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Original Research Article

Diagnostic Value of Pleural Fluid Adenosine Deaminase in Tubercular Pleural Effusion Diagnosis: A Prospective Observational Study

Abhilok Kumar Jha[1], Praveen Kumar Singh[2], Umesh Chandra Jha[3]

[1]Senior Resident, Department of Medicine, Darbhanga Medical College and Hospital, Laheriasarai, Bihar

[2]Assistant Professor, Department of Medicine, Darbhanga Medical College and Hospital, Laheriasarai, Bihar

[3]Associate Professor, Department of Medicine, Darbhanga Medical College and Hospital, Laheriasarai, Bihar

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Corresponding Author: Dr. Praveen Kumar Singh

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Abstract:

Background: In clinical practice, diagnosing Tubercular (TB) pleural effusion remains difficult since conventional diagnostic techniques are helpful but insufficiently sensitive and specific.

Methods: This was a prospective observational study carried out at DMCH, Laheriasarai, Bihar with study population of 76 patients. Etiological diagnosis was based on clinical history with radiological imaging, biochemical and cytological examination of pleural fluid. Pleural fluid ADA was used as a biomarker for the diagnosis of tubercular pleural effusion.

Results: The study included 76 patients with 69.7% (n=53) males and 30.3%(n=23) females. The mean age of patients was 48.97±17.03 years. Of 76 cases of pleural effusion, 62 were exudates and 14 transudates. Tuberculosis was the most common cause among exudates which accounted for 51.3% (n=39) of cases. The sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV), Accuracy of pleural fluid ADA in diagnosing tubercular pleural effusion was 92.3%, 97.3%, 97%, 92% and 94.7% respectively.

Conclusion: Pleural fluid ADA can be one of the most reliable biomarkers for the diagnosis of TB pleural effusion considering its high sensitivity and specificity.

Keywords: Adenosine deaminase, Biomarker, Pleural effusion, Sensitivity, Specificity, Tuberculosis.

Introduction

Pleural effusion is a commonly encountered clinical problem by Pulmonologists as well as physicians. The management strategy to be adopted in pleural effusion depends on whether an effusion is a transudate or an exudate. It is very important to establish accurate etiological diagnosis to treat the patient in an appropriate manner as about 15 to 20% of cases remain undiagnosed. [1]

TB is the most common cause of pleural effusion worldwide (30-60%). It is estimated that between 2 to 3 billion people are infected with Mycobacterium Tuberculosis (MTB) worldwide, of whom 5-15% will develop the tuberculosis (TB) disease during their lifetime. [2] It is important to consider the possibility of tuberculosis in all patients with an undiagnosed pleural effusion. [3]

The stepwise diagnosis of TB pleural effusion is same as for any other exudative pleural effusion. An initial diagnostic thoracentesis is always indicated. Definitive diagnosis of Tubercular pleural effusion can be difficult to make because of low sensitivity and specificity of non-invasive diagnostic tools.

The gold standard for the diagnosis of tuberculous pleuritis remains the detection of Mycobacterium tuberculosis bacilli in pleural fluid or pleural biopsy specimens, either by microscopy/ molecular methods (CB-NAAT) and/or culture, or the histological demonstration of caseating granulomas in the biopsy specimen. Results of pleural fluid staining for Acid Fast Bacilli (AFB) are virtually always negative and pleural fluid cultures for mycobacterium are positive in <25% of cases.

Ultrasound guided pleural biopsy is the most useful diagnostic method as it can establish the diagnosis in 95% of the cases in combination with the culture of biopsy specimen. But pleural biopsy cannot be done in every patient of pleural effusion because of

lack of facilities and inadequate or minimal effusion.

