

ROLE OF ADENOSINE DEAMINASE IN DIAGNOSIS OF TUBERCULOUS PLEURAL EFFUSION

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ABSTRACT

Background: Tuberculosis is a major contributor of exudative pleural effusion which is the second most common extra-pulmonary manifestation of tuberculosis following tubercular lymphadenitis. Tuberculous pleural effusion is lymphocytic and exudative characteristics. The analysis adenosine deaminase (ADA) activity is a very useful diagnostic approach to achieve a more rapid and precise diagnosis in the cases of pulmonary TB.

Aims & Objective: To study the value of adenosine deaminase level in tuberculous pleural effusion.

Materials and Methods: Eighty cases presenting with tuberculous pleural effusion and 20 cases with non tuberculous pleural effusion were included in the study Department of Pathology, Medical College, Baroda ADA estimation in pleural fluid were carried out as per standard techniques of Galanti and Guisti (1974).

Results: There is an increased level of enzyme ADA in pleural fluid of tuberculous patients as compared to non tuberculous effusions.

Conclusion: Estimation of ADA activity may provide the basis for the rapid and efficient diagnosis of pleural TB in different clinical settings. However study should be carried out on large number of patients to reach a better conclusion.

Key Words: Adenosine Deaminase; Tuberculous Effusion; Pleural Fluid; Exudative Pleural Effusions

Introduction

Despite the discovery of the tubercle bacillus more than a hundred years ago, and all the advances in our knowledge of the disease made since then, tuberculosis still remains the major health problem facing mankind, particularly in developing countries. Presently about one third of world's population is infected with mycobacterium tuberculosis.^[1] It is estimated that currently about 8 million new cases of tuberculosis occur every year with 1.66 million deaths occurring worldwide. It is the still commonest cause of death from single infectious disease in the developing countries. Nearly 95% of all tuberculosis cases and 98% death due to tuberculosis are in developing countries and 75% of tuberculosis cases are in economically productive age groups (15-49 years). In India out of a total population of over a billion, each year about 2.2 million person develop tuberculosis of which about 1 million are new smear positive highly infectious cases and up to half a million die. It implies that every minute a death occurs due to tuberculosis in our country. It also imposes a cost on our economy in terms of current and future output losses because of premature death and ill health. To add to the existing burden, the situation is compounded by the large-scale increase of new TB cases associated with increasing HIV infection. India is estimated to have 3.5

million HIV patients and about 1.8 million are co-infected with TB. The incidence of HIV patients among TB patients in India ranges from around 2-20 %, with an estimated 60 % HIV infected persons breaking down with active TB disease in their life time.^[2,3] Pleural effusion is an abnormal collection of fluid in pleural space as a result of excessive transudate or exudate from pleural surface. It constitutes one of the major causes of morbidity in India. Tuberculosis remains as the major cause of pleural effusion in India followed by suppuration and malignancy.

Definitive diagnosis of tuberculosis pleural effusion requires demonstration of acid – fast bacilli in Ziehl – Neelson stained smear of pleural fluid / sputum and/or demonstration of characteristic granuloma in pleural biopsy and/or isolation of bacilli on culture.^[4] However these procedures have their own limitations. The organism is seldom detectable in the pleural fluid (5%) and pleural biopsy is a closed blind procedure difficult to perform routinely and sensitivity is only 30 – 40% .The culture of mycobacterium tuberculosis required 4-6 weeks.^[5,6] Several new techniques introduced for the rapid diagnosis of tuberculosis like Radiometric BACTEC CULTURE^[3] and Polymerase chain reaction^[7] are effective but at present very costly, and require sophisticated equipment and trained personnel. These

can be available in few centres only. Recently various immunological tests have been evaluated based on detection of mycobacterial antigen or antibody. But all these methods also have given disappointing results in clinical settings. So diagnosis of tuberculous pleural effusion is often based on the complete clinical picture, radiological findings, biochemical and cytological analysis of fluid and tuberculin skin testing. Tuberculin skin testing also has lack of specificity (it does not differentiate between past and present infection). In this situation there is need for a single test, which is sensitive and specific, and at the same time, inexpensive and easy to perform. Adenosine deaminase (ADA) estimation is one such test, which fulfils all above requirements. Adenosine Deaminase is an enzyme, which catalyzes the irreversible hydrolytic deamination of adenosine to inosine and ammonia as a step in metabolism of purine. Conway and Cooke first identified ADA in human erythrocyte in 1939.^[8] ADA is present in cytoplasmic fraction of cell and in a certain amount located in nucleus. It's level ten times higher in lymphocyte particularly T- lymphocytes and plays important role in cell-mediated immunity. An increase in ADA activity is found where cell mediated immunity is stimulated.^[9]

A number of studies performed to evaluate the role of ADA estimation in pleural effusion, found significantly high level in tuberculous pleural effusion than in non-tuberculous effusion. Present prospective study is an attempt to evaluate the sensitivity and specificity of ADA estimation in the diagnosis of tuberculous effusion.

