Section: Pulmonary Medicine

Original Research Article

ROLE OF CARTRIDGE BASED NUCLEIC ACID AMPLIFICATION TEST, ADENOSINE DEAMINASE LEVEL AND CYTOLOGY IN DIFFERENTIATION OF TUBERCULAR AND NON-TUBERCULAR PLEURAL EFFUSION IN A TERTIARY CARE HOSPITAL OF CENTRAL INDIA

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Received : 12/10/2023
Received in revised form : 15/11/2023
Accepted : 27/11/2023

Keywords:
CBNAAT, ADA, Tuberculous pleural effusion.

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DOI: 10.47009/jamp.2023.5.6.137

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (6); 667-670

Abstract

Background: Pleural effusion result from surrounding parenchymal disease of lung with tuberculosis being the leading cause in India. It is important to differentiate tuberculous pleural effusion from non-tuberculous effusion with the help of various diagnostic tools available. Therefore, the present study was done to evaluate and compare the role of Gene Xpert, Adenosine deaminase level and cytology in differentiating tuberculous from non-tuberculous pleural effusion. **Materials and Methods:** This study is retrospective analysis done on 200 patients attending respiratory medicine OPD with pleural effusion. This fluid was then subjected to routine analysis, ADA and CBNAAT and thereafter correlation was found out between them to differentiate between tubercular or non- tubercular. **Result:** Out of 200 samples, fluid was exudative in 152 samples (76%). CBNAAT was positive in 53 patients (26.5%) and ADA was raised (>40.0U/L) in 118 patients with mean value of ADA being 75 U/L. Of the total 68 CBNAAT negative patients with raised ADA level, fluid was lymphocyte rich and exudative in 50 samples making it 73.52% of total raised ADA and CBNAAT negative patients and as per defined criteria these patients were considered as tubercular effusions. **Conclusion:** This was a retrospective study based on the differentiation of tubercular and non-tubercular effusion with the help of CBNAAT, ADA and cytology. CBNAAT in addition with ADA is more useful in the rapid diagnosis of tubercular effusion.

INTRODUCTION

Pleural effusion is the collection of fluid in between the parietal and visceral pleura. It can be the result of surrounding parenchymal disease like malignancy, inflammatory or infectious conditions. It accounts for one of the major cause for mortality and morbidity.[1-3]

Among all aetiology, tuberculosis is always the leading cause of pleural effusions in the developing countries.[4] Tuberculous pleural effusion (TPE) is caused by acid fast bacilli Mycobacterium tuberculosis infection of the pleura leading to chronic accumulation of fluid and inflammatory cells in pleural cavity. TPE is the 2nd most common form of extrapulmonary tuberculosis and one of the common causes of pleural effusions in endemic tuberculosis areas.[5] Hence it is important to differentiate tuberculous pleural effusion from non-tuberculous effusion with the help of various diagnostic tools available.

The gold standard for diagnosis of TPE depends on the demonstration of tubercle bacilli in pleural fluid either by AFB positive or culture and granuloma formation in pleural biopsy specimen. Each test has its own limitations.

Culture is found to be positive in less than 5% of cases and direct lung involvement may not occur due to paucibacillary nature of pleural fluid.[6]

Culture of pleural fluid also has low sensitivity (24–58%) and time consuming (approx.2 to 8 weeks).[7,8]

Thoracoscopic pleural biopsy is an invasive, time-consuming procedure and is associated with risk,

with a sensitivity ranging from 93 to 100%.[9–12] Rapid identification is essential for early treatment initiation and improved patient outcome.

Newer methods have been developed to overcome above limitation. One among many is GeneXpert MTB/RIF assay, a real-time semi-nested PCR which can detect Mycobacterium tuberculosis complex directly from clinical samples and also rifampicin susceptibility. It is endorsed by the WHO as a rapid test for both smear-positive and negative (paucibacillary) respiratory samples.[13,14]

Another widely used diagnostic marker for TPE is the pleural fluid adenosine deaminase (ADA) level. ADA is an enzyme in the purine salvage pathway required for converting adenosine to inosine.[15] It is needed for the breakdown of nucleic acids in tissues. ADA value above 40 U/L are known to well correlate with tubercular pathology in various studies and help to differentiate between tubercular and nontubercular pleural effusion.[16]

To treat pleural effusion appropriately, it's important to determine its cause. So, combinations of tests seem to perform better than any single test, especially combinations that include Gene Xpert, adenosine deaminase level and cytology in pleural fluid. Therefore, the present study was done to evaluate and compare the role of Gene Xpert, Adenosine deaminase level and cytology in differentiating tuberculous from non-tuberculous pleural effusion.[16]

MATERIALS AND METHODS

Study Design

This research was a retrospective analysis of routinely collected data. Data were collected at a tertiary referral centre over 12 months, from January 2022 to December 2022 after approval from the Institutional Ethics Committee of the institute.

