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ADENOSINE DEAMINASE (ADA) ANALYSIS AND ITS DIAGNOSTIC ROLE IN TUBERCULOUS PLEURAL EFFUSION

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ABSTRACT

Background: Accurate and early diagnosis of tuberculous pleurisy is essential for its correct treatment and management in clinical practice. Pleural fluid ADA estimation had been proposed as a useful diagnostic biomarker in tuberculous pleurisy. Objective: The study was designed to investigate the diagnostic role of ADA in tuberculous pleurisy in adult Nepalese population. Methods: One hundred and twenty pleural fluid specimens were consecutively selected and divided into two groups: Group I - tuberculous exudates (n=70) and Group II - transudates (n=50), the control cases based on the standard diagnostic criteria. ADA was estimated in pleural fluid in both the groups. Results: The mean \pm SD in the tuberculous pleurisy patient group was 78.83 ± 30.85 U/L and in the transudates, it was 21.59 ± 9.46 U/L (highly significant, $P < 0.001$). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) in the tuberculous pleurisy patients were 100% (CI 94.80 - 100.00), 96.00% (CI 86.64 - 98.90), 97.22% (CI 90.42-99.23) and 100% (CI 92.58-100.00), respectively. Conclusion: The pleural fluid ADA levels were significantly higher in tuberculous pleural effusions as compared with transudates cases. Our findings thus, support the view that pleural fluid ADA estimation is a very useful biomarker in establishing an accurate and early diagnosis in the tuberculous pleurisy patients. In addition, it is a simple, rapid, an inexpensive procedure that could be a useful complimentary test in the diagnostic work-up of tuberculous pleural effusions in clinical practice.

Key Words: *Adenosine Deaminase, Diagnostic Role, Tuberculous Pleural Effusion, Sensitivity and Specificity*

INTRODUCTION

Definitive diagnosis of tuberculous pleural effusion (TPE) is critically essential for its early detection, management and an early institution of specific anti-tubercular therapy. Between 3% and 25% of patients with tuberculosis will have tuberculous pleuritis (Light, 2010). Conventional diagnostic methods had proved their utility for the diagnosis of pulmonary tuberculosis (TB) but have limited application in case of TPE due to its paucibacillary nature. Pleural fluid cultures are positive for *Mycobacterium tuberculosis* in less than 40% and smears are virtually always negative¹. A pleural biopsy has been considered the gold standard in diagnosis of TPE but it is invasive (Ahmed *et al.*, 2011) and pleural fluid sampling is more difficult than simple thoracentesis. The diagnosis cannot be established in 10-20% of the patients with these methods even in the best conditions (Yildiz *et al.*, 2011). Pleural fluid markers of TPE have been extensively evaluated as an attractive alternative to pleural biopsy (Garcia-Zamalloa and Taboada-Gomez, 2012; Krenke and Korczynski, 2010). ELISA and polymerase chain reaction (PCR) are expensive diagnostic tests (Choudhury and Patel, 2010). Variety of new biomarkers have been proposed, viz. interferon gamma (INF- γ), interleukin (IL)-12p40, IL-18, tumour necrosis factor alpha (TNF- α), soluble IL-2 receptors (Sil-2R) and pro-calcitonin (Ambade *et al.*, 2011; Hiraki *et al.*, 2003 and Malekmohammad *et al.*, 2012). Trace elements analysis (such as copper, zinc, magnesium, manganese, selenium and iron) of urine and hair in tuberculous pleurisy have also been investigated and may provide an additional disease correlate for assessing tuberculous pleurisy risk (Liu *et al.*, 2011). Other molecules such as pleural fluid levels of neopterin - a marker of Th1 immune activation, serum leptin - which cross regulates nutritional status and the immune system, lysozyme and products of complement activation such as C3a-desArg and

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SC 5b-9 have been investigated in the diagnosis of TPE but found to have variable sensitivity and specificity (Tung *et al.*, 2011). These diagnostic approaches are technically demanding, needs trained man-power and are expensive as well and thus not cost-effective. There is a need for a single test which is adequately sensitive and specific and at the same time inexpensive and easy to perform (Sharma *et al.*, 2001).

