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Role of Adenosine Deaminase (ADA) in Etiological Classification of Exudative Pleural Effusion

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ABSTRACT

Background: Pleural effusion is an abnormal collection of fluid in the pleural space. On the basis of Light's criteria pleural fluid can be classified as an exudate or transudate. An exudate is almost always secondary to a lung pathology. In India the most common cause of exudative pleural effusion is tuberculosis. Diagnosis of tubercular pleural effusion is not easy as conventional methods have a low diagnostic yield in pleural fluid. Adenosine deaminase (ADA) estimation is a simple biochemical test that can be easily done in the clinical laboratory with quickly available results. A cut off value of >40 IU/L for pleural fluid ADA is said to be diagnostic of tuberculosis. We conducted a prospective observational study of 50 patients presenting with exudative pleural effusion in our hospital in order to study the role of ADA along with biochemical and cytological parameters in the etiological classification of exudative pleural effusion.

Results: Sensitivity of ADA for tuberculosis at the cut off value of >40 IU/L was found to be 96.88%, specificity was 82.61%, positive predictive value for tuberculosis was 88.5%, negative predictive value for tuberculosis was 95%.

Conclusions: We concluded that pleural fluid cytology, Lymphocyte Neutrophil ratio along with serum ADA levels altogether can help in etiological classification of patients with exudative pleural effusion and using a cut off of > 40 U/L for pleural fluid ADA, patients can be classified as tuberculous or non tuberculous with a reasonably high diagnostic accuracy.

Keywords: Adenosine Deaminase, tuberculosis, pleural fluid, exudative.

INTRODUCTION

Pleural effusion is an abnormal collection of fluid in the pleural space resulting from excess fluid production or reduced resorption[1]. Presence of pleural fluid is always abnormal and indicates an underlying disease. A myriad of diseases are there which can cause pleural effusion. That is why a structured diagnostic approach is required to reach at the etiological diagnosis of pleural effusion. The first step in the evaluation of such patients is to see whether the fluid is a transudate or an exudate[2]. The time tested criteria given by Light help in the categorization of fluid as a transudate or exudate. Transudates usually reflect a systemic disease while exudates signify underlying pulmonary or pleural pathology. The criteria given by Light are pleural fluid to serum protein ratio greater than 0.5, pleural fluid to serum LDH ratio greater than 0.6 and pleural fluid LDH more than two thirds the upper limit for serum[3]. Using Light's criteria almost 99% of the effusions can be classified as transudate or exudate. Tuberculosis is endemic in India and is the commonest cause of exudative pleural effusion so much so that in every case of exudative pleural effusion it is imperative to rule out tuberculosis. Diagnosis of tubercular pleural effusion is a common challenge which every clinician faces. Although there are many methods for the diagnosis of tuberculosis like ZN staining, culture, PCR, sensitivity of all of these methods is low in case of pleural fluid[4]. Tuberculin or Mantoux test also has low

sensitivity and specificity. The definitive diagnosis of extrapulmonary tuberculosis is based on demonstration of Mycobacterium tuberculosis in pleural fluid or presence of granulomas can be seen in the pleural biopsy specimen[5]. While culture for M. tuberculosis is time consuming, PCR is expensive with both having low sensitivity for detection of M. tuberculosis in body fluids. Pleural biopsy via thoracoscopy is an invasive and costly procedure. Another test which is being widely used for diagnosing tuberculous effusions is Adenosine Deaminase (ADA) estimation. Adenosine deaminase (ADA) estimation is a simple, cheap and reliable test that can be easily done in the clinical laboratory with quickly available results[6]. ADA, an enzyme that catalyzes the deamination of adenosine to inosine has two isoforms, ADA-1 and ADA-2. ADA-1 is found in many tissues including red blood cells. ADA-2 is found only in macrophages and monocytes[7]. ADA is an important marker of cellular immunity, its sensitivity and specificity for early diagnosis of tuberculosis is high especially in endemic areas[8]. High ADA levels may sometimes be seen in pleural fluid from patients of malignancy, empyema or rheumatoid pleurisy[6]. Nonetheless in areas of moderate to high incidence of tuberculosis empirical antitubercular therapy can be started in any patient presenting with exudative pleural fluid with high pleural fluid ADA with lymphocyte predominance and negative cytology[9].

MATERIAL AND METHODS

We conducted a prospective observational study of 50 patients presenting in our hospital with exudative pleural effusion. The aim was to study the common causes of exudative pleural effusion and assess the biochemical and cytological parameters of different categories of pleural effusion as well as examine the role of ADA in definitively categorizing a patient as tubercular or non tubercular pleural effusion.

