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Original article

Relevance of adenosine deaminase as a marker for tuberculous pleural effusion in developing countries

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Abstract

Objective: Relevance of estimation of pleural adenosine deaminase (PADA) and serum adenosine deaminase (SADA) levels in pleural effusion especially in cases of lymphocytic predominant exudative tubercular effusions. **Methods**: Fifty patients (33 male and 17 female; age: 44.12 ± 11.51 years) with pleural effusions were selected to assay adenosine deaminase (ADA) activity in pleural fluid and serum in adjunct to pleural fluid analysis. Effusions were individually classified as transudates or exudates after careful evaluation of all the biochemical parameters of pleural fluid and serum of patients and on the basis of Light's criteria. Cutoff value for PADA was taken as 60U/L and that for pleural/serum ADA ratio (P/S ADA) was 1.8. **Results**: Fourty-three patients had exudative effusions among which 38 patients had tuberculous pleural effusions and 5 had nontubercular effusions. 7 cases were transudates. Mean PADA levels in tubercular group (78.95 ± 25.32 U/L) were found to be much higher ($P = 0.000\ 0$) than nontubercular (23.00 ± 5.22 U/L) group. SADA levels in tubercular group (31.05 ± 6.42 U/L) were significantly higher ($P = 0.000\ 0$) as compared to nontubercular group (15.58 ± 8.35 U/L). PADA cutoff at 60 U/L yielded sensitivity and specificity of 81.5% and 100% respectively, whereas P/S ADA ratio at 1.8 gave sensitivity and specificity of 84.2% and 75% respectively. A positive correlation ($r = 0.507$, $P = 0.001\ 1$) between PADA and SADA was found in tubercular group but no such correlation ($r = 0.302$, $P = 0.340\ 7$) was observed in nontubercular group. **Conclusion**: The measurement of ADA in tubercular pleural effusions has not only relevance but also a high diagnostic utility when other clinical and laboratory tests are either negative or confusing.

Keywords: Adenosine deaminase (ADA); Pleural adenosine deaminase (PADA); Tubercular pleural effusion; P/S ADA ratio

INTRODUCTION

Definitive diagnosis of tubercular pleural effusion (TPE) is difficult to make because of the low sensitivity and specificity of all the non-invasive diagnostic tools that we have in our hands. Pleural biopsy is conventionally considered as gold standard procedure for the diagnosis of TPE even though pleural fluid contains sensitive biochemical markers like adenosine deaminase (ADA), γ-interferon & tumor necrosis factor. In developing countries, tuberculosis is one of the most common opportunistic infections in people who are seropositive for human immunodeficiency virus – 1 (HIV-1)[1]. The severity of the disease can be judged by the fact that it affects all ages, irrespective of the sex. No other disease has so much of impact on socioeconomic status of an entire nation as India. Recent studies have provided insights into the immunopathogenesis of pleural tuberculosis, including memory T-cell homing and chemokine activation. The measurement of ADA activity is one of the biochemical markers most talked about. The level of serum ADA increases in various diseases in which cell immunization is stimulated[2]. Previous

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studies have confirmed the diagnostic value of ADA activity in effusions due to pleural, pericardial, meningeal, and peritoneal tuberculosis especially in countries with high tuberculosis prevalence[3]. ADA is an enzyme in the purine salvage pathway that catalyses the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine with the release of ammonia. It was determined that PADA level > 70 IU/L is highly suggestive of tuberculosis, while a level < 40 IU/L virtually exclude the diagnosis[4]. The reported cutoff value for ADA varies from 47-60 IU/L. Specificity is increased when the lymphocyte/neutrophil ratio (> 0.75) in the pleural fluid is considered together with an ADA concentration of > 50 U/L[5]. It is found that PADA levels in tubercular effusions were significantly higher than the non-tubercular effusions[6].

The present study was aimed to assess the following objectives:

1. Estimation of PADA and SADA to distinguish between tubercular (TB) and non tubercular (NTB) pleural effusions from the patients belonging to Bijapur, a backward district of Karnataka (India).

