Journal of Academia and Industrial Research (JAIR)
Volume 3, Issue 9 February 2015

ISSN: 2278-5213

RESEARCH ARTICLE

Adenosine Deaminase Activity in Plural Fluid as a Diagnosing Tool for Tubercular Plural Effusion

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Abstract

Conventional methods for the diagnosis of pleural TB have proven inefficient. Direct examination of pleural fluid and Ziehl-Neelsen staining requires bacillar concentrations of 10000/mL, which has a low sensitivity. Adenosine deaminase is a significant indicator of active cellular immunity. In this study, ADA activity was studied for easy diagnosis of tuberculous pleural effusion. The study was conducted on 100 subjects of varying age group and ADA, glucose and protein, haematological parameters and cytological parameters were correlated in diagnosis of tuberculous pleural effusion. From this study, we concluded that using >40 U/L as the cut off value of ADA estimation in tuberculous pleural effusion confirms the high sensitivity (88%) and specificity (90%) of ADA test with glucose and protein levels. Tuberculous pleural effusion is not only simple, but also rapid and cheaper procedure in India where there is a high incidence of tuberculosis but also significant in diagnosis, prognosis and medical management of tuberculous pleural effusion.

Keywords: Tuberculous pleural effusion, adenosine deaminase, tuberculosis, diagnosis, incidence.

Introduction

Tuberculosis (TB) is one of the major global diseases affects millions of people not only in India but also all over the world. It may affect the lungs and all other systems. This infection is caused by *Mycobacterium tuberculosis*. It is second largest infectious disease all over the world after the HIV. Among the extra pulmonary presentations, pleural TB is second in frequency after Tuberculous lymphadenitis. Conventional methods for the diagnosis of pleural TB have proven inefficient and direct examination of pleural fluid and Ziehl-Neelsen staining requires bacillar concentrations of 10000/mL and therefore, has a low sensitivity. Although a culture is more sensitive, it requires 2 to 6 weeks to sensitivity of pleural biopsy specimens is reportedly higher either by culture or histological evaluation. However this procedure requires greater expertise, more invasive and subject to sampling error. The paucity of bacilli and the non-specific cytochemical characteristic of pleural fluid in pleural TB mandate more invasive procedures such as pleural biopsy or thoracotomy for differential diagnosis. Although tubercular pleural effusion may resolve over a period of several months without treatment, a failure to diagnosis and treat pleural TB can result in progressive disease and involvement of other organs. Cellular immunity mediated by T-lymphocytes constitutes a major defense against tuberculosis. Adenosine deaminase, called ADA by Spencer *et al.* (1968) is an enzyme catalyzing conversion of adenosine to inosine in the purine pathway. It has an extremely important physiological role in the lymphoid tissue evidenced by its extremely high concentration in lymphocytes, particularly in T-cells.

Adenosine deaminase has shown promising results in the diagnosis of tubercular pleural, peritoneal and pericardial effusions and tubercular meningitis. Adenosine deaminase is an enzyme widely distributed in human tissues, particularly T-lymphocytes and catalyzes the irreversible hydrolytic deamination of adenosine to produce Inosine and Ammonia (Martin *et al*., 1976). Its plasma activity is high in diseases where cellular immunity is impaired. Thus, increased level of ADA is found in various form of tuberculosis, making it as a marker particularly for pulmonary tuberculosis (Giblett *et al*., 1972). Normal serum level of ADA is <30 U/L while increased level of ADA are found in various forms of tuberculosis particularly TB of lungs with pleural effusion and tubercular meningitis (Mason *et al.,* 2005). A significant decrease was observed in ADA activity after treatment and also in old TB patients as compared to healthy control subjects. This shows that serum ADA activity is increased in pulmonary tuberculosis patients which are a helpful parameter for monitoring the therapy. Thus, the measurement of serum ADA activity is an important parameter for monitoring the therapy in pulmonary tuberculosis patients (Atatas *et al.,* 2003). Adenosine deaminase is involved in the propagation and differentiation of various lymphocytes, particularly T-lymphocytes, so that estimation of its level of activity in various body fluids has been used in the diagnosis of tuberculous effusion especially pleural forms. Conversely its decrease level has been noticed in treated cases. In diagnosis of tuberculosis, microbiologic, genetic and immunologic and biochemical methods are used.

It is a significant indicator of active cellular immunity. Hence in the present study, ADA activity was studied for easy diagnosis of tuberculous pleural effusion.