Nucleic Acid Amplification Tests (NAAT), which amplify MT-specific nucleic acid sequences with a nucleic acid probe [Polymerase Chain Reaction (PCR)], enable direct and rapid detection of MT in clinical samples, including pleural fluid and tissue biopsies. Unfortunately, both in-house and commercialized NAAT lack enough sensitivity for pleural TB, particularly in smear or culture negative specimens. [4] Thus, a negative NAAT should not be used to rule out TB. In a meta-analysis of 14 studies, the pooled sensitivity and specificity of commercially available automated NAAT for identifying pleural TB was found to be 62% and 98%, respectively. [5]

Xpert-MTB/RIF is a rapid, cartridge-based, fully automated real-time PCR test which simultaneously detects MTB and rifampicin resistant strains in less than two hours with minimal hands-on technical time. A meta-analysis of 24 studies totalling 2,846 patients, determined the accuracy of Xpert-MTB/RIF on pleural fluid samples for detecting TB pleurisy where it was found that the pooled sensitivity and specificity of the test was 51.4% and 98.6% when a culture was used as the reference standard. [6]

The diagnosis of pleural tuberculosis has been greatly improved by the use of biochemical markers, which are rapid and more sensitive. Since first reported in 1978, the measurement of pleural fluid ADA has consistently demonstrated a high accuracy for diagnosing pleural TB. [7,8] Many of the meta-analyses conducted have shown that pleural fluid ADA is highly sensitive and specific in diagnosing tubercular pleural effusion. [9-11]

ADA comprises two isoenzymes, namely ADA1 and ADA2. ADA1 is a ubiquitous enzyme that may be produced by many different cell types, including neutrophils, explains most false-positive cases in non-TB effusions. In contrast, ADA2 is secreted only by monocytes and macrophages and is the predominant isoenzyme (85%) in TB pleural effusion. [12] Although ADA2 slightly increases the sensitivity and specificity of the total ADA in diagnosing TB pleuritis, it probably adds little in the majority of cases. Aims and objectives are to assess the significance of Adenosine Deaminase (ADA) level in the diagnosis of pleural effusion and the sensitivity and specificity of ADA activity in tubercular pleural effusion.

Material and Methods

This prospective observational study done at Department of Medicine, Darbhanga Medical College and Hospital, Laheriasarai, Bihar from October 2020 to September 2021. Total 76 patients with pleural effusion were evaluated over a period of 12 months. All Patients aged >18 years diagnosed with pleural effusion were included. Patients who are unwilling to participate in the study, traumatic pleural tap, suspected pulmonary Thromboembolism, chylothorax and hemothorax were excluded in this study.

Demographic data, detailed clinical history and examination, Chest radiograph and Ultrasound (USG) thorax findings of all the patients during the study were recorded.

Additional investigations like Computed Tomography (CT) of Thorax was done wherever indicated.

Diagnostic/Therapeutic Thoracentesis was done to obtain pleural fluid for analysis. USG guided pleural fluid aspiration was carried out in difficult cases with loculated/septated/minimal pleural effusion.

Pleural Fluid Analysis including levels of protein, sugar, Adenosine Deaminase (ADA), LDH with cytological and microbiological examination was done in all patients. Using Pleural fluid analysis reports, effusion was differentiated as exudate or transudate according to standard Light's criteria.

Exudative pleural effusion with lymphocyte predominance (as by fluid cytology) was diagnosed as Tubercular using pleural fluid ADA analysis (Fluid ADA >40 as probable Tubercular and ADA >70 as definite Tubercular effusion). Patients diagnosed with Tubercular pleural effusion were started on Anti Tubercular therapy (ATT) as per RNTCP guidelines.

Statistical software was the Statistical software namely SPSS 15.0, Stata 10.1, MedCalc 9.0.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate tables.

Results

In this prospective observational study, a total of 76 patients were evaluated during the stipulated study period at a Tertiary Care Hospital. Among 76 patients evaluated, 69.7% (n=53) were males and 30.3% (n=23) were females (Table 1).

Mean age among patients was 48.97±17.03 yrs., while patients with tubercular pleural effusion had a mean age of 41.29±17.96 years. 82% (n=62) of analyzed samples of pleural fluid were exudative and 18% (n=14) were transudative (Table 2), classified according to Light's criteria. Light's criteria had a sensitivity of 100% and specificity of 71.4% in differentiating pleural fluid exudates from transudates.

