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Original Article

Estimation of Adenosine Deaminase Levels in Pleural Effusion: It's Role in Differential Diagnosis

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ABSTRACT

Aims: The aim of the present study was to evaluate the efficiency of pleural fluid Adenosine Deaminase (ADA) levels in differential diagnosis of pleural effusions like to differentiate between tuberculous and non tuberculous effusions, transudative and exudative effusion and between inflammatory and non-inflammatory pleural effusions.

Material And Methods: 74 patients of pleural effusion were investigated and divided into four groups based on diagnosis. Group I, II, III and IV had 31 cases of tuberculous effusion, 21 cases of transudative effusion, 14 cases of parapneumonic effusion and 08 cases of malignant effusion respectively. The diagnosis of etiology of pleural effusion was done based on clinical presentation, radiological examination and laboratory investigations. Pleural fluid was analyzed for ADA (Guisti and Galanti's method).

Results: Pleural fluid ADA levels were highest in tuberculous effusion and the difference in ADA levels between tuberculous and other effusion was statistically highly significant (transudative effusion $p<0.001$, PPE $p<0.01$ and malignant effusion $p<0.001$). ADA levels were compared between tuberculous effusion and non tuberculous effusion. Statistically significant increase was seen in tuberculous effusion than non tuberculous effusion ($p<0.001$). Among tuberculous effusion only 03 cases had ADA levels less than 40U/L and among non-tubercular group only 04 cases had ADA levels greater than 40 U/L.

Conclusions: Pleural fluid ADA estimation is a minimally invasive, inexpensive and efficacious method of differentiating pleural effusion etiologies. India has a high prevalence of tuberculosis and the sensitivity and specificity of this test will be high in this population. ADA levels in non-tuberculous exudative pleural effusions rarely exceeded the cutoff; set for tuberculous disease. The pleural fluid ADA levels were significantly higher in tuberculous exudative pleural effusions when compared with non-tuberculous exudative pleural effusions. Therefore ADA estimation being a simple, low cost, rapid and non-invasive test, should become an integral part of the diagnostic work up of pleural effusions.

KEYWORDS: Adenosine deaminase, Pleural effusion, Tubercular, Non-tubercular.

INTRODUCTION

Pleural effusion (PE) occurs due to various factors such as increased pleural membrane permeability, increased capillary pressure, decreased oncotic pressure and lymphatic obstruction.[1] The classification of pleural effusion into exudative and transudative to support the clinical diagnosis has been well established.

There are several causes of exudative pleural effusion including tuberculosis, neoplasms (primary or secondary), pyogenic bacterial infections, fungal infection, sar-coidosis, collagen vascular disease, transplant patients with graft rejection, trauma, and pulmonary embolism.[2] Transudative pleural effusions occur when systemic factors which effect the formation and absorption of pleural fluid are altered.

Tuberculous (TB) pleurisy is the major cause of pleural effusion. [3,4] Tuberculous Pleuritis with effusion as a complication of primary pulmonary tuberculosis has been reported to occur in 2 to 38% of children with pulmonary disease, but it is more likely to occur in adolescents and adults.[5-7]

In India, TB is the commonest cause of pulmonary disease. About 5 lakh people suffering from TB die every year with pulmonary TB often associated with pleural effusion.[2] Tuberculous pleural effusion is the second most common cause of extra pulmonary TB secondary to only lymphoid TB.[8]

Conventional noninvasive diagnostic methods are not always accurate in establishing the diagnosis of pleural effusion. Analysis of pleural fluid yields important information in early differential diagnosis of pleural effusion. Standard workup analysis of pleural fluid includes differentiating whether pleural fluid is transudative or exudative. For many years the most accepted criteria for discriminating transudative from exudative pleural effusion is Light's criteria.1 However Light's criteria may differentiate certain transudative effusion as exudative effusion.[9]

In patients with tuberculous exudative pleural effusion, neutrophils predominate in the early stages of the disease, while abundant mononuclear cells is a classical finding later and is believed to be due to the proliferation and differentiation of lymphocytes which release lymphokines, which in turn activate macrophages for an enhanced bactericidal activity.[10,11] Even pleural fluid cytology takes a back seat while investigating the cause of an exudative pleural effusion, and is usually just evidence supporting our final diagnosis.

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, and C-reactive protein (CRP) have been proposed as alternative noninvasive means of establishing etiology in cases of pleural effusion.[12]

The ADA is an enzyme involved in the purine catabolism. It catalyzes the deamination of adenosine to inosine and of deoxyadenosine to deoxyinosine. Adenosine deaminase is involved in the proliferation and differentiation of lymphocytes, specifically the T-lymphocytes. The T-cells release ADA during the process of activation in the presence of live intracellular pathogens. Thus ADA has been looked upon as a marker of cell mediated immune response and specifically T-cell activation.

