Original Paper

Evaluation of Pleural Fluid Adenosine Deaminase Activity in Tubercular Pleural Effusion in a Military Hospital

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Abstract

Introduction: Tubercular pleural effusion (TPE) is one of the common extra pulmonary tuberculosis which many a times become difficult to make definite diagnosis on account of low sensitivity and/or specificity of non-invasive diagnostic tools. Even pleural biopsy cannot establish all the diagnosis; rather after patient shows unwillingness to invasive procedure. A reliable sensitive and specific marker is required for early diagnosis of TPE as Bangladesh is a high Tuberculosis burden country. Adenine deaminase (ADA) may be a useful surrogate marker.

Objective: To find out a reliable sensitive and specific marker for early diagnosis of TPE as Bangladesh is a high Tuberculosis burden country.

Materials and Methods: This study carried out ADA estimations in 170 cases of pleural effusion at Combined Military Hospital (CMH) Dhaka. Efforts were made to reach diagnosis by other means.

Results: ADA level was found in tubercular pleural effusion ranges from 25 to 180 with a mean 71.51 ±33.1. It ranges 3 U/L to 170U/L with non-tubercular effusion with a mean 20.96±16.71. The sensitivity of ADA is 90.9% and specificity is 95.7% in diagnosing TPE. The positive and negative predictive values are 90.91 % and 95.65% respectively.

Conclusion: ADA is found cheap, sensitive and a useful surrogate marker. However, it is not a confirmatory diagnostic tool but can be used as a reliable adjunct to other investigations.

Key-words: Adenine deaminase (ADA), Tubercular pleural effusion (TPE), Malignant pleural effusion (MPE).

Introduction

Tuberculosis (TB) has existed for millennia and remains a major global health problem. It causes ill-health in millions of people each year and in 2015 was one of the top 10 causes of death worldwide, ranking above HIV/AIDS as one of the leading causes of death from an infectious disease[1]. Among the extra pulmonary tuberculosis tubercular pleural effusion is frequently encountered by physicians. Pleural tuberculosis is a common manifestation of extra pulmonary TB and with or without pulmonary TB is present in around 4% of all TB cases[2]. Histopathological and microbiological analysis of the pleural fluid or tissue may seem to be the most ideal method, but diagnosis cannot be reached in approximately 20% of the patients[3]. ADA is an enzyme catalyzing the conversion of the adenosine and deoxyadenosine to the inosine and deoxyinosine in the purine degradation pathway. Its quantity increases in the immature and non-differentiated T lymphocytes following mitogenic and antigenic stimulation[4].

Materials and Methods

Total 170 patients of either sex with pleural effusion who were admitted to Combined Military Hospital (CMH), Dhaka from January 2014 to November 2015 were studied. This study included these patients who were presented at Pulmonology department and also the patients who were referred to CMH Dhaka from other department as a diagnostic challenge. In all cases pleural effusions were aspirated and examined and were included in the study. Patients on anti-tuberculosis treatment, pregnant, age under 12 years, non aspirable quantity of pleural fluid and previously been diagnosed to have malignant pleural effusion were excluded.

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Physical, biochemical, bacteriological and cytological study were carried out of effusion. Light's criteria was used to decide exudates and transudates and this required a blood sample to be collected on the day of thoracentesis to measure total protein and LDH. All samples were tested for ADA value. ADA activity was determined by standard colorimetric method using adenosine deaminase assay kit (Diazyme ADS kit from Germany) by enzymatic kinetic method at Armed Forces Institute of Pathology (AFIP). ADA levels are calculated and expressed in unit per liter (U/L). The test took 90 minutes from blood collection. The specimen were kept in 2-8ºC if the test was not possible in time.

