

Adenosine Deaminase in CSF and pleural fluid for diagnosis of tubercular meningitis and pulmonary tuberculosis

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ABSTRACT

Tuberculosis (TB) is one of the most common infectious diseases in developing countries including Nepal. Delay in diagnosis and treatment of tuberculosis results in poor prognosis of the disease. This study was conducted to estimate diagnostic cut off values of Adenosine Deaminase (ADA) in cerebrospinal fluid (CSF) and pleural fluid and to evaluate the sensitivity, specificity, positive and negative predictive values of ADA in pleural fluid and CSF from patients with tuberculous and non-tuberculous disease. A total of 98 body fluid (CSF: 24, Pleural fluid: 74) specimens were received for the estimation of ADA. ADA activity was measured at 37°C by spectrophotometric method of Guisti and Galanti, 1984 at 625nm wavelength. Among the patients enrolled for the study subjects for which CSF were received (n=24) included 8 tuberculous meningitis (TBM), and 16 non-tubercular meningitis (NTM). Pleural fluid samples (n=74) were received from 19 pulmonary TB with pleural effusion, 17 PTB without pleural effusion and 37 of non-tuberculous disease patients. CSF ADA activity were (11.16±2.03 IU/L) and (5.35±1.89 IU/L) (p <0.001) in TM and non-NTM groups and Pleural fluid ADA activity were (103±22.18 IU/L) and (23.79 ±11.62 IU/L) (p <0.001) in PTB and non-TB groups respectively. ADA test in body fluids, which is simple, cost-effective and sensitive, specific for the tubercular disease is recommended to perform before forwarding the cumbersome and expensive procedures like culture and PCR for TB diagnosis.

INTRODUCTION

Tuberculosis (TB) is one of the most common infectious diseases in developing countries including Nepal. The World Health Organization (WHO) report (2012) has estimated 8.7 million incident cases of TB and 1.4 million deaths in 2011.^{1,2} Delay in diagnosis and treatment of tuberculosis results in poor prognosis of the disease.^{1,3} Involvement of pleura, peritoneum, meninges and synovial membranes is also common during manifestations of tubercular disease.⁴ Tubercular disease is responded by cellular immune response where accumulation of monocytes, macrophages, lymphocytes and polymorphonuclear cells takes place in lesions.⁵ These responses are initiated on sensitization of T-lymphocytes by the bacterial antigen with the release of cytokines that regulate macrophage function, mechanism of tuberculosis skin testing (TST), used in diagnosis of TB.⁶ TST has low sensitivity and specificity. Other tests for TB diagnosis are Z-N staining, AFB culture, ELISA for antibody detection, PCR and interferon assay etc. Z-N is simple and non-expensive, have low sensitivity. Whereas, others: ELISA, PCR, interferon assay requires standard laboratory setup and expert technician.³ Although sputum culture is highly specific for diagnosis it requires long incubation period.¹

Adenosine Deaminase (ADA) is an enzyme essential in purine metabolism recognized as the marker of cell mediated immunity particularly of T-lymphocyte activation.⁷ It plays important role in differentiating lymphoid cells and is present in abundance in active T lymphocytes whose concentration is inversely proportional to the degree of differentiation.^{4,7} ADA catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine with the release of ammonia.⁸ Its levels are ten times higher in T-lymphocytes than in erythrocytes. The enzyme activity increases during mitogenic and antigenic responses of lymphocytes, and T-lymphocyte blastogenesis can be inhibited by inhibitors of ADA.⁹ Likewise, a deficiency of ADA is associated with severe defects in the cell-mediated and humoral arms of the immune system, predisposing the patient to opportunistic infections.¹⁰ Since ADA is increased in TB effusions and is an easy little-invasive investigation, it is frequently considered as a diagnostic aid in such cases with a sensitivity of 90-100% and specificity 89-100% and ADA levels have also been considered by several researchers to differentiate tubercular disease from non-tubercular.¹¹ Various studies have been conducted demonstrating CSF-ADA estimation as an enzymatic assay in diagnosis

of tubercular meningitis (TBM) and can differentiate TBM from other infectious meningitis.^{12,13} This study was conducted to estimate diagnostic cut off values of Adenosine Deaminase in cerebrospinal fluid (CSF) and pleural fluid and to evaluate the sensitivity, specificity, positive and negative predictive values of ADA in pleural fluid and CSF from patients with tuberculous and non-tuberculous disease.

MATERIALS AND METHODS

The study was carried out in the Department of Biochemistry in collaboration with Microbiology and Medicine in B. P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal from September 2009 to August 2011. A total of 98 body fluid (CSF: 24, Pleural fluid: 74) specimens were received for the estimation of ADA. These specimens were collected aseptically by trained medical doctors after taking patient consent. Ethical clearance was taken as per the guidelines of the Institutional Ethical Review Board (IERB), BPKIHS.

