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The role of genexpert in the diagnosis of tubercular pleural effusion in India

Abstract

Introduction: Tubercular pleural effusion is the second most common extrapulmonary form of tuberculosis in India. Developing nations like India face several health challenges and with limited resources, appropriate planning and channelization of the same is the need of the hour.

Material and methods: The objective of the study was to determine the role of cartridge-based nucleic acid amplification test (CBNAAT) in the diagnosis of tubercular pleural effusion (TPE) and also to study if any association exists between CBNAAT and pleural fluid adenosine deaminase (ADA) and lymphocyte counts. Clinically suspected TPE, lymphocyte predominant (≥ 70%) exudates (according to the Lights criteria) with ADA ≥ 40 U/L and microbiologically confirmed pulmonary tuberculosis patients with a co-existent pleural effusion were included. Pleural fluid CBNAAT was performed on all the samples.

Results: Out of a total of 75 patients, 57 were males and 18 were females. A lymphocyte predominance of ≥ 70% was seen in 73 subjects (97%). Mean ADA was 61.7 U/L ± 16.2 (SD). Pleural fluid CBNAAT was positive for *Mycobacterium tuberculosis* (MTB) in 24 patients (32%). Out of these patients, rifampicin resistance was detected in 2 individuals (8.3%). Sputum smear for acid fast bacilli (AFB) was positive in 3 (4%) patients, whereas in sputum CBNAAT MTB was detected in 8 (10.6%) persons. Association between pleural fluid ADA, lymphocyte count and CBNAAT positivity was evaluated by Student T-test. There was a significant association between higher ADA levels and CBNAAT (p value = 0.001).

Conclusions: Pleural fluid CBNAAT, owing to its low sensitivity, should not be included in the diagnostic protocol of TPE in high prevalence areas. A high ADA ≥ 40 U/L in combination with Light's criteria to define exudates, with lymphocyte predominance is sufficient evidence to diagnose TPE and initiate anti-tubercular therapy, thereby deferring the need to perform an invasive pleural biopsy.

Key words: ADA, pleural fluid genexpert, CBNAAT

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Introduction

Extrapulmonary tuberculosis accounts for about 25% of active TB cases, with increasing incidence in the immunocompromised and children [1]. After PTB, peripheral lymphatic TB predominates (47.2%), followed by tubercular pleural effusion (35.1%) [1]. Difficult sampling and paucibacillary nature of the disease contribute to the diagnostic challenges that physicians face. Gene Xpert Mycobacterium Tuberculosis/ /Rifampicin Assay (Xpert, Cepheid, Sunnyvale, CA, USA), a fully automated cartridge-based nucleic acid amplification test has resulted in a paradigm shift in the management of tuberculosis. This is a rapid, semi-quantitative nucleic acid amplification test (NAAT) with a definite role in the diagnosis of pulmonary tuberculosis. However, its role as a "routine must do test" in the diagnosis of pleural effusions is still unclear. Definitive diagnosis of tubercular pleural effusion (TPE) still depends on demonstration of *Mycobacterium tuberculosis* or caseous granulomas in pleural biopsy. However, they are laborious, time-consuming and insensitive, when done blindly, and hence require expertise. Very high specificity

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of the pleural fluid cartridge-based nucleic acid amplification test (CBNAAT) makes it an excellent rule in test despite its low sensitivity, since it obviates the need for an invasive procedure in at least one-fourth of patients with TPE [2]. However, we are of the opinion that in a country like India where the prevalence of tuberculosis is very high, channelizing the limited resources to diagnose pulmonary tuberculosis, which is more communicable is the need of the hour. We believe that a high adenosine deaminase (ADA) (> 40 U/L) combined with Light's criteria to define exudates in a lymphocyte predominant effusion constitutes enough evidence to diagnose TPE and initiate anti-tubercular therapy (ATT). Pleural biopsies and pleural fluid CBNAAT may be reserved for patients with a low ADA and those in whom an alternate diagnosis seems more likely.