Measurement of ADA in PF has been widely used in the differential diagnosis of lymphocytic exudative pleural effusion as high values have been found in tuberculous pleural effusion.[2,4,8]

Usefulness of adenosine deaminase (ADA) estimation in pleural fluid has been shown as a reliable chemical biomarker specially when there is suspicion of tuberculosis in endemic areas. Sometimes the increase is marked in early stages of the disease and in some other conditions with neutrophillic effusions like in parapneumonic, empyema, rheumatoid arthritis, mesothelioma, bronchial carcinoma, fungal infections etc. Researchers have established that ADA level rarely exceeds the cut-off set for tuberculous effusion in non-tuberculous lymphocytic effusions.[13,14]

The aim of the present study was to evaluate the efficiency of pleural fluid ADA levels in differential diagnosis of pleural effusions like to differentiate between tuberculous and non tuberculous effusions, transudative and exudative effusion and between inflammatory and non-inflammatory pleural effusions.

MATERIALS & METHODS

Present study included 74 patients with pleural effusion of different etiologies, age ranging from 18 to 80 years. Informed consent was taken and the study was approved by ethical and research committee of the institution.

The patients were categorized into four groups based on diagnosis.

GROUP I: Tuberculous pleural effusion (31 cases)
GROUP II: Transudative pleural effusion (21 cases)
GROUP III: Parapneumonic (PPE) (14 cases)
GROUP IV: Malignant effusion (08 cases)

The diagnosis of etiology of pleural effusion was done based on clinical presentation, radiological examination and laboratory investigations. Diagnosis of TB pleural effusion was done when positive for any one of the following test: presence of tubercle bacilli in smear or in culture of pleural fluid, caseating granulomas in histopathological study, radiological findings consistent with TB, response to antitubercular treatment. Malignant pleural effusion was diagnosed when PF cytology showed evidence of malignancy and or neoplastic pleural tissue in pleural biopsy. PPE was diagnosed when patient had fever, pulmonary infiltrates in chest X-Ray and who responded to antibiotic treatment.[2,15]

Transudative and exudative pleural effusion were distinguished based on protein and LDH levels in pleural fluid and serum as per Light's criteria.[3]

Pleural tap was done in all cases and the pleural fluid was analysed for sugar, protein, lactate dehydrogenase (LDH), ADA levels. Pleural fluid sugar, protein and LDH were analysed using Erba reagent kits on EM 200 analyzer. The study parameter ADA in pleural fluid was measured by Guisti and Galanti's method of ADA estimation.[16] The results were expressed as mean ± standard deviation (SD). The statistical analysis was done using unpaired student't' test and probability value (P) value < 0.05 was considered statistically significant. Sensitivity, specificity, positive predictive value and negative predictive value were calculated.

RESULTS

74 cases of pleural effusion were investigated. (Table 1) Pleural fluid was analyzed for Protein, Sugar, LDH, ADA levels and the results are shown in Table 2.

Pleural fluid ADA levels were highest in tuberculous effusion and the difference in ADA levels between tuberculous and other effusion was statistically highly significant (transudative effusion $p<0.001$, PPE $p<0.01$ and malignant effusion $p<0.001$) (Table 3). ADA levels were compared between tuberculous effusion and non tuberculous effusion. Statistically significant increase was seen in tuberculous effusion than non tuberculous effusion ($p<0.001$) (Table 4).

Among tuberculous effusion only 03 cases had ADA levels less than 40U/L and among non-tubercular group only 04 cases had ADA levels greater than 40 U/L (Table 4).

Table 1: Distribution of cases in different groups of pleural effusion

Grou	Diagnosis	No of
I	Tuberculous pleural effusion	31
II	Transudative pleural effusion	21
III	Parapneumonic pleural effusion	14
IV	Malignant pleural effusion	08
Total	All groups	74

Table 2: Pleural fluid protein, sugar and LDH* levels in different groups

Grou p	Diagnosis	Protein (gm%) Mean	Sugar (mg %) Mean	LDH* Mean ±SD
I	Tuberculosis pleural effusion	4.8±1.04	61.3±10.84	134±53.2
II	Transudative pleural effusion	2.2±0.43	66.7±12.83	87.1±25.7
III	Parapneumonic pleural effusion	5.3±0.69	46.8±8.45	174.9±69.2
IV	Malignant pleural effusion	4.6±0.47	42.9±10.92	243.1±112.4

* Lactate Dehydrogenase

Table 3: Pleural fluid ADA levels in different types of pleural effusions

Group	Diagnosis	ADA† Mean ±SD	P value
I	Tuberculous pleural effusion	127.83±77.69	-
II	Transudative pleural effusion	21.87±7.74	0.001*
III	Parapneumonic pleural effusion	52.24±24.96	0.01**
IV	Malignant pleural effusion	34.85±12.79	0.001***

†Adenosine deaminase
*p value between tuberculous effusion and transudative effusion
**p value between tuberculous effusion and parapneumonic effusion
***p value between tuberculous effusion and malignant effusion

Table 4: Pleural fluid ADA† levels in tubercular and non-tubercular pleural effusion.

