DIAGNOSTIC UTILITY OF ADENOSINE DEAMINASE (ADA) ACTIVITY IN PLEURAL FLUID AND SERUM OF TUBERCULOUS AND NON-TUBERCULOUS RESPIRATORY DISEASE PATIENTS

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Abstract. Adenosine deaminase activity (ADA) was assayed in pleural fluid and serum of 42 subjects with pleural effusion. Twenty-nine of them had TB pleural effusion and the remaining 13 had pleural effusion due to non-TB respiratory diseases. Serum adenosine deaminase activity were also measured in 32 pulmonary tuberculosis patients without pleural effusion and equal numbers of healthy controls without systemic diseases for comparative analysis. The patients attending the medicine out-patient department (MOPD) of the B. P. Koirala Institute of Health Sciences, Dharan, Nepal were taken as study subjects. Serum and pleural fluid ADA activities were assayed spectrophotometrically by the method of Guisti and Gallanti. The mean serum ADA activity was significantly increased in patients with tubercular pleural effusion (34.53 ± 10.27 IU/l) compared to pulmonary tuberculosis patients without pleural effusion (26.54 ± 4.76 IU/l), (p=0.004), those with non-TB respiratory disease (16.71 ± 5.16 IU/l), (p=0.0001) and healthy controls (15.53 ± 4.4 IU/l) (p=0.0001). The mean ADA in the pleural fluid of tubercular pleural effusion patients (90.29 ± 54.80 IU/l) was significantly higher compared to those with non-TB respiratory disease (24.43 ± 9.28 IU/l) (p=0.0001). Using the lowest cutoff value for enzyme activity in the serum of patients with TB pleural effusion (25 IU/l), a test sensitivity of 72.41% and specificity of 81.53% were obtained. Using the lowest cutoff value for enzyme activity in pleural fluid of patients with TB pleural effusion (45 IU/l) the sensitivity and specificity for diagnosis were 76.10% and 100%, respectively. Therefore, the measurement of ADA in tubercular pleural effusion has a utility in the diagnosis of tuberculosis when other clinical and laboratory tests are negative.

INTRODUCTION

Tuberculosis (TB) is a bacterial disease caused by the tubercle bacilli which includes *Mycobacterium tuberculosis.* Globally, approximately 16 million people are suffering from active TB, with an estimated 8.5 million developing active TB each year resulting in approximately 2 million deaths yearly (WHO, 2003). Nepal is estimated to have 47,315 TB cases and 21,245 sputum smear for TB positive cases.

The clinical manifestations of tuberculosis are dependent on the cellular immune responses to the tubercle bacilli, characterized by the accumulation of monocytes/macrophages, lymphocytes and polymorphonuclear leukocytes in tuberculosis lesions. These responses are initiated on sensitization of T-lymphocytes by the bacterial antigen with the re-

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lease of cytokines which regulate macrophage function.

Adenosine deaminase (AD; EC 3.5.4.4) is an enzyme required for converting adenosine to inosine in the purine salvage pathway. Its activity is involved in the differentiation and proliferation of lymphocytes and activation of macrophages. This enzyme is important in the rapid proliferation of cells to prevent the accumulation of toxic metabolite. Adenosine diaminase activity (ADA) increases during cellular activation to detoxify toxic metabolite (Piras *et al*, 1978). There is restriction of lymphocytic blastogenesis following the activation of ADA inhibitors through biologic and nonclarified mechanisms, possibly connected with the conversion of dioxyadenosine into dioxy-ATP in lymphocytic cells, which could cause its destruction by inhibition of DNA synthesis (Carson and Seegmiller, 1976).

The sensitivities of ZN staining and culture are 10-40% and 8-49%, respectively in the diagnosis of TB infection (Jay, 1985). Definitive diagnosis of TB requires culture of the suspected organism. Because *Mycobacterium tuberculosis* grows very slowly, it can take up to six weeks to isolate it in culture. Determination of susceptibility to drugs can add another three to six weeks to the process. Meanwhile the disease may progress and be transmitted to others when appropriate treatment is delayed. There is a need of a simple, rapid and reliable test which can be easily carried out in the clinical laboratory. The present study was carried out to evaluate the diagnostic utility of ADA activity in pleural fluid and serum of tuberculosis patients compared to other lung pathology.