Materials and methods

Study population: The study was conducted on 100 subjects of varying age group (20-60 years), both male and female, out of which 50 were newly diagnosed pulmonary tuberculosis cases and 50 were healthy control cases after getting their consent for participation. This study was conducted in Dept. of Physiology, Biochemistry and T.B. and Chest Department, Jhalawar Medical College and SRG Hospital, Jhalawar (Rajasthan). The study subjects were selected from patients coming to OPD and admitted in SRG Hospital and Jhalawar Medical College, Jhalawar. Permission for present study has been taken from ethical committee of Jhalawar Medical College and SRG Hospital, Jhalawar.

Diagnostic criteria for pleural effusion tuberculosis: Physical and biochemical examination was done. Parameters for physical examination were cloudy, clear, bloody, serous and yellow. Biochemical examination was done to check the parameters raised protein >50% serum protiens, glucose <60 mg/dL, cell count >cumm predominant lymphocytes cells, ADA >40 U/L.

Hematological examination: Estimation of Leucocyte count, Hemoglobin and ESR was done by employing standard methods.

Assessment of nutritional status: Anthropometric measurements were done and body mass index was calculated as per the standard procedure (Vasudevan *et al.,* 2013).

Statistical analysis: This study is a prospective case control study. Standard statistical analysis was performed for evaluation of data such as student "t" test and ANOVA test with the help of statistical package for social sciences (SPSS).

Results and discussion

Tuberculosis occurs worldwide and is rampant in many countries. Though curable, its infection is on the rise. The most specific test is the positive bacterial culture of a patient's sputum sample. This is cumbersome and time consumption. X-rays, smears for AFB and tuberculin tests though comparatively rapid are not conclusive. The sign and symptom of tuberculous pleural effusion were fever, cough, weight loss, chest pain, breathlessness and loss of appetite. Generally physical signs and clinical symptoms do not positively help for accurate diagnosis of tuberculous pleural effusion. Adenosine deaminase (ADA) is an enzyme widely distributed in mammalians tissues, particularly in T-lymphocytes. Increased levels of ADA are found in various forms of tuberculosis making it a marker for the same.

Fig. 1. Percentage distribution of age among the study groups.

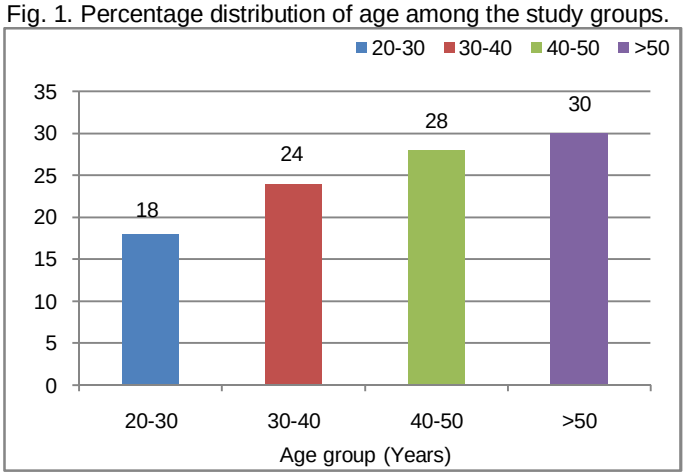

Table 1. Characteristics of control group (n=50).

Parameters	Mean ± SD	Range
Age (years)	33.9 ± 7.85	21-51
Weight (kg)	55.66 ± 7.18	42-77
Height (m)	1.621 ± 0.075	1.48-1.74
BMI (kg/m^2)	21.31 ± 3.05	14.89-5.77
Hb (gm%)	13.82 ± 1.34	11.9-16.0
ESR in 1st hour mm	4.88 ± 1.27	3-7
TLC	7732 ± 1472.5	5100-10800
ADA activity (U/L)	15.5 ± 3.53	9-3
Protein (gm/dL)	6.7 ± 0.67	6-8
Glucose (mg/dL)	83.98 ± 8.22	71-99
Lymphocyte %	20.94 ± 4.80	11-29

Table 2. Characteristics of case group (n=50).