Materials and Methods

The present study was conducted in the Department of Pathology, Medical College (Shri Sayaji General Hospital), Baroda. In this study total 100 patients of age > 12 years and either sex were taken. Patients admitted in various medical wards, cardio thoracic ward or TB sanatorium, Gotri was taken as sample of study. Specific investigations like x-ray chest, USG chest, Mantoux test were carried out. Routine physical, biochemical, cytological and microbiological examination of pleural fluid were performed.

Patients with clinical and radiological findings suggestive of tuberculous and were confirmed by Sputum positive for AFB, Mantoux test, Cytology of pleural effusion, Microbiological examination of pleural fluid. From each patient 2 ml of pleural fluid and 3 ml of blood collected in plain bulb. Samples were assessed within 48 hours of

collection for adenosine deaminase. ADA estimation in pleural fluid ADA was carried out as per standard techniques of Galanti and Guisti (1974).

Statistical Analysis

Results are expressed as Mean \pm SD. The statistical analyses applied included unpaired t test, to analyze the difference between tuberculous (n = 80) and non-tuberculous (n = 20) quantitative variables at 1% level of significance. Table -1 shows mean ADA in tuberculous pleural effusion 62.77 ± 12.3 U/L and non-tuberculous group 26.36 ± 26.0 U/L. The p value was < 0.001, which was highly significant.

Results

In the present study estimation of pleural fluid level were carried out in 100 cases of pleural effusion at Department of Pathology, Medical College, Baroda. Table - 2 shows that out of 100 cases of pleural effusion, 80 cases were of tuberculous pleural effusion and 20 cases were of non-tuberculous pleural effusion. Most of patients were between the age group of 21-30 and 31 - 40 years. Thus they constituted 35 % and 52.5 % respectively. Age group of 41 - 50 and 51 - 60 both included 10 % of the total cases. While only one patient was below the age of 20 years and one above the age of 60 years of age. The mean age is 33.3 years. Considering the sex distribution in tuberculous effusion most of the cases were males comprising 65 % of total cases, while female comprised only 35 % of total cases. Male to female ratio was 1.9: 1.

In non tuberculous effusion CCF cases were between the age group of 31-70 years, cirrhosis between the age group of 21-50 years and both the cases of anemia were between 21-30 years in transudative effusion while in the Exudative effusion, in case of malignant effusion 2 cases were between 21-30 years and 4 cases were between 41-70 years. Cases of empyema were between age group of 41-60 years and pneumonia between 21-40 years. Except two cases of lymphoma all four case of malignancy were above 40 years suggest higher incidence in older age group. Among the sex incidence in the non-tuberculous effusion all the cases of CCF and cirrhosis were male while both cases of anemia were female suggesting anemia is more common in females. In cases of empyema and pneumonia all the cases were males while in case of malignant effusion four cases of malignancy including lung cancer and lymphoma were male and two cases of breast cancer were female. Among

the tuberculous patients, cough was present in 80 % of cases while expectoration and fever were present in 12.5 % and 52.5 % cases respectively. Anorexia was present in 85 % cases and weight loss was present in 91.25 % cases. Breathlessness and chest pain were present in 35 % and 57.5 % cases respectively.

Table no-3 shows that in the present study 11 patients out of 80 had positive past history (13.75%), while 4 patients had positive family history (5%) and 35 patients had habit of smoking. In present study out of 80 patients 33 patients (41.25%) were showing evidence of tuberculosis in X-ray chest in addition to pleural effusion, 52 patients (65%) had positive tuberculin test and 5 were (6.25%) were sputum positive.