Other test from pleural fluid such as RA, ANA were done in appropriate case. Mantoux test (MT), sputum for AFB and malignant cell, sputum for Gene X-pert, fiber optic bronchoscopy, FNAC transthoracic and FNAC lymph node, video assisted thoracic surgery (VATS), pleuroscopy and pleural biopsy were done depending on the probable underlying diseases to reach conclusive diagnosis. Pro BNP, Echo, thyroid hormone test, RA, ANA, anti CCP were used along with renal and liver function test in certain cases which demands to do so. Every effort was taken to reach diagnosis in difficult cases.

Tubercular pleural effusion (TPE) was diagnosed by histopathologically (presence of granuloma in biopsy specimen), microbiologically (presence of AFB in pleural fluid and/or sputum, MTB detected in Gene X-pert, Broncho alveolar lavage revealed AFB, exudative pleural effusion with no alternate explanation) and clinical features compatible with Tubercular pleural effusion and there was clear response to anti-TB drugs.

Malignant pleural effusions (MPE) were diagnosed by clinical features and presence of malignant cell in the pleural fluid/pleural biopsy, bronchial biopsy or BAL fluid, Transthoracic FNAC and Metastatic malignant cell in FNAC or biopsy of lymph node. Parapneumonic effusion and empyema were diagnosed with classical features as cough, sputum, highfever, and pleuritic chest pain, consistent pleural fluid analysis (culture and sensitivity and exclusion of any disease that can explain effusion while collection of pus in pleural space was diagnosed as empyema).

Other diagnosis such as congestive cardiac failure/ Left ventricular failure, chronic kidney disease, chronic liver disease with appropriate clinical findings of the particular disease and hypoalbuminaemia were present and other relevant investigation were consistent such as echo, Brain natriuretic peptide and ultrasonogram.

Undiagnosed was labeled when despite all investigations (Plural fluid analysis, CT scan of chest, biopsy Echo, thyroid hormone profile, connective tissue disease markers) being performed but failed to reach a diagnosis. The data processing and analysis was done by using the SPSS-21 version. Continuous variables were expressed as mean and standard deviations. Categorical variables were compared using the chi-square test. Some values were distributed in percentages by group.

Results

Out of 170 cases 107 (60.3%) were males and 63 (39.7 %) were females (Table-I). The mean age was 45.96 ± 17.89 in tubercular cases and 54.16 ± 13.44 years in non-tubercular cases which is statistically significant (p value < 0.001).

Table-I: Comparison of age of the study subjects between two groups (n=170)

Age (in years)	Group-I (TB) (n=55)	Group-II (Non-TB) (n=115)	p value
< 20	5(9.1%)	1(0.9%)	
21-30	6(10.9%)	7(6.1%)	
31-40	11(20.0%)	13(11.3%)	
41-50	9(16.4%)	24(20.9%)	
51-60	12(21.8%)	40(34.8%)	
61-70	8(14.5%)	18(15.7%)	
71-80	4(7.3%)	12(10.4%)	
Total	55(100.0%)	115(100.0%)	
Mean±SD	45.96±17.89	54.16±13.44	0.001[S]

Note: S= Difference is statistically significant

The most common complaints were cough (76%), shortness of breath (72%), and chest pain (55%). Fever, loss of appetite, loss of body weight and night sweat were complained in 45%, 56%, 15% and 10% respectively. All patient had at least two or more complaints. Lymphadenopathy is found mostly in malignant pleural effusions (14 cases) and only 2 cases in TPE.

Pleural fluid was transudative in 39 patients and was found to exudative in 131 patients. Chest X-ray revealed effusion mild to moderate in tubercular pleural effusion cases and mild to massive pleural effusion in malignant pleural effusion. Three patients required intercostals tube drainage due to massive effusion. Right sided effusion was more common in both TPE (37 cases 67.3%) and MPE (66 cases 57.4%); while bilateral pleural effusion (12 cases 10.4%) is more in congestive cardiac failure and in chronic kidney diseases.

Mantoux test (MT) revealed positive in 55 cases. It was found positive in 30 of TPE and also found positive in 21 cases of MPE. The sensitivity and specificity was found 54.55% and 81.74% respectively and 10 mm or more were considered as positive and < 10 mm as negative.