Parameters	Mean ± SD	Range
Age (years)	43.36 ± 11.33	21-64
Weight (kg)	51.02 ± 8.2	38-69
Height (m)	1.61 ± 0.08	1.45-1.75
BMI (kg/m^2)	19.59 ± 2.10	15.1-22.84
Hb (gm%)	13.7 ± 1.75	9-16.2
ESR in 1st hour mm	21.94 ± 6.49	10-40
TLC case	7762 ± 1432.1	5100-10800
ADA activity (U/L)	79.92 ± 12.50	60-110
Protein (gm/dL)	5.25 ± 0.80	4.05-6.9
Glucose (mg/dL)	38.39 ± 4.80	30-48
TLC in fluid (cu mm)	702.2 ± 212.04	250-1200
Lymphocyte %	73.48 ± 13.10	49-95

Though ADA is also increased in various infectious diseases like infectious mononucleosis, typhoid, viral hepatitis, initial stages of HIV and in cases of malignant tumours, the same can be ruled out clinically. In this study, we have studied 100 cases (50 patients group and 50 healthy controls groups) of male and female between age 20-60 years in patients attending SRG Hospital and Jhalawar Medical College, Jhalawar. Percentage distribution of age in patients of tuberculous pleural effusion of different age groups is shown in Fig. 1. Tables 1-4 show the anthropometric (Height, Weight, BMI), biochemical (ADA, Glucose, Protein), cytological (TLC and lymphocyte %) and haematological (Hb, ESR) data of study groups.

Table 3. Comparison of nutritional status of male and female subjects.

Group	BMI (kg/m^2) Mean ± S.D/ Range	't' value	'p' value
Male (n=36)	19.43 ± 2.21/ 14.02-23.79	-0.85	<0.5
Female (n=14)	20.00 ± 1.79/ 12-20.99	-	-
Significance	-	Significant	Significant

Table 4. Normal values of haematological parameters.

Parameter	Values	Method
Hb (gm%)	Female 12-16 ± 2 Male 14-18 ± 2	Sahli's method
ESR (mm) at the end of 1st hour	Female 0-20 Male 0-9	Wintrobe's method
Total leucocyte count (cu mm)	4000-1100	Manual method dilution principal
Lymphocyte %	30%	-

Table 5. Hematological profile (Control group and tuberculous pleural effusion group).

Parameters	Control group (n=50)	Tuberculous pleural effusion (n=50)
Hb (gm/dL)		
Mean ± S.D.	13.82 ± 1.34	13.7 ± 1.75
Range	11.9-16.0	9.0-16.2
ESR (at the end of 1st hour in mm)		
Mean ± S.D.	4.88 ± 1.27	21.94 ± 6.49
Range	3-7	10-40
TLC (cu mm)		
Mean ± SD	7732 ± 1472.5	7762 ± 1432.1
Range	5100-10800	5100-10800

ESR-Erythrocyte sedimentation rate, TLC-Total leucocytes count.

BMI was calculated in our study as w/h^2 (where w = weight in kg and h = height in meters). The BMI was Mean ± S.D (21.31 ± 3.05) in control group. However, in patients group it was found 19.59 ± 2.10 and showed chronic energy deficiency or underweight). In tuberculous pleural effusion, patients were more nutritionally compromised as compared to healthy control group. Our results were compared and found similar BMI reported by Soe *et al.* (2010). According to vasudevan *et al.* (2013), malnutrition may be of two types, under nutrition and over nutrition. The latter otherwise called obesity. It is used to assess BMI. The most widely used markers for body muscle mass index is 24 h urinary creatinine excretions. Anaemia is a condition in which there is a reduction in the haemoglobin content of blood or in the number of red blood cells or both or defective maturation of the red blood cells (Dacie and Lewis, 2001). Haemoglobin level (gm%) in blood sample of tuberculous pleural effusion subjects and in blood of healthy control groups were estimated.

Both the parameters revealed nutritional status. In healthy subjects, it was found within normal limits with 13.82 ± 1.34 and range was found 11.9-16.0. In pleural fluid of tuberculosis patients, haemoglobin status was 13.7 ± 1.75 and range was 9.0-16.2 (Table 5). In the present study, lower range of haemoglobin demonstrated poor nutritional status and reduced cell mediated immunity (Soe *et al.*, 2010; Agarwal, 2012). The mean value of erythrocyte sedimentation rate (ESR) at the end of 1st h in healthy control group and pleural effusion of tuberculosis patients were performed and found to be 4.88 ± 1.27 with range 3-7 and 21.94 ± 6.49 and range 10-40 respectively. The increased level of ESR in study group of pleural effusion was due to *Mycobacterium tuberculi* infection (Sakuraba *et al*., 2009; Soe *et al.*, 2010) (Table 5). The white blood cell counts (WBC) determines the number of leukocytes per cu mm of whole blood the range and mean value of total leukocyte counts in both the groups (control and case) were within normal limits in blood (5100-10800/cu mm) (Table 5). Alteration in total white cell count indicates the degree of response to a pathological process but it is not specifically diagnostic for any one disorder (Selkurt, 1982).

