive cases had predominatlylymphoctyes in pleural fluid cytology. AFB staining done AFB positive in 6 cases (10%). Pleural biopsy done in 25 cases and epithelioid cell granuloma with caseous necrosis was seen in 13 cases (52%).

TABLE 1: GENDER DISTRIBUTION

TOTAL	MALE	FEMALE
60	38	22

TABLE 2: RESULT OF DIFFERENT PARAMETERS

PARAMETERS	POSITIVE	NEGATIVE
ADA	42	18
CYTOLOGY (PREDOMINANTLY LYMPHOCYTES)	53	7
AFB	6	54
PLEURAL BIOPSY	13	12
CBNAAT	22	38

TABLE 3: CLINICAL FEATURES IN STUDY POPULATION

SYMPTOMS	NO. OF CASES	PERCENTAGE
COUGH	48	80%
CHEST PAIN	43	71.6%
FEVER	38	63.3%
SOB	34	56.6%
LOSS OF APPETITE	31	51.6%
WEIGHT LOSS	22	36.6%

Table 4: ADA level in pleural fluid

PLEURAL FLUID ADA (IU/L)	NO. OF CASES	CBNAAT MTB DETECTED	CBNAAT MTB NOT DETECTED
>40IU/L	42	20	22
<40IU/L	18	2	16
TOTAL	60	22	38

