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Diagnostic and Prognostic Significance of Pleural Fluid Adenosine Deaminase Estimation in Relation to Pulmonary Tuberculosis.

KEYWORDS: *Adenosine deaminase, Pulmonary tuberculosis*

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ABSTRACT *Adenosine deaminase (ADA), an enzyme involved in purine catabolism, catalyses the hydrolytic cleavage of adenosine and 2'deoxyadenosine, irreversible converting them into inosine and 2'deoxyinosine respectively. ADA activity increases during cellular activation for energy demand to detoxify the toxic metabolites. The present study was conducted to evaluate the role of pleural fluid ADA activity in the diagnosis and prognosis of PTB. The pleural fluid ADA values were significantly higher ($p<0.001$) in all age groups of untreated group as compared to the parallel age groups of the subjects of pulmonary tuberculosis, those were under treatment. Enzyme activity slowly decreases in pleural fluid, during the initial months of treatment phase that indicates fast recovery and good response of anti-tubercular therapy and not stabilized during first 3 months of treatment. Therefore it may be used as a prognostic marker for pulmonary tuberculosis in the initial months of treatment.*

INTRODUCTION

Adenosine deaminase (ADA, adenosine aminohydrolase, EC 3.5.4.4), an enzyme involved in purine metabolism, catalyses the hydrolytic cleavage of adenosine and 2'deoxyadenosine, irreversible converting them into inosine and 2'deoxyinosine respectively (1). ADA activity increases during cellular activation for energy demand to detoxify the toxic metabolites. It plays an important role in lymphocyte and monocyte maturation and activity (2). Increased pleural fluid levels of ADA has been reported in several diseases characterized by an enhanced cell mediated immune (CMI) response, such as typhoid fever, bacterial pneumonia, Infectious mononucleosis and tuberculosis (3,4,5,6). There is limited data on the use of pleural fluid ADA levels to diagnose active PTB in adults. Further, the prognostic role of pleural fluid ADA in active PTB has not been studied so far. The purpose of this study was to evaluate the role of pleural fluid ADA activity in the diagnosis and prognosis of PTB.

MATERIAL AND METHODS

142 subjects (indoor and outpatient clinic of either sex) aged 10 to 80 years, suffering from PTB, were included in study. All the patients were examined clinically and investigated. The diagnosis of pulmonary tuberculosis was established by positive sputum smear examination for acid fast bacilli and chest skiagram. Pleural fluid analysis was carried out in all 142 patients suffering from PTB before starting the treatment. The exudates were distinguished from transudates by Pleural fluid protein cut-off level of 3 g/dl or more. Pleural fluid was subjected to routine microscopic and biochemical analysis. The subjects were divided into two groups-Group I (n=142) untreated and recently diagnosed, clinically as well as radiologically established patients suffering from active PTB; and Group II (n=127) included followed up cases of PTB who were receiving effective anti-tuberculosis therapy for a period of three months. All patients were treated with standard chemotherapeutic regimens with isoniazid, rifampin and pyrazinamide and followed-up for a period of three months. Pleural fluid samples were aspirated by thoracocentesis with the help of treating clinicians. About 15 ml of the aspirate was taken in a sterilized container and delivered into 3 vials and brought to the laboratory.

(1) 5ml of the pleural fluid was transferred to a sterilized vial for the estimation of adenosine deaminase (ADA) and total protein.

(2) 5ml of pleural fluid was taken in a clean and dry vial containing 15mg fluoride-oxalate (one part of sodium fluoride and three parts of potassium oxalate) for the estimation of glucose.

(3) 5ml of fluid was taken in EDTA vial (7mg) for microscopic examination (number and types of white cells).

ADA activity in pleural fluid was determined by colorimetric method based on the method of Guisti (1969) (7). ADA-MTB kits were supplied by Microxpress, Tulip diagnostic (P) Ltd. Goa. Manual / literature given with the kit for procedure were followed strictly. The data assembled for different biochemical parameters were subjected to suitable statistical analysis to compute central tendencies (Mean) and accompanying measures of variability statistics (Standard deviation) for groups-I & group-II. The magnitude of inter group difference for each of the parameters was quantified by using student't' test.

RESULTS AND DISCUSSION

In untreated pulmonary tubercular subjects, white cell count levels in pleural fluid were significantly higher than the patients who were receiving anti-tubercular therapy since last three months. Total leukocyte count decreases dramatically during treatment phase; it may be due to repeated thoracocentesis or improvement in the clearance capacity of lungs.

The pleural fluids of pulmonary tubercular subjects in untreated phase were straw coloured and turbid in consistency with lymphocyte predominance. The mean percentage of lymphocytes in pleural fluid was significantly high in untreated pulmonary tuberculosis subjects and rapidly decreases during the initial three months of therapy. A significant decrease in lymphocyte percentage indicated good prognosis and response of anti-tubercular therapy.