Adenosine deaminase (ADA) is an enzyme in the purine salvage pathway required for converting adenosine to inosine. ADA levels are ten times higher in lymphocytes than in erythrocytes, particularly T-lymphocytes (Ramgopal *et al.,* 1982). Table 6 showed significant levels of ADA, protein and glucose in healthy control group and tuberculous pleural effusion patients. White cell counts (Mean ± SD) in tuberculous pleural fluid were recorded and calculated as percentage (Table 7). Determination (Mean ± S.D) of pleural fluid ADA (U/L) and total protein concentration (g/dL) were analyzed in two groups (I. n=40, II. n=10) (Table 8). To differentiated trasudate from exudates, the ratio of pleural fluid and serum protein was calculated. Determination (Mean ± S.D) of pleural fluid ADA (U/L) and glucose concentration (mg/dL) were analyzed in study groups (Table 9). Comparison of biochemical and leukocyte count based on ADA levels in tuberculous pleural effusion patients and control group is shown in Table 10. We investigated the incidence of tuberculous pleural effusion among patients with ADA levels of >40 U/L to 60 U/L as strong suspected and as >60 U/L positive cut off value for the diagnosis of pleural tuberculosis with range 60-110.4 U/L and mean value (79.92 ± 12.50) (Table 6). However, in control group, the ADA cut off value was less than 30 U/L with range 12-19 U/L (Table 10). We have been compared ADA level in both case and control groups (t=35.04, p=0.00) which revealed significant ADA level in present study (Table 10). Increased values of ADA in tuberculous pleural effusion have been studied by several research workers. These results were further confirmed in our study.

Table 6. Biochemical investigation of pleural fluid in tuberculous pleural effusion (n = 50).

Type of examination	Mean ± SD	Range
ADA (U/L)	79.92 ± 12.50	60–110.4
Glucose (mg/dL)	38.39 ± 4.80	30–48
Protein (gm/dL)	5.25 ± 0.80	4.05–6.9

Table 7. Cytological examination of pleural fluid in tuberculous pleural effusion patients (n = 50).

Parameter	Mean ± SD
Total leukocyte count	702.2 ± 212.04
Lymphocyte (%)	73.48 ± 13.10

Table 8. Pleural fluid ADA level in relation to pleural fluid proteins in tuberculous pleural effusion patients (n = 50).

Group	Pleural fluid protein (gm/dL)		ADA (U/L) Mean ± S.D.
	Category	Mean ± S.D.	
Entire series (n = 50)	-	5.25 ± 0.80	79.92 ± 12.50

Table 9. Pleural fluid ADA level in relation to pleural fluid glucose in tuberculous pleural effusion patients (n = 50).

Group	Pleural fluid glucose (mg/dL)		ADA (U/L) Mean ± S.D.
	Category	Mean ± S.D.	
Entire series (n = 50)	-	38.39 ± 4.80	79.92 ± 12.50

Our results showed that ADA is significant marker in diagnosis of tuberculous pleural effusion. Our findings were similar with reports studied by other researchers (Agarwal, 2012). Tubercolous pleural effusion is the result of a cell mediated immune response to the presence of *Mycobacterium tuberculosis* and is characterized by the accumulation of activated T-lymphocytes and macrophages in the pleural space. ADA is reported to be associated with severe form of combined immune deficiency and it's responsible for an increase in toxic nucleotides that prevent the differentiation or proliferation or both of T-lymphocytes and thus, a normal immune function mediated by cells. The raising of the levels of ADA activity under antigenic stimulation shows the importance of this enzyme in the rapid proliferation of cells in order to prevent the accumulation of toxic metabolites. Therefore, an increased ADA activity is present in several circumstances in pleural effusion of a tuberculous nature (Agarwal, 2012).

We observed that glucose levels were below 50 mg/dL in tuberculous pleural effusion with mean value of 38.39 ± 4.80 and range was 30-48 mg/dL (Table 6 and 9). However in control group, glucose was with mean value 83.98 ± 8.22 and range was 75-90 (Table 10). In the present study, low glucose level showed tuberculosis pleuritis in case group in comparison to control group. Statistically data of glucose compared in both groups (I and II) were observed to be t=- 33.83 and p=0.00) (Table 10). Similar findings were reported by Ungerer *et al.* (1988) and Riantawan *et al.* (1999). In this study, pleural fluid protein was 5.25 ± 0.80 within range of 4.05-6.9 gm/dL (Table 6). In control group, the mean serum protein value was 6.77 ± 0.67 within the range of 6-7.30 gm/dL (Table 10). The ratio of pleural fluid to serum protein in the present study was >0.5 applying the Light's criterion. According to Light (2007), the criteria to differentiate pleural fluid transudates from exudates (Light's criterion standards) is

1. Ratio of pleural fluid to serum protein is greater than 0.5.
2. The fluid is considered exudates if ratio of pleural fluid to serum protein is >0.5, ratio of pleural fluid.
3. Ratio of pleural fluid to serum lactate dehydrogenase (LDH) is greater than 0.6
4. Pleural fluid LDH is greater than two thirds of the upper limits of normal serum value.