Diagnosis	ADA† Mean ±SD	No of patients with ADA† > 40U/L	No of patients with ADA† <40U/
Tuberculous effusion	127.83±77.69	28 (90.32%)	03(9.68%)
Non tuberculous effusion	32.89±25.78	04 (9.30%)	25 (90.7%)

†Adenosine deaminase

DISCUSSION

Various biological markers have been investigated in the diagnosis of pleural effusion. Among these pleural fluid ADA, CRP, interferon γ, cytokines, interleukins, tumour markers, vascular endothelial growth factor have been found to be of value in the differential diagnosis of pleural effusion. Nevertheless many of these markers have limited value, either because of low sensitivity & specificity or high cost.

The diagnosis of tuberculous pleural effusion is difficult because of low sensitivity and specificity of various non-invasive tools like acid fast bacilli staining, culture of pleural tap and tuberculin skin testing. Diagnosis increases to 96.2% with pleural biopsy but the disadvantage of this technique is its invasiveness.[2]

Delay in diagnosis and in the start of effective treatment results in poor prognosis and sequalae in upto 25% of cases.[17] Available methods of diagnosis of tuberculosis were evaluated and all of them were found to have low sensitivity and specificity. Direct evidence of acid fast bacilli (AFB) is available only in small percentage of cases.

Adenosine deaminase estimation in pleural fluid has long been taken as a marker for tuberculous pleurisy. Levels above 40 U/L indicate pleural tuberculosis with sensitivity 81 to 100% and specificity 83 to 100%,[18,19] while some other workers have observed that this cut-off indicates a still higher sensitivity of 90 - 100% and specificity of 89 - 100%.[20-23] False positive cases reported could be due to empyema, lymphoma,

malignancy, parapneumonic or collagen vascular disease.[21,24]

In the present study pleural fluid ADA levels were compared between the four groups of pleural effusion. The values were highest in tuberculous effusion and lowest in transudative effusion. The difference in ADA levels between tuberculous and non tuberculous effusion was statistically significant ($p<0.001$). Also the difference in ADA levels between tuberculous effusion and transudative effusion, PPE and malignant effusion were statistically significant. Similar report was also seen by Bharth KG et al[2] and Aliya Nusrath et al.[15]

In the present study, 3 cases of tuberculous pleural effusion had ADA values less than 40 U/L. A similar report was given by Aliya Nusrath et al.[15] and Motoki S et al where they found 12% of the tuberculous pleurisy patients having ADA levels less than 50U/L and out of this 6% of them had less than 35U/L.[3]

The ADA levels at a cutoff value of 40 U/L indicated tubercular pleurisy with a sensitivity of 90-100% and specificity of 89-100%.[25] Wipa R et al reported 80% sensitivity and 80.5% specificity at a cutoff value of 48U/L in diagnosing tuberculous effusion.[26] Burgess LJ et al showed 90% and 89% sensitivity and specificity for identification of TB pleurisy at a cutoff value of 50U/L.[27]

However Rafael L. cautioned the use of pleural fluid ADA assay as an alternative to biopsy and culture, but should rather be considered as a screening test to guide further diagnostic management.[25]

Daniil ZD et al, evaluated multiple biomarkers in discriminating pleural effusion. They concluded the combination of markers like ADA, CRP might be sufficient in discriminating the three different groups of pleural effusion, tubercular, malignant and PPE.[12] We have found the pleural fluid ADA levels to be consistently increased and more than the cut-off (40U/L) in cases of exudative pleural effusions of tuberculous etiology. In cases with non-tubercular exudative pleural effusion the ADA levels were found to be consistently below the cut-off. Therefore, pleural fluid ADA levels can play a very significant role in differentiating cases of exudative pleural effusion into tuberculous and non-tuberculous.

CONCLUSION

Pleural fluid ADA estimation is a minimally invasive, inexpensive and efficacious method of differentiating pleural effusion etiologies. India has a high prevalence of tuberculosis and the sensitivity and specificity of this test will be high in this population.

ADA levels in non-tuberculous exudative pleural effusions rarely exceeded the cutoff; set for tuberculous disease. The pleural fluid ADA levels were significantly higher in tuberculous exudative pleural effusions when compared with non-tuberculous exudative pleural effusions. Therefore ADA estimation being a simple, low cost, rapid and non-invasive test, should become an integral part of the diagnostic work up of pleural effusions.

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