In present study shows 64 % patients had turbid pleural fluid of which 90 % (58/64) had tuberculous etiology of pleural effusion. 14 % patients had hemorrhagic pleural fluid of which 28 % (4/14) patients had malignant pleural effusion. 72 % (58/80) of patients with tuberculous pleural effusion had turbid pleural fluid. In patients of malignant pleural effusion, 66 % (4/6) patients had hemorrhagic pleural effusion. All transudative effusions had clear appearance. In the present study pleural fluid protein was more than 3 gm % in 91% (82/90) of patients with Exudative pleural effusion, with mean of 5.02 gm % in tuberculosis, 4.88 gm % in malignancy, 8.35 gm % in synpneumonic, 3.85 gm % in empyema. This data is comparable with A.P. Jain et al (1982), 20 who found 90% sensitivity. In all Transudate pleural fluid protein was less than 3.0 gm% with mean of 1.7gm%. Among Exudative pleural effusion 77% (70/90) cases shows count >1000/cumm out of which (62/70) 88% were cases of tuberculosis, 5.7% were of malignancy while all cases of empyema and pneumonia had count above 1000/cumm. All cases of Transudative effusion had count below 1000 /cumm. Predominant lymphocyte were found in 76% (76/100) of patients of pleural effusion, of which 94% (72/76) patients had tuberculous effusion.

This column figure - 1 shows mean ADA value in various type of tuberculous effusion. Mean ADA in tuberculosis was 62.77 ± 12.3 U/L. Empyema had 92 U/L, Pneumonia had 30.5 U/L, CCF 9.8 U/L, Cirrhosis had 8.4 U/L, Malignancy 27.8 U/L, and Anemia had 21U/L. Except empyema all other non-tuberculous effusion had mean lower than tuberculosis.

As shown in table-4. 72 patients of tuberculous effusion were showing pleural fluid level above 45 U/L

considered as true positive cases and 8 patients were showing pleural ADA level below 45 U/L considered false negative cases. In non-tuberculous effusion 17 patients were showing pleural fluid ADA level below 45U/L that are true negative cases and 3 patients of non-tuberculous group showing ADA level above 45 U/L considered as false positive cases. Thus in this study the sensitivity and specificity of the test at cut off value 45 U/L were 90% and 85 %respectively.

Table-1: Pleural fluid ADA levels with their significance

Pleural Effusion	No. of cases	Pleural Fluid ADA		
		Mean ± SD	"t"	"p"
Tuberculous	80	62.77 ± 12.3	6.09	< 0.001
Non tuberculous	20	26.36 ± 26.0		

Table-2: Various types of patients selected in test group of study and their table number

Group Code	Category of Patients	No. of Cases
A	Tuberculous pleural effusion	80
B	Non tuberculous pleural effusion	20
	I. Congestive cardiac failure	4
	II. Malignancy	6
	III. Pneumonia	2
	IV. Empyema	2
	V. Cirrhosis of liver	4
	VI. Anaemia with hypoproteinaemia	2
Total		100

Table-3: Correlative findings with tuberculous pleural effusion (N = 80)

Correlative Findings	Positive N (%)	Negative N (%)
Past history	11 (13.75)	69 (86.25)
Family history	4 (5.00)	76 (95.00)
Smoking	35 (43.75)	45 (56.25)
X-ray chest	33 (41.25)	47 (58.75)
Tuberculin test	52 (65.00)	28 (35.00)
Sputum AFB	5 (6.25)	75 (93.75)

Table-4: Number of positive and negative cases according to cut off level (45 U/L) of pleural fluid ADA

Pleural fluid ADA	Tuberculous pleural effusion	Non-tuberculous pleural effusion	Total
Positive (U/L)	72	3	75
Negative (U/L)	8	17	25
Total	80	20	100