2. Estimation of P/S ADA ratio of the same patients to know its diagnostic implications.

MATERIALS AND METHODS

Fifty patients (age: 44.12 ± 11.51 years) of pleural effusion were selected based on history, clinical examination and chest radiographic findings in which 33(66%) were male and 17(34%) were female. Patients who were diagnosed to have either malignancy or connective tissue disorders were excluded from the study. Complete haemogram including erythrocyte sedimentation rate(ESR), renal function tests and liver function tests were done. Sputum for acid fast bacill(AFB), culture & sensitivity was done. Pleural fluid analysis was done with all parameters taken into consideration. Effusions were individually classified as transudates or exudates after careful evaluation of all clinical data, biochemical analysis of pleural fluid and on the basis of Light's criteria. PADA and SADA levels were estimated by colorimetric method of Guisti and Galanti[7]. The means and standard deviations of PADA, SADA and pleural fluid and serum(P/S) ADA ratio were evaluated for both transudative & exudative effusions. Cutoff value for the diagnosis of TPE was taken as >60 U/L and that of P/S ADA ratio was taken as 1.8[8]. Sensitivity and specificity were calculated. Student t' test was performed to calculate the level of significance between different groups. Correlation was done between PADA and SADA of both tuberculous (TB) and non-tuberculous (NTB) groups by using Pearson's correlation co-efficient. Informed consent was taken from all patients. Experimental protocol was ethically cleared by institutional ethical clearance committee as per the declaration of Helsinki (1964).

RESULTS

Out of 50 patients evaluated in this study, 43 (86%) had exudative effusions among which 38 (76%) had tuberculous pleural effusion and 5 (10%) had non tubercular effusions. 7(14%) cases had transudative effusions. Two groups were formed- Group 1 had 38 patients of tuberculous pleural effusions and Group 2 had 12 nontubercular patients. Hemoglobin, total count and random blood sugar levels were compared between the two groups with no much significance (Table 1). Serum creatinine levels were slightly higher in Group 2 (P = 0.016 3) as compared to Group 1 but both the values were much within the normal physiological range. ESR was significantly raised in Group 1 as compared to Group 2 (P = 0.000 0) (Table 1). Pleural fluid analysis was done in which cell count (P = 0.000 0), lymphocyte% (P = 0.000 0) and protein levels (P = 0.000 0) were significantly higher in Group 1 as compared to Group 2 (Table 2). PADA and SADA levels in Group 1 were significantly raised as compared to Group 2 (P = 0.000 0). PADA levels in Group 1(78.95 ± 25.32 U/L) was much higher than PADA levels (t = 7.553, P = 0.000 0) in Group 2(23.00 ± 5.22 U/L). SADA t = 6.761, levels in Group 1(31.05 ± 6.42 U/L) also showed significant elevations as compared to Group 2(15.58 ± 8.35 U/L) (P = 0.000 0). When P/S ADA ratio was compared between the two groups, Group 1(2.57 ± 0.68) showed higher value as compared to Group 2(1.68 ± 0.95) (t = 3.581, P = 0.000 8). When P/S ADA was compared between tuberculous exudative pleural effusions (n = 38) (2.57 ± 0.68) with transudative pleural effusions (n = 7) alone, high levels of significance (t = 4.095, P = 0.002) was observed. Table 3 depicts the sensitivity and specificity as 81.5% and 100% respectively with a ADA cut off at 60 U/L whereas P/S ADA cut off at 1.8 revealed the sensitivity and

specificity to be 84.2% and 75% respectively. Pearson's correlation was done between PADA and SADA in both the tuberculous and non tuberculous groups. A positive correlation ($r = 0.507$, $SE = 0.120$, $df = 36$, $t = 3.532$, $P = 0.001\ 1$) between PADA and SADA was found in Group 1 but no such correlation ($r = 0.302$, $SE = 0.262$, $df = 10$, $t = 1.000$, $P = 0.340\ 7$) was observed in Group 2.

Table 1 Hematological & biochemical parameters (Mean ± SD).

Parameters	Group 1 ($n = 38$)	Group 2 ($n = 12$)	Significance
Hb% (gm %)	10.71 ± 1.53	10.92 ± 3.15	$t = 0.314$ $df = 48$ $P = 0.754\ 9$
ESR (mm/hr)	78.13 ± 17.69	17.67 ± 6.00	$t = 11.560$ $df = 48$ $P = 0.000\ 0$
TC (cells/mm^3)	8 160 ± 2 118	9 641 ± 4 358	$t = 1.600$ $df = 48$ $P = 0.116\ 1$
RBS (mg%)	114 ± 32	130 ± 96	$t = 0.897$ $df = 48$ $P = 0.374\ 2$
Serum creatinine (mg%)	0.95 ± 0.25	1.16 ± 0.27	$t = 2.490$ $df = 48$ $P = 0.016\ 3$

Group 1: tubercular; Group 2: non tubercular

Table 2 Pleural fluid analysis (Mean ± SD).

Parameters	Cell count (cells/mm^3)	Lymphocyte (%)	Protein (gm/dL)	Sugar (mg/dL)
Group 1 ($n = 38$)	631 ± 220	85 ± 11	4.47 ± 1.11	46 ± 23
Group 2 ($n = 12$)	145 ± 72	39 ± 15	2.64 ± 0.89	37 ± 20
Significance	$t = 7.480$, $df = 48$, $P = 0.000\ 0$	$t = 11.543$, $df = 48$, $P = 0.000\ 0$	$t = 5.196$, $df = 48$, $P = 0.000\ 0$	$t = 1.216$, $df = 48$, $P = 0.229\ 9$

Group 1: tubercular; Group 2: non tubercular

Table 3 Sensitivity & specificity of adenosine deaminase (ADA) test in pleural fluid and P/S ADA.