Inclusion criteria taken were :-

1) Age > 14 years
2) Pleural fluid protein > 3.5 g/dl

Exclusion criteria taken were:-

1) Age < 14 years
2) Transudative pleural effusion

These patients presented with pleural effusion in the OPD or IPD department of our hospital. Thoracocentesis was done after thorough clinical and radiological examination and the aspirated pleural fluid was sent to the laboratory for biochemical, cytological and microbiological examination. Biochemical examination including pleural fluid protein, glucose and ADA estimation were done by spectrophotometry in fully automated biochemistry analyser AU-480 by Beckman coulter. Total and differential cell count in pleural fluid were done using body fluid mode on hematology analyser XN 1000i. Giemsa stained smears were examined by the pathologist. ZN and Gram stain were done in all cases. PCR and AFB culture were done in patients with strong suspicion of tuberculosis. Chest X ray was done in all patients. Mantoux and CT scan were done in some patients. Bronchoscopy and pleural biopsy were done where it was thought to be indicated.

All findings were analysed.

A definite diagnosis of tuberculosis was taken in patients fulfilling one or more of the following criteria:-

1) Acid Fast Bacilli (AFB) seen in pleural fluid smear or AFB grown in culture or PCR positivity.
2) Radiological evidence of tuberculosis along with clinical symptoms of tuberculosis with or without Mantoux positivity.
3) Presence of granulomas in pleural biopsy or lymph node aspirate from mediastinal lymph nodes.

Presence of atypical cells in pleural fluid was taken as evidence of malignancy. Radiological findings, past history of such patients were taken into consideration.

Parapneumonic effusion referred to exudative pleural effusion associated with acute pneumonia or lung abscess while patients with presence of frank pus in pleural cavity with pleural fluid glucose <40 mg/dl were referred to as empyema patients. Patients of chronic renal failure with exudative pleural effusion with no history or finding suggestive of tuberculosis or malignancy were referred to as pleural effusion secondary to uremia.

RESULTS

Table I:- Sex and category wise distribution of patients

S.N.O.	Diagnosis	No. Of cases(N=50)	M(N=50)	F(N=50)
1.	Tuberculosis	31 (62%)	20 (40%)	11 (22%)
2.	Malignancy	10 (20%)	8 (16%)	2 (4%)
3.	Uremia	3 (6%)	3 (6%)	0
4.	Empyema	4 (8%)	4 (8%)	0
5.	Parapneumonic	2 (4%)	1 (2%)	1 (2%)
	Total	**50 (100%)**	**36 (72%)**	**14 (28%)**

Table II:- Age wise distribution of patients

Age group(yrs)	Tuberculosis (N=50)	Malignancy (N=50)	Uremia (N=50)	Empyema (N=50)	Parapneumonic (N=50)	Total (N=50)
15-25	7 (14%)	2 (4%)	0 (0%)	0 (0%)	0 (0%)	9 (18%)
26-35	8 (16%)	0 (0%)	0 (0%)	0 (0%)	1 (2%)	9 (18%)
36-45	2 (4%)	1 (2%)	1 (2%)	2 (4%)	0 (0%)	6 (12%)
46-55	6 (12%)	3 (6%)	1 (2%)	0 (0%)	1 (2%)	11 (22%)
56-65	4 (8%)	0 (0%)	1 (2%)	2 (4%)	0 (0%)	7 (14%)
66-75	4 (8%)	4 (8%)	0 (0%)	0 (0%)	0 (0%)	8 (16%)
Total	**31 (62%)**	**10 (20%)**	**3 (6%)**	**4 (8%)**	**2 (4%)**	**50 (100%)**

Table III :- Pleural fluid findings expressed as Mean±SD levels in different groups of patients

Diagnosis	No. Of cases N=50	Mean±SD Protein (g/dl)	Mean±SD Glucose mg/dl	Mean±SD total cell count Cells/cmm	Predominant cell type	Atypical cells	Mean±SD ADA
Tuberculosis	31(62%)	5.5±0.5	108±70	2572±1796	Lymphocytes	No	57.7±21
Malignancy(epithelial)	8 (16%)	4.5±0.9	64.2±37	696±360	Lymphocytes	Yes	13.3±3.7
Lymphoma	2 (4%)	5.7±0.4	52±4.2	9750±2474	Lymphocytes	Yes	332±214
Empyema	4 (8%)	4.3±1	4.1±2.5	12800±7038	Neutrophils	No	89±78
Uremia	3 (6%)	4.9±1	122.6±28.4	583±361	Lymphocytes	No	18.2±6.7
Parapneumonic	2 (4%)	3.85±0.5	73±32	2341±508	Neutrophils	No	16.9±14