MATERIALS AND METHODS

A total of 74 cases enrolled during July 2004-July 2005 were considered for the study. This population included 42 cases with pleural effusion and 32 diagnosed cases of pulmonary tuberculosis without pleural effusion. Thirty-two healthy subjects were also included for comparative analysis. The study subjects and controls were interviewed for background characteristics using a questionnaire. Informed consent was obtained prior to inclusion in the study. The institute research and ethical review committee of the B.P. Koirala Institutes of Health Sciences (BPKIHS) approved the study. Trained medical officers were involved in performing the thoracocenteses.

The study population categorized as:

Controls (Con): Thirty-two healthy subjects without any systemic diseases belonging to similar geographical areas from where cases of TB are enrolled.

Group I patients (n=29): tubercular pleural effusion (TB/PE) on the basis of clinical history, good response to anti-tubercular treatment (ATT) on isoniazid (INH 5mgkg^{-1}day^{-1}), pyrazinamide (PZA, 25 mgkg^{1}day^{-1}), rifampin (RFP, 10 mgkg^{-1}day^{-1}), ethambutal (ETH, 15 mgkg^{-1}day^{-1}) (n=25) and smears positivity for pleural fluid (n=4).

Group II patients (n=13): diagnosed with non-tuberculosis respiratory diseases (NTBRD/PE) such as malignancy (n=7), COPD (n=3), empyemas (n=2) or pneumonia (n=1).

Group III patients (n=32): newly diagnosed pulmonary tuberculosis patients without pleural effusion (PTB/w/o PE) on the basis of smear positivity for acid-fast bacilli on there consecutive sputum samples and Lowenstein-Jensen culture used to confirm TB.

Sample collection

The pleural fluid sample was obtained through thoracocentesis from patients attending the medicine out-patient department of the B. P. Koirala institutes of Health Sciences (BPKIHS), Dharan, Nepal. A blood sample (2ml) was collected in a plain vial from patients with TB-pleural effusion, non-TB respiratory diseases, TB without pleural effusion and healthy individuals. Serum was separated by

centrifugation at 2,500 rpm for 15 minutes at room temperature in a Remi Research centrifuge model R-23. Serum and pleural fluid were separated into clean, dry, sterile vials and stored at -20ºC until use.

Adenosine deaminase activity assay

ADA was assayed on the same day as collection of samples. ADA activity was measured by a spectrophotometric method described by Guisti and Galanti (1984). Ammonia forms under conversion of adenosine to adenosine deaminase causing an intensely blue indophenol with sodium hypochlorite and phenol in an alkaline solution as determined by modification of a Berthelot's reaction. Sodium nitroprusside was used as the catalyst. The ammonia concentration was directly proportional to the absorbance of the indophenol measured at a wavelength of 620 nm. The reaction catalyzed by ADA was stopped at the end of one hour incubation at 37ºC by the addition of phenol nitroprusside solution.

ADA activity was expressed in international units (IU) using the formula as follows: (△ Absorbance of sample/ △ Absorbance of standard) x 50 IU/l.

Adenosine was obtained from SRL chemicals, India and all the other chemicals were of analytical grade and obtained from MERCK Company.

Data analysis

The data were analyzed using SPSS-10 tools. The results were expressed as mean ± SD. Statistical comparison was carried out using the Student's *t*-test for unpaired data. A p-value less than 0.05 was considered significant.

RESULTS

The total number of patients studied was 74. Of these, 29 were diagnosed with tubercular pleural effusion, 13 with non-tuberculosis respiratory diseases and 32 as confirmed cases of pulmonary tuberculosis without pleural effusion. Table 1 depicts the mean age and sex ratio among the study group to be