Materials and methods

The study was conducted in the Department of Pulmonary Medicine, SDS TRC and RGICD on 75 patients with tubercular pleural effusion. Patient's demographic and anthropometric data was collected. Functional status assessment was done using the Karnofsky score. Diagnostic thoracocentesis was performed and pleural fluid was sent for biochemical, cytological and microbiological (smear for acid fast bacilli-AFB) analysis. 10 mL of pleural fluid was sent for CB-NAAT. All patients were started on anti-tubercular therapy and were followed up with a chest X-ray to document the response to ATT at an interval of two months till completion of their treatment. On a chest X-ray, pleural effusions were categorized into mild (less than one-third of the hemithorax), moderate (one-third to two-third of the hemithorax) and massive (more than two-thirds of the hemithorax). Two experienced pulmonologists with six years of experience each, reviewed the radiographs independently and reached a decision on the final interpretation by consensus. Ethical clearance was obtained from the institutional ethical committee. Descriptive and inferential statistical analysis was carried out. The results of continuous measurements like age are presented on Mean SD (min-max) and Median (IQR). The outcomes of categorical measurements are presented as numbers and percentages (%). Student t test was done to determine the association between CBNAAT and pleural fluid ADA, lymphocyte count individually. A p value of < 0.05 was considered significant. The statistical software, namely SPSS 18.0 was used for the analysis of the data.

Inclusion criteria

1. Patients willing to give a written informed consent.
2. All patients aged > 18 with a history compatible with tuberculosis and radiological evidence of pleural effusion on a chest X-ray.
3. Exudates with lymphocyte predominance (70%) (exudates defined according to the Light's criteria) with ADA ≥ 40 U/L.
4. Sputum AFB positive patients or sputum CBNAAT positive patients with a co-existent pleural effusion.

Exclusion criteria

1. Exudative pleural effusions with neutrophil predominance or lymphocyte predominant with ADA <40 U/L.
2. Transudative pleural effusions.
3. Unresolved pleural effusions at the end of six months of ATT.

Objectives

1. To determine the role of genexpert in the diagnosis of tubercular pleural effusion.
2. To study the association between pleural fluid CBNAAT and pleural fluid ADA.
3. To study the association between pleural fluid CBNAAT and pleural fluid lymphocyte counts (%).

Results

A total of 75 patients were selected for the study, and all of them had a complete resolution of pleural effusion at the end of six months of ATT. There were 57 male and 18 female patients, with a mean age of 36 years ± 13 (SD). The mean body mass index (BMI) was 19.58kg/m^2 ± 3.88 (SD) which, as per the World Health Organization (WHO) BMI recommendation for Asians was within the normal range [3]. The functional status of the patients was assessed using the Karnofsky score and the mean score was 83.64 ± 10.3 (SD), indicating good functionality. The score was low in patients with comorbid conditions like chronic obstructive pulmonary disease (COPD) and ischemic heart disease (IHD). Anemia was the most commonly associated comorbidity. Other comorbidities are listed in Table 1.

67 (89%) patients presented with cough as the predominant symptom. Only 14 (18.6%) subjects complained of minimal expectoration, whereas 53 (81.4%) had a dry cough. 46 (61%) patients had chest pain and 42 (56%) had dyspnea. None of the patients presented with hemoptysis. Among

Table 1. Demographic profile of the patients

Total number of patients	75
Males	57 (76%)
Females	18 (24%)
Mean age at presentation	36 ± 13 (SD)
Mean BMI	19.58 kg/m^2 ± 3.88 (SD)
Mean Karnofsky score	84 ± 10.3 (SD)
Co-morbid conditions	35 (80.6%)
Anaemia (haemoglobin < 10 g/dL)	18 (51.4%)
Chronic obstructive pulmonary disease	5 (14.2%)
Diabetes mellitus	2 (5%)
Hypertension	2 (5%)
Ishemic heart disease	2 (5%)
Immunocompromised state	3 (8%)
Cor-pulmonale	2 (5%)
Chronic liver disease	1 (2%)

BMI — body mass index

the constitutional symptoms, fever was the most common symptom, seen in 57 (76%) persons. 31 (41%) and 28 (37%) patients experienced loss of appetite and weight, respectively (Table 2).