Adenine deaminase studies from pleural fluid were done in all patients in this study. The mean ADA value was found 37.31 ± 33.21 U/L. ADA level was found in tubercular pleural effusion ranges from 25 to 180 with a mean 71.51 ±33.1. It ranged 3 U/L to 170U/L with non-tubercular effusion with a mean 20.96±16.71. In 55 patients, it was more than 40 U/L. Among the positive cases 40 cases were diagnosed as tubercular pleural effusion, 5 were diagnosed positive in non-tubercular effusions (2 cases in MPE in cases, 2 in empyema and one in Rheumatoid arthritis).

Table-II: ADA level in pleural fluid of study groups

	Group-I (TB) (n=55) Mean ±SD	Group-II(Non-TB) (n=115) Mean±SD	P value
ADA	71.51±33.21 (25-180)	20.96±16.71 (3-170)	<0.0001[S]

Note: S= Difference is statistically significant

The receiver operating characteristic curve (ROC) curve is shown in the figure-1. Through analysis of a ROC curve, the optimal cut off value was determined 40U/L. The area under the curve was 0.974 and the standard error was 0.011 (95%CI: 0.952-0.996). Basing upon this cut off value ADA sensitivity and specificity were 90.91 and 95.65% respectively. If the cut of value was taken 30U/L then the sensitivity and specificity became 96.4 and 92.2 respectively. Again 50 U/L was considered the cut off value then the sensitivity and specificity become 67.3% and 96.5% respectively.

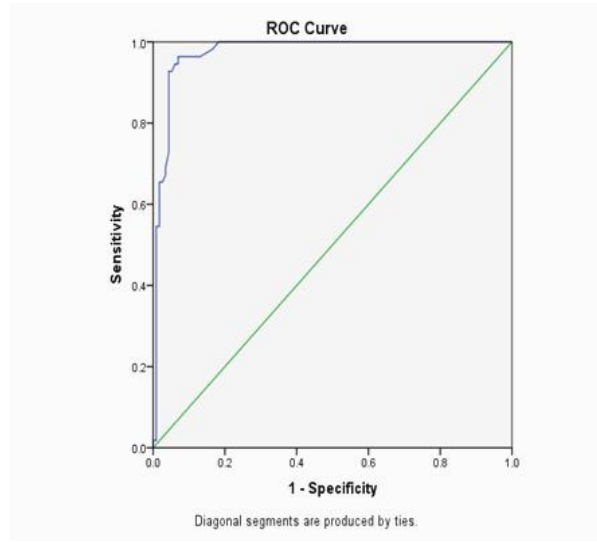

Fig-1: ROC curve from pleural effusion ADA values

Table-III: Area under the curve

Area Under the Curve				
Test Result Variable(s): ADA in U/L				
		P	*95% Confidence Interval*	
AUC	*Std. Error*	*value*	*Lower Bound*	*Upper Bound*
0.974	*0.011*	*0.000*	*0.952*	*0.996*

The test result variable(s): ADA in U/L has at least one tie between the positive
Actual state group and the negative actual state group. Statistics may be biased.
a. Under the non-parametric assumption
b. Null hypothesis: true area=0.5

Sensitivity of ADA value was 90.91% and specificity was 95.65%. The positive and negative predictive value was found 90.91% and 95.65% respectively.