Table 1: Gender distribution of patients

Gender	No. of patients (n=76)	Percentage
Male	53	69.7%
Female	23	30.3%

Table 2: Type of pleural effusion

Gender	No. of patients (n=76)	Percentage
Exudate	62	81.6%
Transudate	14	18.4%

Table 3: Analysis of pleural fluid parameters in exudative pleural effusions

Variables	Mean±SD
PF glucose	107.92
PF protein	4.24±1.41

ANOVA test, Mean ± SD

Table 4: Analysis of pleural fluid parameters in transudative pleural effusions

Variables	Mean±SD
PF glucose	116.57±69.57
PF protein	1.84±0.66

Mean pleural fluid glucose in exudative effusion was 107.92 and transudative pleural effusion was 116.57± 69.57, while mean pleural fluid protein was 4.24±1.41 and 1.84±0.66 in exudative and transudative effusions (Table 3, 4).

Table 5: Analysis of pleural fluid parameters in exudative pleural effusions

Variables	Type of effusion					p-value
	Tubercular	Parapneumon-ic	Empyema	Malignant	Paramalig-nant	
PF glucose	79.36±19.5	92.58±50.44	2.00±0.71	68.33±14.16	112.2±16.81	<0.001**
PF protein	4.92±0.82	4.31±0.80	5.16±1.09	4.47±1.54	4.46±0.53	<0.001**
PF LDH	623.61±90.67	375.21±22.95	2642.2±101.62	2298±306.87	267.6±53.10	<0.001**
PF TLC	427.70±94.98	1826.09±272.50	1913.80±257.54	135.33±97.08	72.40±12.81	<0.001**
PF Lympho-cytes (%)	83.24±12.20	54.55±34.09	46.60±30.95	80.00±10.00	83.00±10.95	<0.001**
PF neutro-phils (%)	14.33±9.84	41.82±34.80	51.00±33.62	20.00±10.00	17.00±10.95	<0.001**

ANOVA test, Mean ± SD

Mean LDH in exudative and transudative effusions was 772.52±144.56 and 116.05±32.89 respectively (Table 5, 6). The mean pleural fluid glucose and protein in tubercular pleural effusion was 79.36±19.5 and 4.92+0.82 respectively. As per the fluid cytological analysis, pleural effusion secondary to infections (tuberculosis, parapneumonic) were more cellular than malignant and transudative effusions suggesting acute process.

Table 6: Analysis of pleural fluid parameters in transudative pleural effusions

Variables	Transudate
PF glucose	116.57±69.57
PF protein	1.84±0.66
PF LDH	116.06±32.89
PF TLC	88.43±76.01
PF Lymphocytes	73.93±11.12
PF Neutrophils	21.36±10.10

Lymphocytes were the predominant cell type in tubercular and malignant effusion whereas neutrophils predominated in empyema and parapneumonic effusions according to differential cytological analysis of the pleural fluid. Tubercular effusions were highly lymphocyte predominant

with lymphocytes accounting to 83.24±12.20 % of total cell count (Table 5).

The mean pleural fluid ADA in tubercular effusion was 62.61±24.50, while it was 21.20±11.27 in parapneumonic /14.77±5.87 in malignant effusions /16.98±11.08 in Para malignant /6.42±4.54 in transudative effusions respectively (Table 7, 8). Fluid ADA was statistically strongly significant (p<0.001) in diagnosing tubercular effusions (Table 7).

Table 7: Analysis of pleural fluid ADA in exudative pleural effusions

Variables	Type of effusion					p-value
	Tubercular	Parapneumonic	Empyema	Malignant	Paramalignant	
PF ADA	62.61±24.50	21.20±11.27	88.88±48.44	14.77±5.87	16.98±11.08	<0.001**

Table 8: Analysis of pleural fluid ADA in transudative pleural effusions

Variables	Transudate
PF ADA	6.42±5.54

The Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV), Accuracy of pleural fluid ADA in diagnosing tubercular pleural effusion was 92.3%, 97.3%, 97%, 92% and 94.7% (Table 9).Majority of patients in this study (50%, n=38) were diagnosed with tubercular pleural effusion followed by parapneumonic effusion (15.8%, n=12), para malignant effusion (6.6%, n=5), empyema (6.6%, n=4) and malignant effusion(3.9%, n=3).