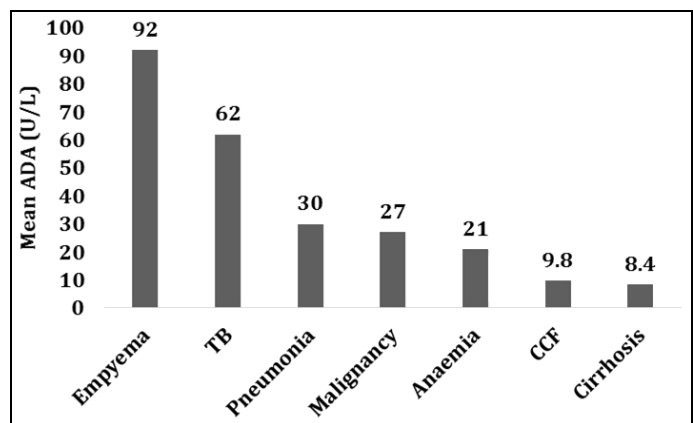


Figure-1: Mean ADA in pleural effusion of various etiologies

Discussion

Present study confirms that ADA level in tuberculous pleural effusion is increased than non tuberculous effusion. In present study mean ADA in tuberculous pleural effusion 62.77 ± 12.3 U/L and non-tuberculous group 26.36 ± 26.0 U/L comparing with various workers mean ADA in tuberculous pleural effusion like J. Blake et al (1982)^[10] (46 ± 13 U/L), Ocana et al (1983)^[11] (92.43 ± 29.43 U/L), Inma Ocana et al^[12] (93.8 ± 29.56 U/L), Jose Banales et al (1991)^[13] (mean 123.25 ± 39.4 U/L) Lesley Burgess et al (1995)^[14] 111.1 U/L.

In non tuberculous group except empyema all have lower mean value of ADA the tuberculous group. This finding also compared with other worker studies Esther San Jose et al (1992)^[15], Luis Valdes et al (1993)^[16]. They concluded that in empyema, the immune response involve polymorphnuclear cells and macrophages rather than lymphocytes which results that high pleural fluid ADA results chiefly from large phagocyte population.

In this study the sensitivity and specificity of the test at cut off value 45 U/L were 90% and 85 % respectively. Among the false positive cases, two cases were of empyema and one case of lymphoma, which were easily excluded by clinical and laboratory findings. Eight cases of tuberculosis had value below 45 U/L (true negative), possibly due to their suppressed cellular immunity comparable with other worker study. Ocana et al (1983)^[11] studied specificity of 97 % and sensitivity of 100 % of the test in tuberculosis at cutoff 45 U/L, Inma Ocana et al^[12] sensitivity of and specificity of 0.97 at cut off 50 U/L, Jose Banales et al (1991)^[13] studied 98% sensitivity and 96% specificity at cut off 70 U/L for the diagnosis of tuberculosis. Esther San Jose et al (1992)^[15] found sensitivity 100 % and specificity 93 % at cut off 43 U/L. Luis Valdes et al (1993)^[16] found sensitivity 100 % and specificity 95 % at cut off 47 U/L. Y. Aoki et al (1994)^[17] found ADA sensitivity and specificity were 81 % and 89 % at a cut off 45 U/L. Lesley Burgess et al (1995)^[14] found at cut of 47 U/L, 100 % sensitivity and 87.5 % specificity. SK Sharma et al (2001)^[18] studied at a cut of 35 U/L, sensitivity 83.3 % and specificity of 66.7 %. Danielle M. Lima et al (2003)^[19] studied ADA activity and PCR on pleural fluid of tuberculosis and non-tuberculous patients. They found at cut of 40 U/L sensitivity and specificity of ADA were 68.8 % & 72.4 %, where combined used of ADA and PCR improves sensitivity (87.5 %) and specificity (72.4 %). Wu-Huei Hsu et al (1993)^[20] found mean ADA 137.9 ± 30.7 U/L in

immunocompetent tuberculosis patients, 65.8 ± 49.9 U/L in immunocompromised tuberculous patients and 36.9 ± 25.6 U/L in non-tuberculous patients and concluded that diagnostic value of ADA in immunocompromised hosts with tuberculous pleural effusion is not as significant as in immunocompetent hosts. This is due to impaired cellular immunity in immunocompromised host ADA secreted by T4 lymphocyte. The sensitivity of the ADA as a serological marker for tuberculous exudative pleural effusion depends on the prevalence of the disease in the population. In western countries where the prevalence of tuberculosis is low, the positive predictive value of the test decreases because of the high false positives, but the negative predictive value remains high. Thus ADA can be used for ruling out suspected cases of tuberculosis and can be a very effective screening test. India has a high prevalence of tuberculosis and the sensitivity and specificity of this test will be high in this population. Therefore ADA estimation being a simple, low cost, rapid and non-invasive test, should become an integral part of the diagnostic work up of exudative pleural effusions in suspected cases of tuberculosis.

Conclusion

This study was completed with the remarks that estimation of adenosine deaminase in pleural fluid is a simple, rapid and less expensive laboratory investigation for the diagnosis of tuberculous pleural effusion when the diagnosis is uncertain by other investigations. Though it cannot be used as a general screening test or as a full proof standard test for the diagnosis of tuberculous pleural effusion, it has a valuable role as an adjuvant investigation for other diagnostic investigations of tuberculosis.

Limitation of Study: Number of patients studied is small. So definitive criteria can't be established on this sample size. A large number of patients are required to confirm our findings further and establish the definitive criteria.

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