Participants

The present study includes 200 random samples of pleural fluid collected from patients presenting with symptoms, medical history and radiological picture suggestive of pleural effusion, either admitted or attending OPD of Department of respiratory medicine.

Inclusion Criteria

All clinically diagnosed cases of pleural effusion irrespective of age and sex were included in the study.

Exclusion Criteria

1. All patients who were diagnosed as a case of active tuberculosis
2. On anti-tubercular treatment
3. Diagnosed case of carcinoma of any site
4. Patients not giving consent for thoracocentesis.

Procedure

All patients underwent chest ultrasonography to assess the characteristic of pleural effusion and to mark most readily approachable thoracic site for thoracocentesis. Pleural fluid was collected after informed consent from each patient from point of aspiration marked by chest ultrasonography taking all aseptic precautions. The pleural fluid samples were submitted for CBNAAT, routine microscopy examination including ADA level, pleural fluid smear microscopy after concentration for presence of acid-fast bacilli under light emitting diode based fluorescent microscopy (LED-FM) and cytological analysis.

The CBNAAT was performed using aspirated pleural fluid. The material was mixed with buffer in ratio 1:2 in a pre-sterilized falcon tube and incubated at room temperature for 25 to 30 min. Two mL of this sample was then transferred to an Xpert cartridge using a Pasteur pipette, and the cartridge was loaded onto Xpert (Cepheid, Dx System Version 4.0c) machine. Results were reported as positive or negative for M. tuberculosis. CBNAAT gives a semi-quantitative estimate of the concentration of bacilli as defined by the cycle threshold range (very low – greater than 28; low – 22 to 28; medium – 16 to 22; high – less than 16). Rifampicin resistance results were reported as susceptible and resistant depending upon the presence of a mutation in the rpoB gene. Strains harbouring mutations in the rpoB gene were resistant to Rifampicin.

Adenosine deaminase activity in pleural fluid was determined by colorimetric technique using the user defined method on Transasia EM 360 fully automated biochemistry analyser. Pleural fluid ADA levels less than 40 U/L were considered normal, 40 to 62 U/L were considered as suspected for MTB and greater than 62 U/L were considered as strong suspect for MTB.[16,17]

For Cytology the slides were made from centrifuged deposits and stained with Leishman and Papanicolaou stain. The total count was done by fully automated cell counter and differential count was done on Leishman-stained slides. Fluorescent staining for MTB results were also documented.

In the study, presence of below mentioned criteria was adopted to label a case as tuberculous effusion

1. Bacteriological confirmation of the presence of Mycobacterium tuberculosis in pleural fluid by CBNAAT or Fluorescent stain.
2. Exudative (according to Light's criteria), lymphocytic pleural effusion with ADA>40 U/L.
3. Clinical presentation consistent with TB with the exclusion of other clinical considerations;

Statistical Analysis

Data collected include age, gender, sensitivity & specificity values of ADA, CBNAAT and positivity on microscopy were calculated using standard formulae and using SPSS software version –29.0.10.

RESULTS

Data were collected from 200 patients who had undergone diagnostic thoracocentesis. There were 146 males and 54 females with age ranging from 15 years to 82 years and mean age of 40.43 years. The

ISSN (O): 2687-5365; ISSN (P): 2753-6556

predominant age group affected is 15 to 30 years as shown in the [Table 1].

Out of 200 samples, fluid was exudative in 152 samples (76%) (protein > 3.0 gm/dl). Out of 200 samples, CBNAAT was positive in 53 patients (26.5%) [Table 2] and ADA was raised (>40.0 U/L) in 118 patients [Table 3] with mean value of ADA being 75.75 U/L.

Out of 53 patients who were positive for CBNAAT, ADA level was raised in 50 patients making it 94.3% of total CBNAAT positive patients while 3 patients (5.7%) were having normal ADA level. [Table 4]

Out of 53 patients who were positive for CBNAAT, fluid was polymorph rich in 28 patients (52.8%) and lymphocyte rich in remaining 25 patients (47.2%).

Out of 200 patients CBNAAT was negative in 147 patients. Among these 147 patients ADA was raised in 68 patients making it 46.3% of the total CBNAAT negative patients and ADA level was normal in remaining 79 patients. [Table 5]

Of the total 68 CBNAAT negative patients with raised ADA level, fluid was lymphocyte rich and exudative in 50 samples making it 73.52% of total raised ADA and CBNAAT negative patients and as per defined criteria these patients were considered as tubercular effusions. While in remaining 18 samples, fluid was polymorph rich making it 26.5% of total raised ADA and CBNAAT negative patients and these patients were considered as Empyema due to other bacterial infections.