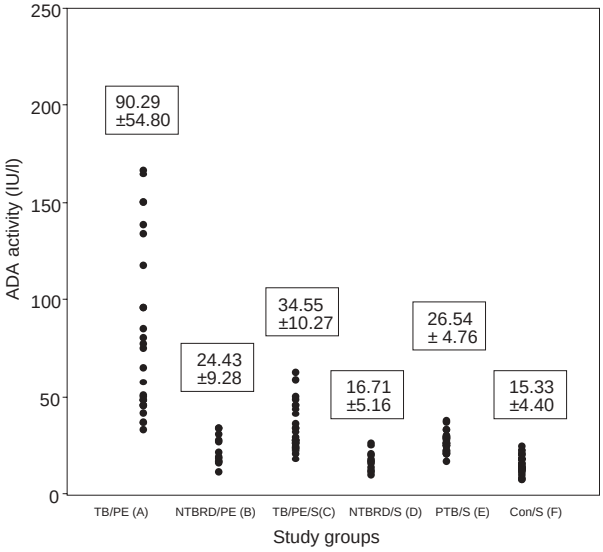

Fig 1–Distribution of ADA activity (IU/l) in pleural fluid (A&B) and serum(C-F) of tuberculosis patients, non-TB respiratory diseases and control subjects. The ADA activities inscribed in the respective boxes are represented as mean±SD.

Table 1
Basic characteristics of study groups.

Variables	Age (yrs) (Mean ± SD)	Sex ratio (M:F)
Control (C) (n=32)	36.22 ± 13	1.8:1
Gr I- TB/PE (n=29)	40.69 ± 20.17	1.9:1
Gr II- NTBRD/PE (n=13)	45.46 ± 23.59	1.7:1
Gr III- PTB/w/o PE (n=32)	40.11 ± 15.33	2.0:1

homogenously distributed. Fig 1 depicts the mean serum ADA activity in TB/PE (34.55 ± 10.27) which was significantly greater than the control (15.33 ± 4.40); p<0.001, NTBRD/PE group, (16.71± 5.16); p<0.005 and the PTB/ w/o PE (26.54 ± 4.56); p<0.001. ADA activity was found to be increased in the following order GrI>GrIII>Gr II, with the lowest activity in the control. The activity of ADA in the pleural fluid was found to be significantly greater in the TB/PE (Group I: 90.29±54.80) compared to the NTBRD/PE (Group II: 24.43±9.28), p<0.001.

Table 2 shows the comparative ADA activity in the pleural fluid and serum of the Gr I and Gr II patients. Pleural fluid ADA activity was significantly greater in Gr I (90.29 ± 54.80) compared to Gr II (24.43 ± 9.28); p<0.0001. The ADA activity in the pleural fluid compared to serum was in ratios of 3.1:1 and 2.0:1 in Gr I and Gr II, respectively.

Table 3 shows the sensitivity and specificity of serum ADA activity and pleural fluid ADA activity in Gr I and TB Gr III. In Gr I patients a higher sensitivity (76%) and specificity (100%) were found in keeping with a lower cutoff limit for pleural fluid ADA of 45 IU/l compared to the cutoff serum ADA activity at 25 IU/l with a sensitivity and specificity of 72.41% and 81.53%, respectively.

DISCUSSION

The raised ADA activity under antigenic stimulation is found in infections, such as tuberculosis and typhoid fever, where cell-mediated immunity (CMI) is stimulated (Mishra *et al*, 1994). Other earlier studies have also shown increased levels of serum ADA in a number of diseases where CMI is stimulated like Behcet's disease (Kose *et al,* 2001), typhoid (Ungerer *et al*, 1996), tuberculosis

Table 2
ADA activity in pleural fluid and the serum of study groups (values expressed as mean ± SD).

Variables	PF ADA (IU/l)	Serum ADA (IU/l)	ADA ratio (PF/Serum)
Gr I- TB/PE (n=29)	90.29 ± 54.80 [a,f] [b,e]	34.55 ± 10.27	3.1:1
Gr II- NTBRD/PE (n=13)	24.43 ± 9.28 [c,d]	16.71 ± 5.16	2.0:1

[f]p< 0.0001, [e]p< 0.001, [d]p<0.01
a) Pleural fluid ADA activity Gr-I *vs* Gr- II
b) Pleural fluid ADA activity *vs* Serum ADA activity in Gr I
c) Pleural fluid ADA activity *vs* Serum ADA activity in Gr II

Table-3
Sensitivity of serum ADA activity and pleural fluid ADA activity in tubercular pleural effusion at lower cut off limits of 25 IU/l and 45 IU/l respectively.

Variables		Serum ADA (25 IU/l)	Pleural effusion ADA (45 IU/l)
Gr-I TB/PE	Sensitivity	72.41%	76.10%
	Specificity	81.53%	100%
Gr III- PTB/w/o PE	Sensitivity	69.0%	NA
	Specificity	78.0%	NA

(Sharma *et al,* 2001), acute nephrotic syndrome (Mishra *et al*, 1997) and cancers (Ergoly *et al*, 1984).