Light's criteria were applied to all the pleural effusions to determine their nature. The mean pleural fluid protein and lactate dehydrogenase (LDH) was 5.07 g/dL ± 0.82 and 690 U/L ± 316.5, respectively. Median pleural fluid total cell count was 645 cells/cu.mm (IQR=726). A lymphocyte predominance of ≥70% was seen in 73 patients (97%). The mean pleural fluid sugar was 80.5 mg/dL. Mean ADA was 61.7 U/L ± 16.2. Pleural fluid CBNAAT was positive for MTB in 24 subjects (32%). Out of these patients, rifampicin resistance was detected in 2 individuals (8.3%).

Sputum AFB smear was positive in 3 (4%) patients, whereas in sputum, CBNAAT MTB was detected in 8 (10.6%) subjects. Among them, one had rifampicin resistance (Table 3, 4).

Radiological quantification using the aforementioned descriptors revealed mild effusion in 34 (45.3%) and moderate in 35 (46.6%) people. Massive effusion was seen only in 6 (8%) patients. Right-sided effusion were predominant and was observed in 44 (58.6) patients. 26 (34.6 %) subjects had left-sided effusion, whereas 5 (6.6%) had bilateral effusion. On reviewing the chest X-rays for other radiological abnormalities, consolidation was found in 11 (14.7%) patients and 2 (2.6%) individuals had miliary nodules.

Table 2. Symptoms at presentation

Symptoms	Number of patients
Cough	67 (89%)
Sputum	14 (18.6%)
Dyspnea	42 (56%)
Chest pain	46 (61%)
Fever	57 (76%)
Loss of weight	28 (37%)
Loss of appetite	31 (41%)

Table 3. The mean ± SD values of pleural fluid parameters (biochemical and cytology)

Pleural fluid parameter	Mean	± SD
Protein	5 g/dL	0.82
LDH	690 U/L	316.5
ADA	61.7 U/L	16.2
% of lymphocytes	95	13.35

ADA — adenosine deaminase; LDH — lactate dehydrogenase

Table 4. Pleural fluid and sputum CBNAAT status and rifampicin resistance

CBNAAT	MTB detected (%)	Rifampicin resistance (%)
Pleural fluid	24 (32%)	2 (6%)
Sputum	8 (10.6%)	1 (12.5%)

CBNAAT — cartridge-based nucleic acid amplification test; MTB — *Mycobacterium tuberculosis*

Association between pleural fluid ADA, lymphocyte count each individually with CBNAAT positivity was evaluated. The mean ADA value in the group where CBNAAT was positive for MTB was 70.79 ± 15.69, and it was significantly higher with a p value of 0.001. In case of CBNAAT positivity and pleural fluid lymphocyte count, there was no significant association (Table 5).

Discussion

The sensitivity of CBNAAT depends on the type of sample on which it is performed. Considering the cost and resources involved in performing the test, its judicious use lies in the hands of the treating physicians. The role of ADA in diagnosing TPE has been proved beyond doubt and is used as the sole criteria with other bioche-

Table 5. Comparison of ADA and lymphocyte count with CBNAAT positivity in pleural fluid

Variables	CBNNAT		Total	P-value
	No	Yes		
ADA	57.44 ± 15.12	70.79 ± 15.69	61.83 ± 16.46	0.001
LDH	594.69 ± 222.47	816.08 ± 460.98	667.48 ± 334.43	0.007
Lymphocyte count (%)	94.94 ± 16.53	95.13 ± 8.98	95.00 ± 14.42	0.959

ADA — adenosine deaminase; CBNAAT — cartridge-based nucleic acid amplification test; MTB — *Mycobacterium tuberculosis*
LDH — lactate dehydrogenase

mical and cytological characteristics. Owing to its high negative predictive value (NPV) and positive predictive value (PPV) in areas of high prevalence, it remains a test of great clinical utility. It seldom rises beyond 40 U/L in non-tubercular effusions, thereby increasing its specificity. That is, ADA < 40 excluded tuberculosis in 99% of cases [5]. Specificity of ADA in low prevalence areas has also been estimated. In a study done in north-western Europe, 338 patients with effusions were analyzed, and when an ADA cut-off of 35 U/L was used in combination with lymphocytic effusions, sensitivity was 90.9% and specificity was 98.9% [6].