Pleural fluid glucose levels were significantly lower in untreated pulmonary tubercular subjects and its level increas-

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es during the treatment phase. After treatment of three months, pleural fluid glucose levels rises that indicate improvement in the functions of pleura.

In all the cases, pleural fluid of pulmonary tuberculosis patients was exudative in character with high protein content (8). In untreated patients pleural fluid protein concentrations were significantly high as compared to those, who were followed up for three months.

The pleural fluid adenosine deaminase activities were significantly higher in all the age groups of untreated pulmonary tubercular subjects as compared to the parallel age groups of follow up patients. The pleural fluid ADA activity in untreated PTB subjects was 100.0±19.48 U/L. In pathological conditions, the clearance capacity of lungs is decreased leading to increased number of cells in the pleural fluid and the recirculation of the activated lymphocytes or endocavitary production of ADA for detoxification of toxic metabolites may cause a high pleural fluid ADA activity. In the subjects of group-II, the levels of pleural fluid ADA were significantly lower as compared to the subjects of group-I. This could be due to repeated thoracocentesis, improvement in the clearance capacity of lungs and normalization of the altered lymphocytes turnover. There was no statistical significant variation observed in the mean pleural fluid ADA levels in relation to age and sex in the pulmonary tubercular subjects.

CONCLUSIONS:

Pleural fluid ADA estimation can be used as a diagnostic marker for pulmonary tuberculosis, because adenosine deaminase activity increases in pleural fluid of untreated patients with pulmonary tuberculosis. The adenosine deaminase activity slowly decreases in pleural fluid, during the initial months of treatment phase that indicates fast recovery and good response of anti tubercular therapy and not stabilized during first 3 months of treatment. Therefore it can be used as a prognostic marker for pulmonary tuberculosis.

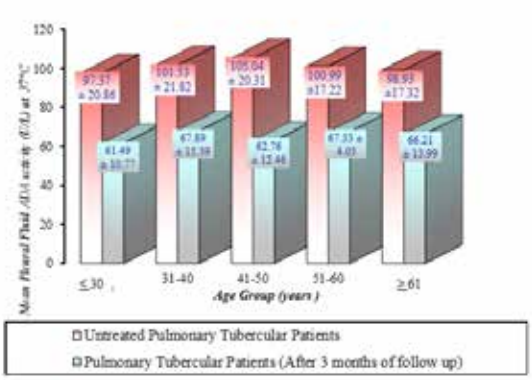
TABLE: 1

COMPARATIVE STUDY OF STATISTICAL ANALYSIS OF PLEURAL FLUID GLUCOSE, PROTEINS, LYMPHOCYTE PERCENTILE AND PLEURAL FLUID ADA LEVELS IN GROUP-I (BEFORE TREATMENT) AND GROUP - II (AFTER TREATMENT OF THREE MONTHS) OF PULMONARY TUBERCULAR SUBJECTS IN VARIOUS AGE GROUPS.

Group Compared	Statistical Value	Age Group (years)				
		≤ 30	31 - 40	41 - 50	51 - 60	≥ 61
Group - I v/s Group - II	**PLEURAL FLUID GLUCOSE**					
	t - value	3.8757	6.5993	3.0572	3.4282	1.2148
	p - value	< 0.001 ***	< 0.001 ***	< 0.01 **	< 0.01 **	> 0.05
	PLEURAL FLUID PROTEINS					
	t - value	5.1666	6.0338	2.6698	2.7045	3.5588
	p - value	< 0.001 ***	< 0.001 ***	< 0.05 *	< 0.01 **	< 0.01 **
	PLEURAL FLUID ADENOSINE DEAMINASE					
	t - value	6.5271	6.2398	6.1302	7.480	4.2546
	p - value	< 0.001 ***	< 0.001 ***	< 0.001 ***	< 0.001 ***	< 0.001 ***
	PLEURAL FLUID LYMPHOCYTE PERCENTILE					
	t - value	10.1124	9.9831	5.2813	6.5909	9.2135
	p - value	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***	<0.001 ***
	PLEURAL FLUID POLYMORPHS PERCENTILE					
	t - value	1.7457	1.8885	2.4797	1.8791	2.8571
	p - value	>0.05	>0.05	<0.05 *	>0.05	<0.01 **
	PLEURAL FLUID TOAL CELL COUNT					
	t - value	4.9692	6.3879	2.1508	6.1015	3.5138
	p - value	< 0.001 ***	< 0.001 ***	<0.05 *	< 0.001 ***	< 0.01 **

* Statistical Significant ** Statistical Very Significant *** Statistical Highly Significant Rest Non Significant

Mean Pleural Fluid Adenosine Deaminase activity in different age groups of Pulmonary Tubercular Subjects



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