Adenosine Deaminase and Other Conventional Diagnostic Parameters in Diagnosis of Tuberculous Pleural Effusion

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The diagnosis of pleural tuberculosis (TB) continues to be a challenge in clinical practice. Traditional diagnostic methods are very useful for the diagnosis of pulmonary TB but have a low yield when applied to pleural fluid. Recognition of the difficulty in diagnosing pleural TB led to a search for methods that would optimize the workup of pleural effusion patients with suspected TB. Among these new techniques adenosine deaminase (ADA) levels in pleural fluid is a simple, inexpensive and rapid test for early diagnosis of pleural tuberculosis which may improve the prognosis and reduce spread of disease and sequelae. ADA activity rises predominantly in response to stimulation by tubercular antigen. So, the aim of this study was to evaluate the diagnostic value of ADA level in plural fluid and other conventional methods for diagnosis of tubercular plural effusion. This was a cross sectional study. This study was carried out in 64 patients suffering from plural effusion and were consecutively selected and divided into two groups: tuberculous (n =40) and non-tuberculous (n = 24), depending upon the etiology. Details clinical history, physical examination, routine and other relevant investigations including ADA estimation was measured. The mean value of ADA in the tuberculosis group was 64.11±19.5 U/L, which was significantly higher ($p<0.05$). Cut off value of ADA was ≥40 U/L with 97 % sensitivity and 93% specificity. In this study, ADA levels in nontuberculous pleural effusions rarely exceeded the cut-off value set for tuberculous disease. The pleural fluid ADA levels were significantly higher in tuberculous pleural effusions when compared with non-tuberculous pleural effusions.

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Key words: Adenosine deaminase, tuberculosis, pleural effusion, diagnosis

Introduction

Tuberculosis (TB) remains a global health problem and is one of the commonest causes of exudative pleural effusion in many regions. Although TB pleural effusion may resolve spontaneously, if untreated up to 65% of patients with tubercular pleural effusions will eventually develop active TB in extra-pleural sites.[1] Tuberculous pleural effusion is the second frequent form of extra-pulmonary presentation after tuberculous lymphadenitis.[2] and occurs in approximately 5% of patients with tuberculosis.[3] A prompt accurate differential diagnosis of pleural exudates is vital for initiating treatment, particularly for cases with tuberculosis.

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Pulmonary tuberculosis is the common presentation of Mycobacterium tuberculosis infection. Extra-pulmonary tuberculosis can also manifest that occurs outside the lungs. During primary infection with tuberculosis and during any subsequent secondary active disease Mycobacterium tuberculosis can spread by blood or the lymphatic system to other parts of the body. The majority of the extra pulmonary forms of TB affect organs with suboptimal conditions for bacillary growth, for this reason, the extra pulmonary disease generally has an insidious presentation, a slow evolution and paucibacillary lesions. Tuberculous pleural effusion has been considered mostly as a manifestation of primary tuberculosis. Gold standard for diagnosis of TB pleural effusion is the identification of mycobacterium tuberculosis in pleural fluid or tissue by microscopic examination and culture.[4] But this is very difficult to identify because of low bacillary load.[5] Moreover culture requires more than 100 viable bacilli[3] and it is also a time consuming procedure, requires 4-6 weeks to yield growth of Mycobacterium, even with radiometric Mycobacterium culture system (BACTEC), which takes 18 days.[6]. Microscopy of the pleural fluid for acid fast bacilli is positive in less than 5% TB pleuritis cases.[7] Moreover, direct examination with Z-N staining requires bacillary concentration of 100/ml to be positive, but Mycobacterium load in pleural fluid is very low.[3]

Biopsy of parietal pleura has become a sensitive diagnostic test for tuberculous pleural effusion.[3] Histological examination of tissue from the pleural biopsy may demonstrate granulomatous inflammation, caseasous necrosis or acid fast bacilli. Pleural biopsy specimen demonstrate granulomatous pleuritis in 50-80% of patient with tuberculous pleural effusion.[8,9]. Furthermore, pleural biopsy is a blind, invasive procedure, complicated and depends on the skill of the physician because it is technically difficult.[7] It does not always guarantee the collection of representative sample. Sensitivity also depends on multiple pieces of tissues which yield more positive results.[10] Other methods, such as thoracoscopy or polymerase chain reaction, may enhance diagnostic sensitivity and specificity and may shorten the time to differential diagnosis.[11] Yet, these procedures are invasive or may be costly in terms of required equipment and trained personnel are needed. Therefore, these methods may not be readily available, particularly for resource-poor clinics or hospitals in our country.