ADA ACTIVITY

ADA activity was measured at 37°C by spectrophotometric method of Guisti and Galanti, 1984 at 625nm wavelength. Enzymatic activity (1 IU) of ADA was defined as amount of enzyme required to liberate 1 micro moles of Ammonia per minute from adenosine under standard conditions at 37 °C. 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 of same batch obtained from MERCK, India.

DATA ANALYSIS

Data was analysed in SPSS version 16. Sensitivity, specificity, positive and negative predictive values, of ADA were calculated for CSF and Pleural fluid samples of tuberculous and non-tuberculous disease respectively. ADA values were expressed in mean±SD and statistical tests were employed according to the nature of the data. P value of less than 0.05 was considered as statistically significant.

RESULTS

Among the patients enrolled for the study subjects for which CSF were received (n=24) included 8 tuberculous meningitis (TBM), and 16 non-tubercular meningitis (NTM). Pleural fluid samples (n=74) were received from 19 pulmonary TB with pleural effusion, 17 PTB without pleural effusion and 37 of non-tuberculous disease patients, including exudative and transudative effusions. Diagnosis of above mentioned diseases were based on

combination of the followings: history, findings

on Z-N stain, TST interpretation, chest X-ray findings, cytology reports and culture respectively.

Table-1 shows the sensitivity, specificity, positive and negative predictive values of CSF and pleural fluid ADA activity in tuberculous disease and non-tubercular disease. Table-2 depicts the mean±SD values of ADA activity in different disease categories. CSF ADA activity were (11.16 ± 2.03) and (5.35 ± 1.89) in TM and non-TM groups and Pleural fluid ADA activity were (103 ± 22.18) and (23.79 ± 11.62) in PTB and non-TB groups respectively. Significant differences in CSF ADA levels were observed between TM and NTM (p < 0.001) and pleural fluid ADA levels between tubercular disease and non-tubercular disease (p < 0.001)

Table-1: Predictive Values of body fluid ADA

Values	CSF ADA	Pleural Fluid ADA
Disease	Tubercular disease (n=8)	Tubercular disease (n=38)
	Non-tubercular disease (n=16)	Non-tubercular disease (n=36)
Sensitivity	100%	82.30%
Specificity	93.80%	97.20%
Cut off values	8.83	44
PPV	90.90%	94.70%
NPV	100%	94.40%
Area under curve	1	0.96

DISCUSSION:

Immune response in tuberculosis is mainly cell mediated, while both cell-mediated and humoral immune responses are elicited by most non-tuberculous infections in human body.¹¹ Thus ADA activity, a marker of T-cell activation and cell-mediated immune response help to differentiate tubercular etiology from nontubercular. Other earlier studies have also shown increased levels of serum ADA in a number of diseases where CMI is stimulated like Behcet's disease¹⁴ (Kose et al, 2001), typhoid¹⁵ (Ungerer et al, 1996), and tuberculosis.^{13, 16} Sharma SK et al 2001 showed cut off value of ADA 35 IU/L and found sensitivity and specificity of pleural fluid ADA to be 83% (CI 0.76-0.9) and 66% (0.56-0.76) respectively.⁷ Rohani et al 1995 showed mean CSF value higher than 9 IU/L and sensitivity and specificity of 1.0 and 8.76 respectively.⁹ Gautam et. al. 2007 showed sensitivity and specificity of CSF ADA activity 85.0% and 88.0% respectively at cut-off value of 6.97 IU/L to diagnose TBM in cerebrospinal fluid (CSF).¹³ Similarly Gautam et al 2007 showed higher sensitivity (76%) and specificity (100%) were found in keeping with a lower cutoff limit for pleural fluid ADA of 45 IU/L.^{13,}

Table-2: ADA activity of pleural fluid and CSF in tuberculous disease and non-disease in various age groups

Variables	CSF Group		Pleural Fluid Group	
	TM (n=10)	NTM (n=16)	PT(n=38)	NT(n=32)
Age years (Mean±SD)	34.0±19.1	42.0±16.11	42.13±19.03	42.97±21.91
ADA Activity (IU/L)	10.93±1.85**	5.34±1.89	103.35±54.33**	23.79±11.61

16 Our study comparable to previous studies has shown sensitivity and specificity 100% and 93.80% in CSF and sensitivity and specificity of 82.3% and 97.2% in Pleural Fluid respectively. The cut off values for CSF and ADA values in tubercular diseases were 8.83 IU/L and 44 IU/L respectively.

LIMITATIONS :

ADA is a diagnostic marker for tubercular disease. However, several cut-off values could be obtained for each tubercular disease. Few number of sample size limits our study to calculate separate cut offs and predictive values for each tubercular diseases. Also, the invasive techniques might be painful to the patients, so proper information should be provided to the patient regarding effects of the invasive techniques.

ADA is a diagnostic marker for tuberculous diseases as shown by its high sensitivity (100%) and specificity (82.3%) for the tubercular diseases with CSF and, specific (97.2%) and sensitive (93.8%) with pleural fluid samples. ADA test in body fluids, which is simple, cost-effective and sensitive, specific for the tubercular disease is recommended to perform before forwarding the cumbersome and expensive procedures like culture and PCR for TB diagnosis.

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