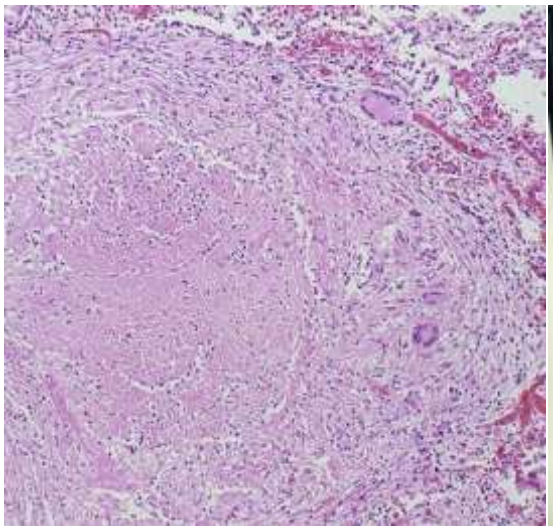

IMAGE 1: CASEOUS NECROSIS

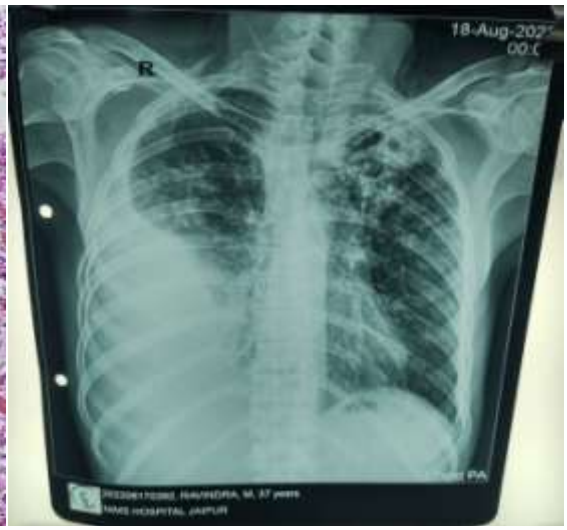

IMAGE 2: CHEST X-RAY RT.SIDE PLEURAL EFFUSION

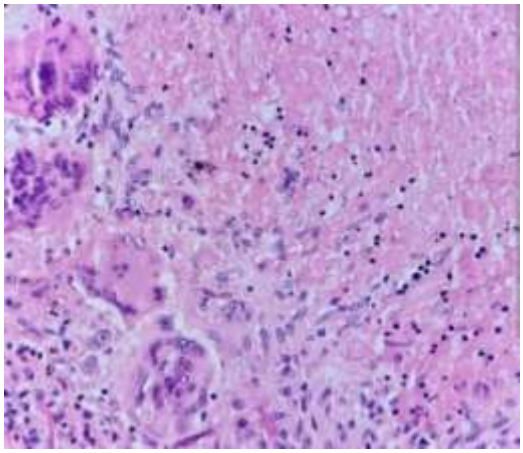

IMAGE 3: EPITHELIOD CELLS GRANULOMA

V. Discussion

TB remains one of the most frequent causes of pleural effusions in developing countries like India. The gold standard for the diagnosis of tuberculouspleuritis remains the detection of M. 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 AFB. The results of present study show that Gene Xpert MTB assay play significant role in routine tuberculous pleural effusion diagnosis. The result is available in same day with high specificity. But it cannot be used alone for the diagnosis of TPE, given its low sensitivity. So it cannot be used alone. Globally the use of GeneXpert assay has resulted in an increase in the number of positive results by 16.5% and this increase has been more important for the extra-pulmonary specimens especially the body fluids[14].

In this study of 60 patients, 38 (63.3%) are males and 22(36.6%) females with sex ratio 1.7:1(M: F). The male predominance seen in our study (63.3%) is similar to studies done by Modi et al.[17](73.33%), AnushreeChakraborthy et al.[16](76%), Shubham Kumar Sharma et al.[15](78%) and Kate et al.[20] (72%). Male predominance is nearer to the study done by Sharma et al.[15]

The most common presenting symptom is cough (80%) in the study, similar to that of AnushreeChakraborthy et al.[16](89%), Lokeswara Reddy et al.[18] (73.3%) whereas the most common presenting symptom is a fever in Shukla et al.[15] and Sachinkate et al.[20] The second most common symptom in our study is chest pain (71.6%), similar to Sharma et al. study(71.5%). In the present study, fever accounts for (63.3%), which is comparable to Lokeswarareddy et al.[18](70%). The other symptoms, like loss of weight and loss of appetite are similar to AnushreeChakraborthyet al.[16], indicating that the disease is a chronic process.

Lymphocyte predominance in the study (88.3%)is similar to study by Kate et al.[20] (90%), and chakraborthy et al.[16] (97%). The lymphocyte percentage in our study is closer to kate et al.[20] study. High lymphocyte predominance in exudative effusions favors tubercular etiology, which is further confirmed by ADA levels.

The present study has 70% of cases with ADA>40IU/L, similar to Modiet al.[17], with 72.38%. Kate et al.[20] showed a high value of 93.33% and in A. LokeswaraReddy et al.[18] study 88.3%

CBNAAT positivity detected in our study is 36.6%, Chakraborthy et al.[16] (32%), Sharma et al (18.5%), Zainul et al.[22](31%),Shukla et al.[15] (20.58%).

In our study, pleural fluid CBNAAT was found to be positive in 36.66% of cases. In a well-structured meta-analysis of 24 studies from India, it was seen that the sensitivity of CBNAAT in TPE was between 22.7–51.4% .[21]Shukla et al.[15] found that sensitivity of genexpert in TPE was 20.58% in their study. Rifampicin resistance was detected in 21% of cases. They found a positive correlation with high ADA values, pleural fluid lymphocyte counts and MTB detection by genexpert. [15]

VI. Conclusion

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.

Estimation of ADA in pleural fluid is a simple, rapid, and less expensive laboratory investigation. The sensitivity of ADA, when combined with lymphocytepredominant exudates, helps to diagnose tubercular effusions. However,Low levels of ADA in the pleural fluid may be found, giving rise to a false negative result. Conversely, raised ADA levels may be observed in a number of conditions potentially leading to a false positive

diagnosis of TB. These include rheumatoid effusion, empyema due to other bacteria, mesothelioma, lung cancer, parapneumonic effusion, and haematological malignancies

Rapid identification is essential for early treatment initiation and improved patient outcome. For this purpose we can use CBNAAT but it has low sensitivity.

Pleural biopsy should be a routine complementary diagnostic procedure to be taken up in patients with exudative pleural effusions. Because of its low complication rate, high diagnostic yield, simplicity of operation and minimal discomfort to the patient, pleural biopsy may be included as one of the initial diagnostic procedures for the diagnosis of tubercular pleural effusions.

No single test available at present is able to diagnose tubercular effusions. Thus combining,clinical, radiological, pleural fluid cytology along with ADA levels, CBNAAT, Pleural biopsy and AFB detectioncan diagnose most of the tubercular effusions.

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Conflict of Interest
None

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