Of the total 79 samples, who were negative for CBNAAT with normal ADA level, 13 patients were positive for malignant cells in cytology, while remaining 66 patients were negative for malignant cells and considered as parapneumonic effusion.

Out of total 200 patients, 6 patients were positive for fluorescent staining and all these 6 patients were also positive for CBNAAT.

[Table 6] shows the distribution of patients as per the etiological diagnosis.

Table 1: Age wise distribution of study population

Age group	No. of cases	Percent
15-30	76	38.0
31-45	43	21.5
46-60	55	27.5
61-75	21	10.5
76-90	5	2.5
Total	200	100.0

Table 2: CBNAAT Positivity in pleural fluid

CBNAAT	No. of cases	Percent
POSITIVE	53	26.5
NEGATIVE	147	73.5
Total	200	100.0

Table 3: ADA level in pleural fluid

ADA GROUP	No. of cases	Percent
> 40U/L	118	59.0
<40U/L	82	41.0
Total	200	100.0

Table 4: ADA and CBNAAT in pleural fluid

	No. of cases	CBNAAT MTB detected	CBNAAT MTB not detected
ADA > 40 U/L	118	50	68
ADA < 40 U/L	82	3	79
Total	200	53	147

Table 5: Showing correlation between protein level, ADA and CBNAAT.

		Protein level											
		>3 gm/dl;						<3 gm/dl					
		ADA group						ADAGroup					
		> 40 U/L			<40 U/L			> 40 U/L			<40 U/L		
		Lymphocyte group			Lymphocyte group			Lymphocyte group			Lymphocyte group		
		>50 %	<50%	Total	>50 %	<50%	Total	>50 %	<50%	Total	>50 %	<50%	Total
Cbnaat	Positive	24	22	46	1	0	1	0	4	4	0	2	2
	Negative	49	16	65	29	11	40	1	2	3	22	17	39
	Total	73	38	111	30	11	41	1	6	7	22	19	41

Table 6: Distribution of patients according to etiological diagnosis

Aetiology	No. of cases	Percent
Tubercular Effusion	103	51.5%
Malignant Effusion	13	6.5%
Empyema	18	9%
Parapneumonic Effusion	66	33%
Total	200	100.0

ISSN (O): 2687-5365; ISSN (P): 2753-6556

DISCUSSION

Pleural effusion is common pathology of underlying disease of pleura of which TB remains one of the most frequent causes in developing countries like India.

This was a retrospective study based on the differentiation of tubercular and non-tubercular effusion with the help of CBNAAT, ADA and cytology. CBNAAT in addition with ADA is more useful in the rapid diagnosis of tubercular effusion.

In this study of 200 patients, 146 (76%) are males and 54 (24%) are females with sex ratio 2.7:1(M: F). The male predominance seen in our study (76%) is similar to studies done by K. Mathangi et al,[19] (80%) and Anushree Chakraborthy et al (76%).[20] Male predominance is nearer to the study done by Anushree Chakraborthy et al. This indicates that pleural effusion particularly due to tuberculosis is more common in males due to the presence of confounding factors like smoking, migration to high prevalent areas, exposure to outdoor pollution.

The mean age of the present study group is 40.43+ 18.05years. Modi et al study has mean age 43.19±1[21], K. Mathangi et al. has a mean age of 39.88±14.43[19] and Mohammed Zainul et al has mean age of 45.17±14.69.[22] From all these study findings, including the present study, it can be emphasized that TB forms an unavoidable differential diagnosis among 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.

Lymphocyte predominance in the study among the patients with CBNAAT negative and raised ADA level is 73.52% which is similar to study by K. Mathangi et al,[19] (74%) and Modi et al. (67.87%).[21] In our study, all 13 patients with malignant effusion have normal ADA level. Similar finding was seen in study done by Lokeswara Reddy et al.[23]

CBNAAT positivity detected in our study is 26.5% which is similar to study by K. Mathangi et al. (30%),[19] Chakraborthy et al,[20] (32%) and Zainul et al (31%).[22]

33% are parapneumonic effusion in the study similar to K. Mathangi et al (24%) and Modi et al. (21.9%).[19,20]

CONCLUSION

Pleural effusions are the most commonly encountered disease. In developing countries like India, TB is the most common cause of pleural effusions. It is concluded from our study that to know the exact cause of effusion, combination of CBNAAT, ADA level and Cytology plays an important role.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556