Since the conventional diagnostic tools are incapable of pinpointing the cause, so several bio-markers like ADA, interferon (IFN)-γ, a variety of tumor markers and cytokines have been proposed as alternative noninvasive methods of establishing tuberculous etiology in cases of exudative pleural effusion. Adenosine deaminase estimation in pleural fluid has long been taken as a marker for tuberculous pleurisy. The purpose of this present study was to determine the value of ADA in the diagnosis of tuberculous pleural effusion, to compare the result with culture and to assess its accuracy.

Methods

This was a cross sectional study. All adult patients of both sexes with pleural effusion who were admitted during March, 2011 to February, 2012 in National Institute of Diseases of Chest and Hospital and subsequently underwent thoracocentesis were included in the present study with inclusion and exclusion criteria. Clinically and radiologically diagnosed pleural effusion cases that had undergone thoracocentesis were included. Multiple pathology of pleural effusion that is patients with more than one disease, patients with haemothorax secondary to trauma, empyema thoracis and patient with anti-TB drug therapy were excluded from this

study. Patients were divided into the following groups:

Group-I: Included patients with clinically suspected tuberculous pleural effusion. Tuberculous pleural effusion was diagnosed on the basis of the presence of either positive staining or culture for Mycobacterium tuberculosis in the pleural fluid or presence of caseating granuloma on pleural biopsy or clinical features compatible with tuberculosis and response to anti-tubercular treatment.

Group-II: Included patients with non tuberculous respiratory disease who were available in our study period such as malignancy, pneumonia, parapneumonic effusion, chronic pleuritis etc. Non tuberculous patients were diagnosed by the attending physician. Malignant effusions were diagnosed by cytology, pleural biopsy or fiber-optic bronchoscopy. Para pneumonic effusion was identified by the presence of pulmonary infections associated with acute febrile illness, pulmonary infiltrates, purulent sputum and response to antibiotic treatment. Other non tuberculous patients were diagnosed by standard clinical criteria and investigations.

Pleural fluid analysis was done for protein, sugar, LDH, total WBC count, differential WBC count, Gram stain, Ziehl Nelsen staining and Cytology. Pleural fluid ADA was estimated in all patients by colorimetric method at BSMMU (Bangabandhu Sheikh Mujib Medical University). Pleural biopsy, CT chest, bronchoscopy, and other tests were done wherever indicated.

Results

This cross sectional study was carried out in 64 patients suffering from plural effusion. Tuberculous pleural effusion was diagnosed in 40 patients (62.5%). Another 19 patients (29.68%) had malignancies, 5 patients (7.81%) had other etiologies including; 3 pneumonia, 2 with miscellaneous causes. Table I summarizes the diagnostic criteria among the patients of tuberculous pleural effusion. Out of 40 tuberculous pleural effusion cases, only culture positive for M. tuberculosis were five patients. Fifteen patients were diagnosed by granulomatous inflammation in pleural biopsy. Chronic inflammatory lesions were found in thirteen cases on pleural tissue biopsy for whom anti-TB drugs were advised based on clinical features and radiological evidence .They were diagnosed as tuberculous pleural effusion by response to anti-tuberculous treatment. Seven patients were combindly diagnosed by AFB positive in microscopic examination, positive culture and granulomatous inflammation in pleural biopsy. ADA level in pleural fluid was measured in all the patients. The mean value of ADA in the tuberculosis group was 64.11±19.5 U/L, which was significantly higher than for the non-tuberculous groups ($p<0.05$) (Table II). The mean values of ADA in the non-tuberculous group were 34.31±14.73 U/L. Cut off value of ADA was ≥40 U/L, which was calculated by ROC curve (Fig-1). Sensitivity of the test in tuberculous pleural effusion was 95% which was higher than microscopy (7.5%), culture (30%) histopathology of pleural tissue biopsy (52.2%). Specificity was 83.3%, the positive and negative predictive values at this cut-off value were 91.0% and 90.0%, respectively (Table-VII).