Table-IV: Sensitivity and specificity of ADA for tubercular effusion

ADA	TB	Non-TB	Total
Positive	(TP) 50	(FP) 5	55
Negative	(FN) 5	(TN) 110	115
Total	55	115	170

Table-V: Determination of likelihood ratio, positive and negative predictive value

	Value	95% CI		
Sensitivity	90.91%	80.03%	to	96.95%
Specificity	95.65%	90.14%	to	98.56%
Positive Likelihood Ratio	20.91	8.84	to	49.48
Negative Likelihood Ratio	0.10	0.04	to	0.22
Disease prevalence	32.35%	25.39%	to	39.94%
Positive Predictive Value	90.91%	80.03%	to	96.95%
Negative Predictive Value	95.65%	90.14%	to	98.56%

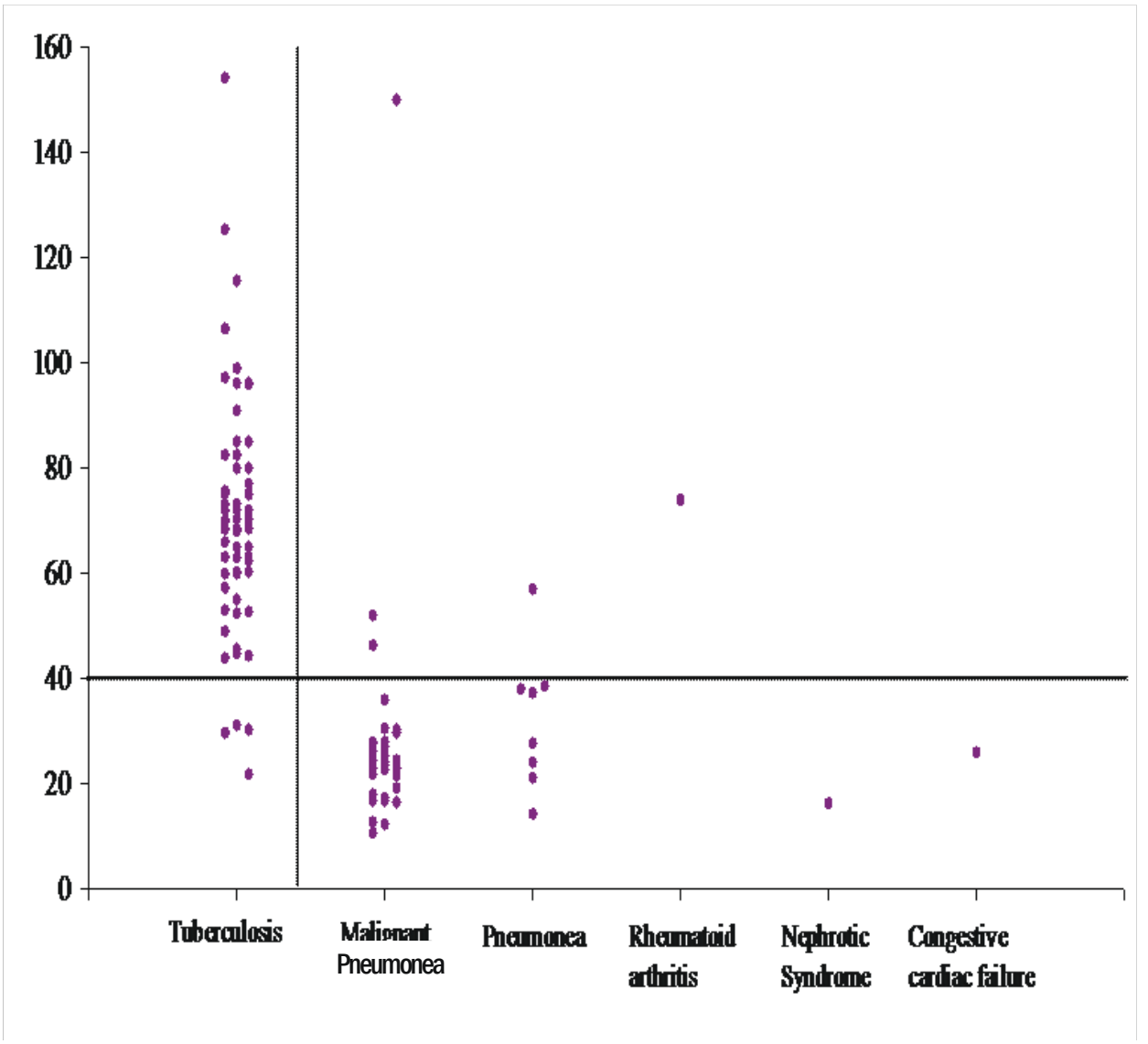

Fig-2: Adenosine deaminase levels in pleural fluids of the studied groups

Fiber optic bronchoscopy, transthoracic FNAC, FNAC from supra/cervical lymph node FNAC, video assisted thoracic surgery, pleuroscopy/pleural biopsy, pleural fluid cytology carried out 73, 50, 16, 11, 19 and 170 cases respectively.

Table-VI: Invasive procedures* and their contributions to establishing diagnosis

	FOB diagnostic/total*	Pleural/pleuroscopy biopsy diagnostic/total	VATS diagnostic/total	Lymphnode biopsy diagnostic/ total	CT guided FNAC iagnostic/total	Pleural fluid cytology, bacteriological examination*
Tuberculosis	5/23	5/7	5/5	2/2	3/8	1/55
Lung cancer/metastatic cancer	12/41	5/7	4/5	12/14	17/38	5/57
Parapneumonic	1/9	1/3	-	-	3	18/18
Others	9	2	1	-	1	5/16
Total	18/73	11/19	9/11	14/16	20/50	29/170

FOB: fiber optic bronchoscopy LN: lymph nodeFNAC: fine needle aspiration cytology VATS: video assisted throacoscopic surgery * some cases underwent more than one invasive procedure and all patient had thoracentesis