Tuberculosis was the most common cause of exudative pleural effusion and chronic kidney disease among transudative effusions (Table 10, 11).

Table 9: Sensitivity, specificity, PPV, NPV, accuracy of Pleural Fluid ADA

Variable	Observation					Correlation (in %)					p-value
	TP	FP	FN	TN	Total	SE	SP	PPV	NPV	Accuracy	
Pleural Fluid ADA	36	1	3	36	76	92.3	97.3	92	97	94.7	<0.001

Table 10: Type of exudative pleural effusion

	No. of patients (n=76)	Percentage
Tubercular	39	51.3%
Parapneumonic	13	17.1%
Paramalignant	5	6.6%
Malignant	3	3.9%
Undiagnosed	2	2.6%

(NOTE: The term Para malignant effusion refers to effusions which are not the result of direct neoplastic involvement of pleura but are still related to the primary tumor).

Table 11: Type of transudative pleural effusion

	No. of patients (n=76)	Percentage
CKD	10	13.15%
CLD	2	2.6%
CCF	2	2.6%

Discussion

ADA is one of the most studied methods developed in the past three decades for the diagnosis of pleural TB (Extra pulmonary TB) and it is widely considered a reliable test. [13-16] However, it is not routinely performed at most centers, because of expensive commercially available kits. The low specificity of ADA is a greater concern in countries where TB is uncommon. However, where TB prevalence is high in countries like India, ADA is found to be particularly useful in the diagnosis of Pleural TB.

In this study the most common cause of pleural effusion was tuberculosis (46%, n=35) followed by parapneumonic effusion (15.9%, n=12), empyema (6.6%, n=5), para malignant effusion (6.6%, n=5) and malignant effusion (3.9%, n=3). No cause could be determined in 2.6% (n=2) patients. This study is consistent with other studies which showed tuberculosis as the most common cause of pleural effusion. [17-19] India has high prevalence of TB and hence tuberculous effusions appear to be the most common type of effusion. Patients with tuberculous pleural effusion had a mean age of 41.29±17.96 which was lower compared to the mean age in patients with malignant and para

malignant pleural effusion (55.67±11.85 and 58.4±17.79). A study conducted by Chan et al, on patients with tuberculous pleural effusion revealed mean age of 44 years. [20] The higher prevalence of tuberculosis in low age group is probably due to living or working conditions and poor socioeconomic conditions. This is consistent with previous studies which had shown that men are more predisposed to tuberculosis and malignancy. [21]In this study lymphocytes were the predominant cell type in tubercular and malignant effusion whereas neutrophils predominated in empyema and parapneumonic effusion. This is consistent with various other studies which showed lymphocyte rich effusion occurring in tuberculosis and neutrophilic rich effusion in empyema and parapneumonic effusion. [22,23] In this study, mean ADA among tubercular pleural effusion was 62.61±24.50 while in parapneumonic effusion, empyema, malignant and para malignant effusions it was 21.20±11.27, 88.88±44.44, 14.77±5.87, 16.98±11.08 respectively. Mean ADA among transudative pleural effusions was 6.42±4.54.

The Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV), Accuracy of pleural fluid ADA was 92.3%, 97.3%, 97%, 92% and 94.7% respectively. In a similar study by Sachin Kate et al, which included 75 patients, the sensitivity/specificity/PPV/NPV of pleural fluid ADA in diagnosing TB pleural effusion was 93.3%/90%/93%/90%. [24] Another study by A. Trajman et al, who evaluated 132 patients (95 Tubercular) the sensitivity and specificity of pleural fluid ADA was 91% and 93% respectively. [4] In a meta-analysis by Goto et al, who analyzed nearly 40 studies (1966-1999) from Cochrane and Medline database concluded that pleural fluid ADA was an efficient diagnostic tool in diagnosing TB pleural effusions and had sensitivity of 47.1-100% with specificity of 50-100%.[25]

Conclusion

From the study, it is concluded that Pleural Fluid Adenosine deaminase is a highly sensitive and specific biomarker in diagnosing Tubercular pleural effusion and can be a very useful aid in day to day clinical practice.

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