Table I: Distribution of tuberculous pleural effusion patients as per diagnostic criteria (n=40)

Diagnostic criteria	Positive Result
Pleural Fluid	
Culture for M. tuberculosis only	5
Pleural tissue biopsy for histopathology	
Granulomatous inflammation	15
Chronic inflammatory lesion	13
Combine diagnosed by microscopic examination, culture and biopsy	7

Table II: Distribution of the study patients according to ADA level in pleural fluid (IU/L) (n=64)

ADA in Pleural fluid (IU/L)	Group I (n=40)	Group II (n=24)	p
	N (%)	N (%)	
< 40	2(5.0)	20(83.3)	
≥40	38(95.0)	4(16.7)	
Mean± SD	64.11±19.5	34.31±14.73	0.001 s
Range (min-max)	(28.2-116.2)	(8-66.4)	

s= significant

Table III: ADA level in pleural fluid for prediction of tuberculous pleural effusion (N=64)

ADA in Pleural fluid	Pleural effusion		Total
	Tuberculous	Non-tuberculous	
Positive (≥40)	38(TP)	4(FP)	42
Negative (<40)	2(FN)	20(TN)	22
Total	40	24	64

Table IV: Presence of AFB in microscopy for prediction of tuberculous pleural effusion (n=64)

AFB in microscopy	Tuberculous		Total
	Positive	Negative	
Positive	3(TP)	0(FP)	3
Negative	37(FN)	24(TN)	61
Total	40	24	64

Table V: Culture of pleural fluid for prediction of Tuberculous pleural effusion (n=64)

Culture of Pleural fluid	Tuberculous		Total
	Positive	Negative	
Positive	12(TP)	0(FP)	12
Negative	28(FN)	24(TN)	52
Total	40	24	64

Table VI: Pleural tissue biopsy for histopathological examinations for prediction of Tuberculous pleural effusion (n=64)

Pleural biopsy	Tuberculous		Total
	Positive	Negative	
Granuloma Positive	21(TP)	0(FP)	21
Granuloma Negative	19(FN)	24(TN)	43
Total	40	24	64

Table VII: Sensitivity, specificity, accuracy, positive and negative predictive values of the ADA level, microscopy, culture, biopsy, for prediction of tuberculous pleural effusion

Validity test	ADA	AFB in Pleural fluid	Culture of Pleural fluid	Pleural biopsy
Sensitivity	95.0	7.5	30.0	52.5
Specificity	83.3	100.0	100.0	100.0
Accuracy	90.6	42.2	56.3	70.3
Positive predictive value	91.0	100.0	100.0	100.0
Negative predictive value	90.0	39.3	46.2	55.8

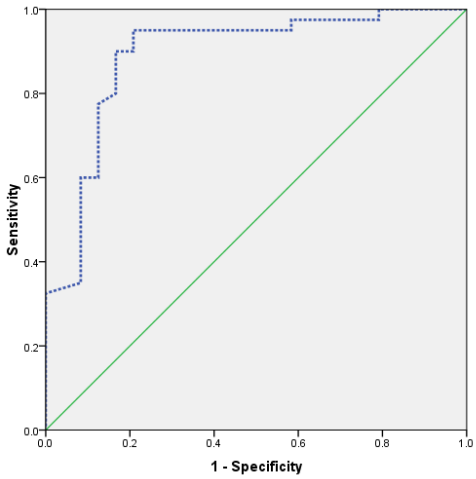

Figure 1- Receiver-operator characteristic (ROC) curve of ADA in Pleural fluid for prediction of tuberculous pleural effusion

Discussion

Our findings seem to confirm that pleural fluid ADA is a very good parameter for diagnosis of tuberculous pleural effusion than other conventional methods. In this study, tuberculous pleural effusion (TPE) patients had a bimodal age distribution. One peak was in the age group of 21-30 years and another was in the 41-50 years. In this study, seventy five percent (75%) of TB pleural effusion cases were male and twenty five percent (25%) were females with a male female ratio of 3:1. Similar male predominance was also observed in other studies.[12]

The number of tubercle bacilli in the pleural fluid is actually very small, resulting in the difficult direct detection of the bacilli. Acid-fast bacilli are reported to be seen in less than 10% of pleural aspirates.[13,14] In this study, Ziehl Neelsen stain of pleural fluid revealed positivity in 7.5 % cases, which was consistent with other studies.[15-18]