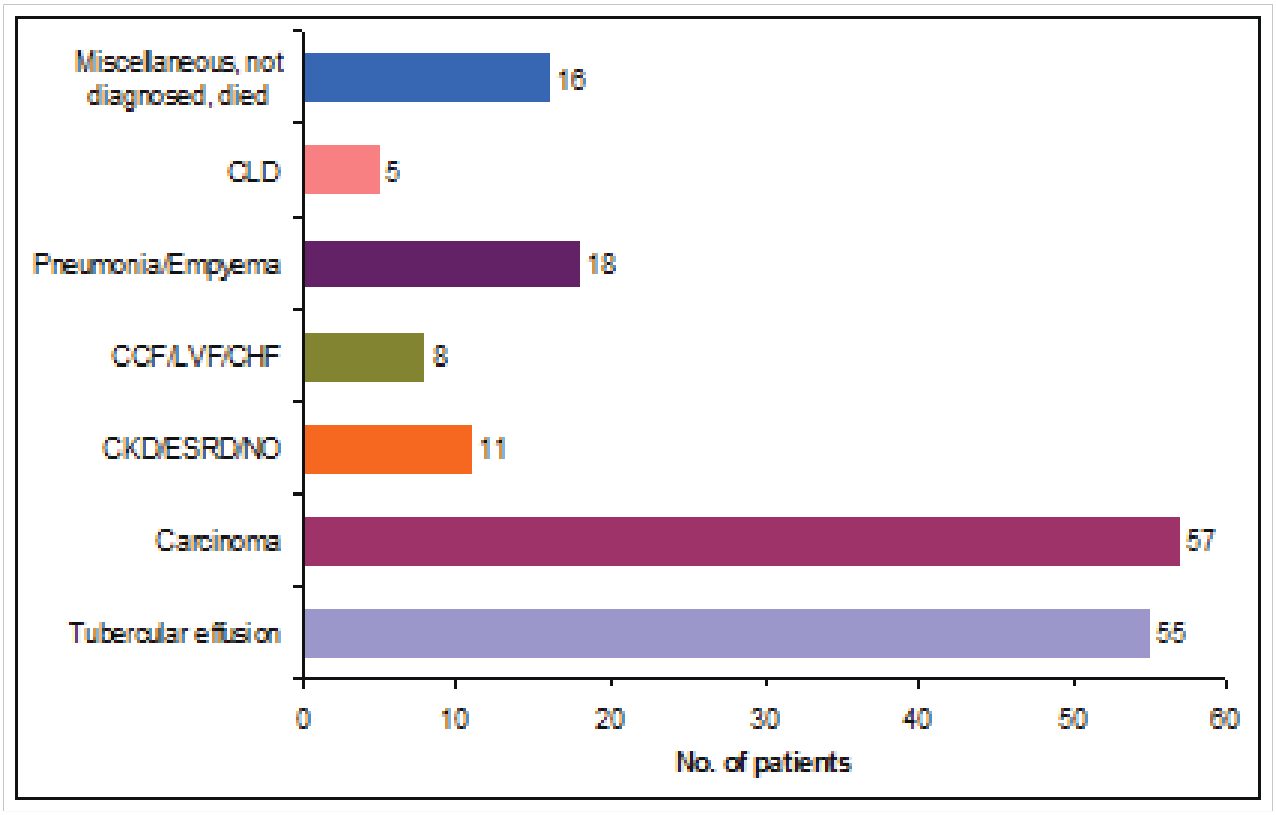

Fig-3: Bar diagram of different diseases

CLD: chronic liver disease; LVF: left ventricular failure; CHF: chronic heart failure; CKD: chronic kidney disease; ESRD: end stage renal disease; NS: nephrotic syndrome

Among the transudative pleural effusion chronic kidney disease/end stage renal disease, nephrotic syndrome were common (11 cases 6.47%), followed by left ventricular and chronic heart failure (8 cases, 4.71%). In this study the final diagnosis could be reached all but 6 cases. The distribution of the diagnosis is shown in the Figure-3. The most common diseases was malignancy followed by tubercular pleural effusion. The diagnosis which came out finally in this study revealed malignancy was the most common cause 57 (33.52%). TPE was revealed in 55 (32.35%) cases where as para pneumonic effusions were found in 18 (10.59%) cases. Among the transudative pleural effusion chronic kidney disease/end stage renal disease, nephrotic syndrome were common (11 cases, 6.47%), followed by left ventricular and chronic heart failure (8 cases, 4.71%). In miscellaneous group, two patients were found in each rheumatoid arthritis and systemic lupus erythematosus, one patient of hypothyroidism, vasculitis and dengue fever. Two patient died and one patient left to other hospital.

Discussion

Many markers that may be helpful in the differential diagnosis were studied in the pleural fluid. Two of these, ADA and interferon gamma are the most widely used and currently the most expected tests[5]. Especially ADA has been more commonly preferred for the diagnostics algorithms in the countries with a moderate to high incidence of tuberculosis because it is more inexpensive methods that can be accessed more quickly[6].

This study showed malignancies were most common cause (57cases); Tubercular pleural effusion ranked second (55 cases) and para pneumonic effusion (18 cases) third most frequent cause. This differs from the study carried out at National Institute of diseases of chest and Hospital (NIDCH) by SK Bhoumik et al[7] and NIDCH and Bangabandhu Sheikh Mujib Medical University (BSMMU) by Ahmed et al[8] where tubercular effusion was most common(60.1% n-106) and also from study in India[9]; where tubercular effusion was 66% (n-50). This is due to the fact that present study was conducted in a multi-disciplinary hospital where as other study of Bangladesh and India conducted at Chest diseases hospital and chest department respectively. More so as a tertiary care hospital all tubercular pleural effusion cases were not referred as dependent hospitals are equipped with TB and isolation ward. But this study result is similar to Turkey study[10] where malignancies (26%,n-240) was most common second to tubercular effusion (19%, n-240). Turkeys study showed that despite extensive investigations some causes of effusion remained unanswered in 6 cases. In this study, 6 cases remain undiagnosed in 6 cases. In the United States of America first three diseases in pleural effusion incidence were CHF, parapneumonic effusion and malignant effusion[11].