Table IV:- ADA level in different patients

ADA (U/L)	TuberculosisN=50	Malignancy	Uremia	Empyema	Parapneumonic	Total
0-20	0 (0%)	8 (16%)	1 (2%)	1 (2%)	1 (2%)	10 (20%)
21-40	1 (2%)	0 (0%)	2 (4%)	1 (2%)	1 (2%)	5 (10%)
41-60	23 (46%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	24 (48%)
61-80	4 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (8%)
81-100	2 (4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (4%)
>100	1 (2%)	2 (4%)	0 (0%)	2 (4%)	0 (0%)	5 (10%)
Total	**31 (62%)**	**10 (20%)**	**3 (6%)**	**4 (8%)**	**2 (4%)**	**50 (100%)**

A total of 50 patients of exudative pleural effusion were evaluated. Patients were of different age groups ranging from 15-75 years. Males were more commonly affected as compared to females. Male to female ratio was 2.5:1 (Table I). The incidence of pleural effusion was more on the right side of the pleural cavity (54%). Based on the etiology of pleural effusion patients could be divided into 5 groups namely tuberculosis, malignancy, empyema, parapneumonic effusion and uremic effusion. The leading cause of exudative pleural effusion in our study was tuberculosis (62%) followed by malignancy (20%). The incidence of tuberculosis was highest in the age group 26-35 years while malignancy was most commonly seen in the age group 66-75 years (Table II). No significant age preference was seen for the other three groups (Table I).

There were 31 cases of tuberculosis in our study. In 7 patients pleural biopsy was done which showed presence of

necrotizing granulomas. In 10 patients mediastinal lymph node aspirate was done which showed presence of necrotizing granulomas. Mantoux positivity along with suggestive radiological features of tuberculosis was seen in one clinical suspect. GeneXpert positivity in pleural fluid was seen in one patient. In the remaining 10 patients definitive diagnosis of tuberculosis could be made on the basis of clinical and radiological features.

Tuberculous pleural fluid was lymphocyte rich with mean ± SD protein level of 5.5±0.5, cell count of 2572±1796. Mean ± SD of ADA in this group of patients was 57.7±21. Tuberculous effusion was more common on the left side of pleural cavity in our study (Table III).

In the second group of patients having malignancy, 8 patients had pleural fluid metastasis from carcinoma. Mean ± SD levels of ADA in these patients was 13.3±3.7. Two patients were of Non Hodgkins Lymphoma having atypical lymphoid cells in the pleural fluid. Mean± SD levels of ADA values in the lymphoma subgroup were 332±214 IU/L. In the third group of patients diagnosed as empyema mean glucose levels were low being 4.1±2.5mg/dl. Mean total cell count was 12800±7038 cells/cmm with predominance of neutrophils. Mean ADA levels were 332±214 IU/L. In the fourth group of patients diagnosed as uremic pleural effusion mean total protein was 4.9±1 g/dl, mean glucose levels were 122.6±28.4mg/dl. Mean total cell count was 122.6±28.4 cells/cmm with predominance of lymphocytes. Mean ADA levels were 18.2±6.7 IU/L. In the fifth group of patients diagnosed as parapneumonic effusion mean total protein was 3.85±0.5 g/dl, mean glucose levels were 73±32 mg/dl. Mean total cell count was 2341±508 cells/cmm with predominance of neutrophils. Mean ADA levels were 16.9±14 IU/L. (Table III)

ADA levels were > 40 IU/L in 30 out of 31 (96.7%) patients of tuberculosis. In our study four patients in the non tuberculosis group had ADA values >40 IU/L. (Table IV) These were patients of empyema and lymphoma and had ADA values >100 IU/L.

Sensitivity of ADA for tuberculosis at the cut off value of >40 IU/L was found to be 96.88%, specificity was 82.61%, positive predictive value for tuberculosis was 88.5%, negative predictive value for tuberculosis was 95%.