In our study, pleural fluid CBNAAT was found to be positive in only 32% of cases, and rifampicin resistance was seen in 8.3%. 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% using a composite reference standard (CRS) and pleural fluid culture as the reference standard [2].

In another meta-analysis determining the role of genexpert in the diagnosis of extrapulmonary tuberculosis, a total of 18 studies were analyzed with 4461 samples. Pooled sensitivity for pleural fluid was 46.4% against culture and 21.4% against CRS (composite reference standards — another NAAT, histology, smear, biochemical testing, response to ATT) — 14 studies and 841 samples were included in the data. They attributed the poor sensitivity to the paucibacillary nature of the disease and the presence of polymerase chain reaction (PCR) inhibitors in fluid and blood contamination. They also suggested that concentration of the pleural fluid prior to analysis increased the sensitivity [4, 7, 8]. However, in another meta-analysis, Meldau *et al*. compared the diagnostic utility of ADA, genexpert and gamma interferon (IFN-γ), and put forth conflicting findings. They found that poor sensitivity of Xpert was neither due to sub-optimal detection (requires ≥ 75 CFU/mL) compared to other types of biological samples nor due to increased PCR inhibition (as there was no evidence of PCR inhibition using internal positive control). These data confirmed the notion that TPE is a paucibacillary disease and concentration of fluid for Xpert did not make any difference in improving its sensitivity. They proposed IFN-γ to be a more sensitive rule in and rule out test than ADA in high prevalence settings, and finally, concluded that either of the two could be used to guide therapy, as routine pleural biopsy may be challenging in high prevalent, resource-limited countries [9].

We also studied the association between pleural fluid CBNAAT MTB detected, with pleural fluid ADA and lymphocyte counts. There was a significant connection between CBNAAT positivity and ADA level (p-value = 0.001). In a study from India, sensitivity of genexpert in TPE was 20.58%. 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 ($r = 0.92$, $r = 0.97$, respectively with $p < 0.05$) [10].

In our study, sputum AFB smear was positive in 3 (4%) patients, whereas in sputum, CBNAAT MTB was detected in 8 (10.6%) individuals. Among these 8 patients, MTB was discovered by CBNAAT in pleural fluid in 6 (75%), and 7 (87.5%) had consolidation in their chest X-rays. Hence we are of the opinion that patients having consolidation along with pleural effusion and sputum MTB detected in CBNAAT may have higher possibility of MTB detection in pleural fluid CBNAAT. Similar observation has been made in an Indian study done on 106 subjects. They found that in patients with pleural effusion with underlying lung consolidation, the overall sensitivity of PCR for MTB was 92.8% for diagnosing TB pleural effusion [11].

Conclusions

Lymphocyte predominant effusions with high ADA levels (> 40 U/L) with a compatible

clinical presentation, where alternate diagnosis seems unlikely are treated as tubercular pleural effusions in India. This remains the standard of care even to this day. Given the fact that performing a pleural biopsy is not feasible at all health care centers, and also considering the invasive nature of this diagnostic procedure, it is not put into routine clinical practice. Sensitivity of ADA when combined with lymphocyte predominant exudates, in high prevalence areas has stood the test of time in deciding the initiation of ATT. Low and variable sensitivity of pleural fluid CBNAAT, as shown again in our study, should probably make us reconsider our decision about performing this test routinely (or including it in diagnostic protocol). Timely diagnosis of PTB (drug sensitive and resistant) is the need of the hour, and we are of the honest opinion that the available resources have to be channelized in doing the same. We suggest that pleural fluid genexpert should be performed only when the diagnosis is in doubt and can change the patient management.

Conflict of interest

None declared.

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