Culture requires 100 viable bacilli and therefore, is more sensitive than Z-N staining.[3] In our study, pleural fluid culture yielded Mycobacterium tuberculosis in 30 % cases. This finding was consistent with other study.[16,18] Lower rate of culture positivity ranging 4-16% were also reported by other workers.[17,19]

Our results drew attention to the well-recognized fact that biopsy methods play a crucial role in the diagnostic algorithm in patients with Tubercular pleural effusion.[16] In this study, granuloma was positive in 52.5% of tuberculous pleural effusion cases which was almost consistent with other study.[17]

In our study, we investigated 64 pleural effusion cases. Our findings seem to confirm that ADA activity is a useful parameter for the diagnosis of tuberculous effusion. The mean levels of pleural ADA in tuberculous effusion were higher than in any other group. The cut off value utilized in this study was (40 U/L) which was consistent to other study where ADA has been evaluated in the differential diagnosis of Tuberculous pleural effusion.[20,21] In this study, we found sensitivity of ADA in tuberculous pleural effusion 95%.Choudhury and Patel (2010); Kim et al., (2009) found sensitivity of ADA 97% and 94.4% respectively in their study which was consistent with present study. Specificity was 83.3% in present study. Ogata et el (2011); Mohammadtaheri et el (2005) found specificity of ADA 86% and 82% respectively which was also consistent with this study.[22, 23] We found positive predictive value (PPV) of ADA for diagnosis of tuberculous pleural effusion was 91% which was consistent with the study of Liu et al (2010), where PPV was 90%.[24] Negative predictive value in this study was 90%, which was also consistent with the other study,[2,17] where NPV were 88.5% and 87% respectively. Accuracy for detection of tuberculous pleural effusion was 90.6% in present study. Chen et al (2004); Mohammadtaheri et al. (2005) found accuracy of ADA 90.5% and 88% respectively, which was almost consistent with this study.[25] In this study, a ROC curve, a graphic approach, was employed which is preferable when there are many possible cut off value. Based on the ROC curve, an ADA in pleural effusion levels of $\geq$ 40 U/l was the most suitable cut-off value yielding a sensitivity of 95% and a specificity of 83.3% for diagnosis of patients with tuberculous pleural effusion. The mean ADA activity level in patients of TPE was 64.11 ± 19.50 U/L while in the group of nontuberculous patients it was 34.31 ±14.73 U/L ($P<0.05$), which was consistent with other study.[24]

Out of 40 patients with tuberculous pleural effusion in our study, two patients showed pleural fluid ADA activities below the threshold value. False negative results were

associated with malignant condition, may be they had immunosuppression. False negative cases were frequently found in studies conducted for the diagnosis of TPE by ADA activity.[26] In Spain a study was conducted by Villena et al (1995), where false negative cases were five. They could not confirm the reason but emphasized on the relation to the HIV infection.

In non-tuberculous pleural effusions cases, ADA activity was low and only four patients with cancer presented an ADA activity higher than 40 U/L. This Increased activity is probably due to malignant proliferation of undifferentiated T lymphocyte.[27] Despite increased ADA levels, etiological diagnosis was established of these cases by pleural fluid cytology, pleural biopsy, chest X-ray demonstrated a pulmonary mass, FNAC, fiber optic bronchoscopy, which led to a definitive diagnosis.

Lymphocytosis was found in 27(67.5%) cases of TB pleural effusion in this study. Lymphocyte count was also increased in non-tuberculous malignant group. This findings of tuberculous pleural effusion was consistent with other study.[4] The mean percentage of lymphocytes in tuberculous effusion did not differ significantly from that caused by malignant diseases as evidenced by Antonangelo et al.[4]

Our findings seem to confirm that pleural fluid ADA is a very good parameter for diagnosis of tuberculous pleural effusion. Early diagnosis and treatment is important by using a sensitive test regarding high prevalence of TB in our country.

Conclusion

The result indicated that the analysis of ADA levels in pleural effusion constitute a very useful marker for the diagnosis of tuberculous pleural effusion which, in addition, can be made quickly and cheaply. The conventional methods are less sensitive, time consuming, expensive and not easily available. So, we may conclude that a simple, less expensive, highly sensitive test like ADA estimation should be employed routinely to differentiate between tubercular and non-tubercular etiology. In places where prevalence of tuberculosis is still high, like in our country, ADA assay can be considered as an important diagnostic tool for tuberculous pleural effusion.

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