DISCUSSION

An exudative pleural effusion is a common clinical presentation in a patient with underlying lung disease. An exudate usually results from local lung diseases like tuberculosis, pneumonia, malignancy while a transudate is due to systemic diseases such as congestive heart failure, cirrhosis or nephritis[10]. In the present study we found greater incidence of pleural effusion in males as compared to females. Male to female ratio being 2.5:1. Past studies have also shown that pleural effusion is more common in males as compared to females[11] .Tuberculosis is global health problem which is endemic disease in developing countries and one of the most frequent causes of exudative pleural effusion[12]. In our study also we found tuberculosis to be the leading cause of exudative pleural effusion, its incidence being 62%. Kate et al[2] found the incidence of tuberculosis to be 60% in their study which is almost similar to our findings. However establishing the diagnosis of tuberculosis in pleural fluid is not always an easy task. Conventional diagnostic modalities like AFB smear, PCR have low detection rates in pleural fluid[13]. Thoracoscopy has high diagnostic yield however it is invasive as well as not freely available at all centers[14]. Herein simple biochemical assays like ADA estimation play a significant role. In our study we found that at the cut off value of >40 IU/L, the sensitivity of ADA was found to be 96.88%, specificity was 82.61%, positive predictive value for tuberculosis was 88.5%, negative predictive value for tuberculosis was 95%. In various previous studies sensitivity of 81-100 % and specificity of

89-100% has been observed at cut off levels of >40 IU/L for pleural fluid ADA[15,16]. In our study 30 out of 31(96.7%) patients of tuberculosis had ADA levels >40 IU/L. Other than tuberculosis patients of lymphoma and empyema also showed high ADA levels. Two of the four (50%) patients of empyema had ADA levels >100 IU/L. There were two patients of lymphoma in our study. Both these patients had a high cell count in the pleural fluid with presence of atypical lymphoid cells and ADA values >100 IU/L. Porcel et al found that in patients with extremely high ADA (>250 U/L), empyema or lymphoma rather than tuberculosis should be considered[5] . We also had similar findings in our study as all four patients having ADA levels of >100 IU/L in our study were either of empyema or lymphoma. In our study only one patient of TPE had ADA level of >100IU/L. This patient had high pleural fluid cell count with predominance of neutrophils. GeneXpert for tuberculosis was positive in this patient. Past studies have shown that neutrophils predominate in early stages of TPE and the likelihood of isolating Mycobacterium tuberculosis increases significantly if the fluid is neutrophil rich[17,18]. Similar finding was seen in our study as genexpert test for M. tuberculosis was positive in this patient. Pleural fluid cytology in addition to ADA levels also plays an important role in correct etiological classification of pleural fluid. Tuberculous pleural fluid is considered to have high cell count usually >1000 cells/cmm with a differential lymphocyte count of >70% lymphocytes. In our study lymphocytic predominant pleural effusion was seen in 30 out of 31 patients (96.7%) of TPE. In one patient of TPE predominance of neutrophils was seen. Our finding is similar to the study done by Valdes et al[19] as they found the incidence of neutrophil predominant pleural effusion in TPE to be 6.7% as against 3.3% in our study. In our study we found that in addition to tuberculous effusion, uremic and malignant effusions were also lymphocyte predominant. However in uremic pleural effusion total cell count was much lower as compared to tubercular pleural effusion. Exudative pleural effusions with predominantly mononuclear cells indicate chronic diseases like tuberculosis, malignant disease, pulmonary embolism, pleural effusion following coronary artery bypass surgery[20].In addition to TPE, high ADA levels with high cell count and predominance of lymphocytes was also seen pleural effusion due to lymphoma. However in lymphomatous pleural effusion atypical lymphoid cells were seen. Malignant pleural effusions due to metastasis from carcinoma showed presence of atypical cells. In our study atypical cells were seen in all patients of suspected malignancy. Cytological analysis of multiple samples by an expert cytopathologist is very important in case a malignant neoplasm is suspected[21]. Parapneumonic effusions and pleural effusion due to empyema showed predominance of neutrophils (>80%). High ADA levels were seen in 2 out of 4 (50%) empyema patients. In empyema patients cell count was very high and there was a predominance of neutrophils. Pleural fluid glucose levels were found to be extremely low in patients of empyema. Both these findings in empyema patients are in conformance with the standard diagnostic criterias prescribed for empyema patients[22]. Thus it can be concluded from our study that pleural fluid cytology, Lymphocyte Neutrophil ratio along with serum ADA levels altogether can help in etiological classification of patients with exudative pleural effusion and using a cut off of > 40 U/L for pleural fluid ADA, patients can be classified as tuberculous or non tuberculous with a reasonably high diagnostic accuracy.

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Conflicts of interests: - None

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