Adenosine deaminase (ADA) (EC.3.5.4.4) is an enzyme involved in the breakdown of adenosine to urea and ADA levels were found to be elevated in the pleural fluid of patients with TPE way back in 1978 by Piras *et al.*, (1978). Since then, several studies had been carried out that have shown the usefulness of ADA estimation in pleural fluid for the rapid diagnosis of TPE (Ocana *et al.*, 1983; Gupta *et al.*, 1990; Burgess *et al.*, 1995; Kaisemann *et al.*, 2004; Verma *et al.*, 2008; Gupta, 2010 and Haque, 2012). At present, ADA is the most cost-effective pleural fluid biomarker and is routinely employed as a screening tool, in particular in countries where TB is endemic (Yildiz *et al.*, 2011 and Porcel, 2009). Limited studies had been reported from Nepal that had investigated ADA role in the diagnosis of TPE (Lamsal *et al.*, 2007). The aim of this investigation was to assess the significance of ADA activity in pleural fluid for the diagnosis of TPE in Nepalese population.

MATERIALS AND METHODS

Setting

This prospective study was carried out on patients admitted in the medical ward of a centrally located tertiary care hospital in Kathmandu, Nepal from January 2009 to December 2010. The ethical review committee of the hospital permitted to carry out this study and informed consent was taken from the patients before inclusion in the study. Their results were dispatched immediately after the tests were performed, so that the patients get appropriate treatment.

Patients

120 consecutive cases of patients admitted in the medical ward of the hospital on account of pleural effusion were selected and a final diagnosis was made based on standard criteria. These were divided into two different patient groups. Group I - tuberculous exudates -70 cases - based on presence of acid-fast bacilli in pleural fluid or biopsy tissue and radiological findings consistent with TB, clinical presentation consistent with TB with exclusion of other clinical conditions, definite clinical and radiological improvement in two months of administration of anti-tubercular treatment. Group II - transudates - 50 cases - congestive heart failure (38 cases), end-stage liver disease (5 cases), nephritic syndrome (5 cases) and hypoproteinemia (2 cases) and were diagnosed by standard clinical and diagnostic procedures.

Laboratory Tests

Pleural tap was done in all the cases and blood samples were also taken at the same time. A battery of laboratory investigations was done i.e. pH, glucose, protein, lactate dehydrogenase (LDH), Ziehl - Neelsen staining and total ADA. Light's criteria (Light *et al.*, 1972) - plural fluid protein serum protein > 0.5; fluid LDH / serum LDH > 0.6 was used to ensure exudative pleural effusions in tuberculous cases.

ADA Estimation

ADA estimation was carried out by spectrophotometry method based on the principle of Guisti and Galanti method of enzymatic analysis (Guisti and Galanti, 1984). ADA MTB diagnostic kit from Microexpress - a division of Tulip Diagnostics Pvt. Ltd., India was used according to the manufacturer's instructions. Briefly, ADA hydrolyzes adenosine to ammonia and inosine. The ammonia formed further reacts with phenol and hypochlorite in an alkaline medium to form a blue iodophenol complex with sodium nitroprusside acting as a catalyst. Intensity of the blue coloured iodophenol complex formed is directly proportional to the amount of ADA present in the specimen, and is measured with the aid of a colorimeter at a wavelength of 623 nm.

Adenosine + H₂O $\xrightarrow{\text{ADA}}$ Ammonia + Inosine

Ammonia + Phenol + Hypochlorite $\xrightarrow{\text{Alkaline medium}}$ Blue iodophenol complex

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Control for each specimen was performed alongside with the test specimen. The reading was taken by technicians who were blinded as to the origin of the pleural fluid specimens (from which group of patients). The readings were converted to U/L in order to make the statistical calculations. Reference values of ADA levels above 40 U/L in pleural fluid were taken as positive for calculating the diagnostic parameters of sensitivity and specificity.

Statistical Analysis

The results were expressed as mean \pm SD. Statistical comparison was carried out by using the Student's t test. A two-tailed P value of < 0.05 was taken as statistically significant. Diagnostic test 2×2 contingency tables were made. Sensitivity, specificity, positive and negative predictive value were calculated. All parameters were estimated with 95% confidence interval using the Stata 10.1 statistical software package (Stata Corp. College Station, Tx).

RESULTS

Table 1 shows age and sex ratio of the different population groups that were investigated in this study. It comprised a total of 120 consecutive pleural effusions in - patients in the age group of 17 to 80 years. TPE group included 70 cases with age in years, (Mean \pm SD) of 39.50 ± 17.63 and male to female ratio of 4:1. The control group comprised a total of 50 cases with age in years, (Mean \pm SD) of 45.40 ± 17.57 and a male to female ratio of 2.12:1.

Table 2 depicts pleural fluid ADA levels (Mean \pm SD) in TPE cases as 78.83 ± 30.85 U/L and in transudates cases, it was 21.59 ± 9.46 (highly significant, $P < 0.001$)

Table 3 shows the different diagnostic parameters in TPE cases with a sensitivity, specificity, PPV and NPV of 100% (CI 94.80 - 100.00), 96.00% (CI 86.64 - 98.90), 97.22% (CI 90.42-99.23) and 100% (CI 92.58-100.00), respectively. None of the pleural fluid specimens in TPE cases showed an ADA level of < 40 U/L and only two pleural fluid specimens showed an ADA value of > 40 U/L in the transudates cases.