The above criteria are called light's criteria of pleural disease. In our study, the nature of tuberculous pleural effusion was that of an exudates which is easily revealed by estimating protein in serum and pleural fluid (>0.5 gm/dL), applying the Light criteria. Our results were similar to other researchers (Light, 2002).

Mean ± S.D of ADA (U/L) and Total Leukocyte (Count/ cu mm) in tuberculous pleural fluid of study groups is shown in Table 11. In group I, total leukocyte count/cumm was <500 and in group II, it was >500. The main type of pleural fluid leukocyte in tuberculous pleural effusion was lymphocyte. Cellular immunity mediated by T-lymphocytes constitutes a major defense against tuberculosis. In our study, increased level of lymphocyte count indicates infection or inflammation and in most cases are associated withan exudates (Soe *et al*., 2010; Agarwal, 2012).

Table 10. Comparison of biochemical and leukocyte count based on ADA levels in tuberculous pleural effusion patients and control group.

	Mean ± SD		
	ADA (U/L)	Protein (gm/dL)	Glucose (mg/dL)
Pleural fluid patients (I)	79.92 ± 12.50	5.25 ± 0.80	38.39 ± 4.80
Control group (II)	15.5 ± 3.52	6.77 ± 0.67	83.98 ± 8.22
't' value I Vs II	35.04	-10.25	-33.83
'p' value I Vs II	0.00	0.00	0.00
Significance	Significant	Significant	Significant

Table 11. Tuberculous pleural fluid ADA levels in relation to leukocyte count.

Group	Total leukocyte count/cu mm		ADA (U/L)
	Category	Mean ± S.D.	Mean ± S.D
I (n =10)	<500	401.0 ± 74.30	81.1 ± 15.21
II (n =40)	>500	777.5 ± 161.68	79.62 ± 11.94
Entire series (n = 50)	–	702.2 ± 212.04	75.32 ± 40.12

Conclusion

Determination of Adenosine deaminase, glucose and protein is important in diagnosis and medical management of tuberculosis in pleural fluid. In the present study, we have estimated ADA level, glucose, protein and haematological parameters in pleural fluid of tuberculosis and serum of healthy control group in Jhalawar area of Hadoti region of Rajasthan. In our study, we had correlated ADA, glucose and protein, haematological parameters and cytological parameters in diagnosis of tuberculous pleural effusion. Suitable statistical tool was applied to find out the significance of this study in relation with diagnosis, prognosis and severity of tuberculous disease in pleural effusion. About 100 cases and control of different case groups, male and female were studied. Study population was categorized taking BMI as a factor for nutritional status. Lower level of Haemoglobin in tuberculous pleural effusion cases demonstrated anemia due to malnutrition. Increased level of ESR showed *Mycobacterium tuberculosis* infection in patients of tuberculous effusion. Total leukocyte count was within normal limits in both case and control in study population. However, differential leukocyte counts particularly lymphocyte count in pleural fluid of tuberculous effusion was significantly increased. ADA estimation was planned as the screening test with cut off value >40 U/L for the diagnosis of tuberculous pleural effusion. In our study, we concluded that the best cut off level of ADA activity was tested (79.92 ± 12.50 and suggested that ADA estimation was a test for the diagnosis of tuberculous pleural effusion. Glucose and protein levels in present study in relation with ADA level in tuberculous pleural effusion differentiate pleural fluid from trasudates from exudates. Low glucose levels <60 mg/dL and ratio of pleural fluid to serum protein (>0.5) full fill the Light's criteria of tuberculous pleural effusion. From this study we concluded that using >40 U/L as the cut off value of ADA estimation in tuberculous pleural effusion confirms the high sensitivity (88%) and specificity (90%) of ADA test with glucose and protein level in tuberculous pleural effusion not only a simple, rapid and cheaper procedure in India where there is a high incidence of Tuberculosis but also significant in diagnosis, prognosis and medical management of tuberculous pleural effusion.

Acknowledgements

Author would like to acknowledge Dr. M. Hamid and Dr. Atul Tiwari for their constant encouragement for the study.

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