Significantly increased ADA activity in the serum of tubercular pleural effusion patients compared to healthy controls is due to activation of cell mediated immunity. In tuberculosis there are increased numbers of T-lymphocytes and macrophages in pleural fluid which may be associated with highly elevated ADA activity in such patients. The ADA activity is greater in lymphocytes and is related to differentiation of lymphocytes. In pathological conditions, the clearance capacity of lungs is decreased leading to increased numbers of cells in pleural fluid and the recirculation of activated T-cells may cause a high serum ADA activity in patients with pulmonary disease.

The ratio of ADA activity in the pleural fluid to serum was 3.1:1 in TB/PE compared to 2.0:1 in NTBRD/PE. This could be used in differentiating TB/PE from NTBRD/PE. It enables the diagnosis of disease if seen in parallel with pleural fluid and serum when other clinical and laboratory tests are negative.

A previous study showed that levels of pleural fluid ADA were significantly higher than serum ADA levels in both tuberculous and non-tuberculous pleural effusions, suggesting a localized intrapleural production of ADA (Sharma *et al,* 2001). Increased serum ADA activity has been observed in bacillary and paucibacillary PTB (Lakshmi *et al*, 1992). Serum ADA activity and lysozyme levels have been noted to be significantly elevated in children with different forms of tuberculosis in comparison to controls (Mishra *et al,* 2000). Some researchers have studied ADA activity in sputum and serum of PTB patients and found higher ADA activity in those patients with respect to other lung pathology (Dilmac *et al,* 2000). High levels of ADA activity have been observed in pleural fluid and serum of patients with tuberculous effusion compared to neoplastic effusion (Baganha *et al*, 1990). A previous study of serum ADA in pulmonary tuberculosis, malignancy and non-tubercular respiratory diseases showed significantly higher levels in pulmonary tuberculosis patients than other groups (Bansal *et al,* 1991). Our results are consistent with their findings.

Using the cutoff levels of 25 IU/l in serum and 45 IU/l in pleural fluid, ADA had sensitivities of 72.41% and 76.10% with specificities of 81.53% and 100%, respectively, in TB/PE patients. It has been reported that with an ADA activity cutoff value of 54 IU/l, the sensitivity is 82% and specificity is 97% for the diagnosis of tuberculosis (Baganha *et al,* 1990). Reported cutoff values for ADA (total) vary from 47 to 60 U/l (Valdes *et al*, 1993; Perez-Rodriguez et *al*, 1999; Roth *et al*, 1999). Several studies have suggested that an elevated pleural fluid ADA level predicts tuberculous pleuritis with a sensitivity of 90-100% and a specificity of 89-100% when the Guisti method is used (Guisti, 1970; Burgess et *al*, 1995; Valdes *et al*, 1995; Villena *et al*, 1996). Fluid culture results are positive in <25%. Without treatment, the natural history of tuberculous pleuritis has a high rate of recurrence (65%) of active tuberculosis (Valdes *et al*, 1993).

There are two principal isoenzymes of ADA, ADA-1 and ADA-2. ADA-2 is released by monocyte macrophages (Gakis *et al*, 1989) when they are stimulated by the presence of live microorganisms in their interior. An increase in total ADA activity may be characterized by *M. tuberculosis* infecting the macrophages. In a few cases with pulmonary tuberculosis a low ADA activity was obtained in spite of positive sputum was for TB. The most likely mechanisms could be suppressed or immature cell mediated immunity especially affecting the differentiation of the T-lymphocyte population.

Recirculation of activated T-cells and macrophages may cause higher serum ADA activity in patients with tuberculosis induced pleural fluid for detoxification to avoid accu-

mulation of toxic metabolites. Pleural fluid ADA was found to be three times serum ADA activity in tuberculous disease and two times in non-tuberculous disease.

Although many sensitive tests involving molecular diagnostics are available for the rapid diagnosis of TB, such technology is not available in developing countries. The colorimetric method for the measurement of total ADA described by Guisti and Galanti (1984) has an advantage over other methods because of its low cost, simplicity of technique and rapid-turn around time. The estimation of ADA activity in body fluid therefore serves as a resonable tool in the diagnosis of TB pleural effusion, especially when other clinical laboratory tests are negative.

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