In this study the mean level of ADA was 37.31± 33.21; in TPE ADA level was found in tubercular pleural fluid ranges from 25 to 180 with a mean 71.51±33.1. It ranges 3 U/L to 170U/L with non-tubercular effusion with a mean 20.96±16.71. It was found ADA level of the pleural fluid is significantly higher in TPE (p< 0.001) Similar type of result was found in other study conducted at Bangladesh[7,8] and in India[9] and Egypt[12]. Bhoumik SK et al studied pleural fluid ADA level along with histopathology of pleural biopsy in 103 cases at NIDCHand found a cut off ADA level of 40U/L in pleural fluid which has sensitivity 94% and specificity 88%. Sharmeen Ahmed et al also carried study at BSMMU and NIDCH among 50 patient with pleural biopsy/clinical trial among biopsy negative patient and found sensitivity 94% and specificity 88%. They considered biopsy proven tubercular pleural effusion and biopsy negative but responded to ATT as tubercular case. The negative predictive value is 90% and positive predictive value is 92%. Even comparison between tubercular and malignant pleural effusion by pleural fluid ADA activity also showed statistical significance ($p < 0.0001$).

In the Indian study by Mathur PC et al[9] ADA is effective and cheap test with sensitivity 100% and specificity 94.6% with positive and negative predictive value of 95.5% and 100% respectively. Whereas in Egyptian study[12] by revealed ADA positive (44 U/L) in number of cases 39 patients (18%). Of these patients 24(62%) were diagnosed with TB, 9(23%) with par pneumonic effusion, 2(5%) with idiopathic effusion, 1 with malignant mesothelioma, 1 with lymphoma and one with rheumatoid pleurisy.

With a cut off value 40U/L of pleural fluid ADA, ROC curve analysis showed high diagnostic sensitivity 89.47% andspecificity 95.58%. Patients of non-tubercular group showed >40U/L in 5 cases including 2 in malignancy, oneeach in parapneumonic effusion and in empyema and one in rheumatoid arthritis. Out of 55 TPE 6 patient had ADA value <40U/L. If ADA cut off value is considered 50U/L then the sensitivity become 66.1% and specificity becomes 96.5% whereas ADA has the sensitivity and specificity 96.4% and 92.1% in case of cut off value 32U/L. In the following table comparison of the different studies regarding utility of pleural fluid is shown.

Table-VII: Utility of Pleural Fluid ADA in the diagnosis of tubercular pleural effusion

Study	YEAR	No of Patients	Threshold U/L	Sensitivity %	Specificity %
Bhoumik et al[7]	2008	62	40	94	88
Sharmeen et al[8]	2011	62	40	94	88
Valdes et al	1993	405	47	100	95
Fatma Tokgoz[10]et al	2013	240	47	100	91
Helmy et al[12]	2012	30	30	80	85
Mathur et al[9]	2006	50	100	100	100
Kabir et al	2016	170	40	90.9	95.7

The diagnosis which came out finally in this study revealed malignancy was the most common cause 57(33.52%). TPE was revealed in 55 (32.35%) cases where as para pneumonic effusions were found in 18(10.59%) cases. Among the transudative pleural effusion chronic kidney disease/end stage renal disease, nephrotic syndrome are common (11 cases 6.47%), followed by left ventricular and chronic heart failure (8 cases 4.71%). In comparison the study at western India (n-50) by Bhavsar Kushal[13] found TPE 66%, Malignancy 18%, parapneumonic 10%, congestive cardiac failure 2% and hupoproteinemia 4% .

Conclusion

This study revealed that ADA levels are significantly high in tubercular pleural effusion as against non-tubercular causes. The method of ADA estimation is easy, simple and doesn't require expensive equipment. So, the study shown a simple, inexpensive, highly sensitive and specific test like ADA estimation should be employed to differentiate between tubercular and non-tubercular pleural effusion. So, finally this can be concluded that pleural fluid ADA is an useful surrogate marker for the diagnosis of tubercular pleural effusion.

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