Parameter		Group1	Group2	Total
ADA (U/L)	>60	31	0	31
	<60	7	12	19
Total		38	12	50
DISADA	>1.8	32	3	35
	<1.8	6	9	15
Total		38	12	50

DISCUSSION

In clinical practice the pleural effusions are frequent and often constitute difficult diagnostic problems. In spite of careful diagnostic evaluation the etiology of effusion cannot be established in many patients. In our study the mean pleural ADA in tubercular effusions is significantly higher as compared to other causes of effusion which may be considered as a helpful tool in differential diagnosis of exudative and transudative pleural effusions. In our study, higher lymphocyte predominance was noted in tuberculous pleural effusion but no correlation was found between the pleural fluid ADA and T-lymphocyte count. The molecular forms of ADA were studied in pleural effusions using the technique of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)[9]. Two isoenzymes have been identified namely ADA-1 and ADA-2. ADA-l isoenzymes have been found to be present in all the cells with highest activity observed in lymphocytes and monocytes whereas ADA-2 is found only in monocytes[10]. ADA-2 is the predominant isoform in tubercular pleural effusions, accounting for most of the total ADA activity, however in clinical practice the difference in the use of total ADA or the isoform ADA-2 is not considered to be significant and furthermore the isoenzyme assay is more expensive and not readily available[11]. Determination of isoenzyme would only enhance the diagnostic utility of ADA activity in the determination of pleural effusion. Pleural fluid culture takes 6-8 weeks for isolation of the organisms and pleural biopsy is an invasive procedure which carries a definitive risk of complications like intercostal neuralgia, pneumothorax and hemothorax. Looking into the disadvantages of pleural biopsy and delay in procuring culture report of tubercular organisms, estimation of PADA has become a simple, rapid, safe, reliable and noninvasive test for the diagnosis of tubercular pleural effusion. It was determined that PADA levels > 70 U/L is highly suggestive of tuberculosis, while a level < 40 U/L virtually excludes such diagnosis[4]. A metaanalysis[12] of 40 studies published from 1966 to 1999 concluded that the test performance of ADA (sensitivity range 47.1% to 100%, and specificity range 0% to 100%) in diagnosing tuberculous pleural effusions is reasonably a good one. It is adequate enough to avoid pleural biopsy

especially in young patients from areas with high prevalence of tuberculosis. Specificity is increased when the lymphocyte/neutrophil ratio in the pleural fluid (>0.75) is considered together with an ADA concentration of >50 U/L[5]. Reported cutoff value for ADA varies from 47 to 60 U/L[13]. The discrepancies in the results among the reported studies can be attributed to the use of different methods of ADA analysis, with the most frequent being the colorimetric assay by Guisti and Galanti[7]. The analysis of PCR along with ADA activity is found to be a very useful diagnostic approach to achieve a more rapid and precise diagnosis in the cases of pulmonary tuberculosis[14]. The significant increase of PADA in tubercular effusions as compared to non tubercular effusions in our study corroborate with other observations[6]. Recent study on P/S ADA ratio for diagnosis of tuberculous pleural effusion showed sensitivity and specificity of 82.6% and 84.8% respectively at the threshold value of 1.8[8]. Another study showed that ADA effusion/serum ratio reached a cutoff in tubercular pleural effusions of 1.7 with a sensitivity and specificity of 84.6% and 72.2% respectively[15]. The cutoff value of the P/S ADA ratio in our study taken as 1.8 showed a sensitivity and specificity of 84.2% and 75% respectively which reflects ADA as a useful biochemical marker to suggest exudative effusions[16]. As pleural cells become activated and produce cytokines as a response to mycobacteria, hence evaluation of the level of ADA in the pleural fluid may be considered as the most useful tool for diagnosis of tuberculous pleural effusions[17]. From the current study, a positive correlation between PADA and SADA in patients with tubercular effusions and no such correlation in case of nontubercular effusions may be considered as a secondary marker for differentiation of tubercular with nontubercular effusions. It has to be remembered that the diagnostic value of ADA is independent of HIV serologic status[18].

Pleural ADA may be considered as a highly sensitive marker for evaluating tubercular pleural effusions and we can practically exclude tuberculosis as the cause of effusion in case of low pleural ADA values. Estimation of ADA activity (PADA and SADA) may provide the basis for rapid and efficient diagnosis of pleural tuberculosis but other variables like ESR, lactate dehydrogenase and lymphocyte to neutrophil ratio should also be kept in mind at the time of diagnosis.

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