Table 1: Age and Sex ratio of the different study population groups

Study Group	No. of patients	Age (years) (Mean \pm SD)	Sex Ratio (M:F)
TPE	70	39.50 ± 17.63	4 : 1
Non-TPE (transudates)	50	45.40 ± 17.57	2.12 : 1

Table 2: CSF ADA levels in different study populations groups

Study Group	No. of patients	Mean \pm S.D (U/L)	P value comparison TPE & Non-TPE
TPE	70	78.83 ± 30.85	$p < 0.001$
Non-TPE (transudates)	35	21.59 ± 9.46	

Table 3: Validity of CSF ADA as a diagnostic test in suspected cases of tuberculous pleurisy cases

Study Group	Sensitivity % (CI)	Specificity % (CI)	PPV % (CI)	NPV % (CI)
TPE	100.00 (94.80 - 100.00)	96.00 (86.64-98.90)	97.22 (90.42- 99.23)	100.00 (92.58- 100.00)

PPV= positive predictive value, NPV= negative predictive value, CI = 95% confidence interval

DISCUSSION

Since conventional diagnostic methods have their limitations in detecting TPE, several alternative diagnostic approaches have been extensively evaluated (Udwadia and Sen, 2010). However only ADA

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and IFN- γ have become reliable for diagnosing TPE. The long history of the successful use of the ADA test, its simplicity, low cost and quickly available results, makes it the preferred option 4.

ADA is an essential enzyme in the metabolism of purine nucleosides (Garcia-Zamalloa and Taboada-Gomez, 2012). ADA acts in proliferation and differentiation of lymphocyte, especially T lymphocyte. It is a significant indicator of active cellular immunity. Furthermore, it has been proposed to be a useful surrogate marker for TB because it can be detected in body fluid, such as pleural, pericardial and peritoneal fluid (Dinnes *et al.*, 2007). The levels of ADA increase in TB because of the stimulation of T cells by mycobacterial antigens (Boonyagars and Kiertiburanakul, 2010). Apart from TB, the main disease that causes an elevated ADA is parapneumonic pleural effusion, one-third of cases of UPE and two-thirds of those of CPE/empyema may have a high ADA level but both conditions are easily distinguished from TPE because they develop neutrophilic effusions (Krenke and Korczynski, 2010; Porcel, 2009). ADA has been found to be a reliable marker of TPE in HIV-positive patients, even in those with a low CD4 - cell count (Baba *et al.*, 2008).

This research was carried out on a total of 120 cases of pleural effusions, in which 70 were of TPE and 50 were the transudates, the control group. The ADA level (Mean \pm SD) in TPE cases was 78.83 ± 30.85 while in the transudates it was 21.59 ± 9.46 (highly significant, $P < 0.001$). With a cut off value for ADA of 40 U/L, the sensitivity and specificity for diagnosing TPE was 100% (CI 94.80 - 100) and 96% (CI 86.64 - 98.90) with PPV and NPV of 92.22% (CI 90.42 - 99.23) and 100% (CI 92.58 - 100) respectively in this study. Almost all research workers have shown sensitivity and specificity of 90% to 100% for the value of ADA in pleural fluid using different cut off levels (Mathur *et al.*, 2006). The most widely accepted cut off level of ADA for the diagnosis of TPE is 40 U/L (Garcia-Zamalloa and Taboada-Gomez, 2012; Liang *et al.*, 2008). However, Kaur *et al.*, (1992) showed a poor diagnostic value of ADA in pleural, peritoneal and cerebrospinal fluid in tuberculosis. One limitation of this study is that all the cases in the TPE group were not confirmed by the pleural biopsy diagnostic method, which is considered to be the gold standard method for its confirmatory detection. This could have introduced some bias in the selection of this patient group. Though other standard diagnostic procedures were scrupulously followed up to confirm these cases were of tuberculous pleural effusions.

Krenke *et al.*, (2008) studied 94 patients (28 cases of TPE and 66 cases of non-TPE group). The ADA activity was significantly higher in TPE than in non - TPE (614.1 ± 324.5 vs. 15.1 ± 36.0 pg/ml, $P < 0.0001$). The diagnostic sensitivity and specificity were 100% and 93.9% at the cut off value of 40.3 U/L). Patel and Choudhury (2011) analyzed 53 patients with TPE and 96.67% had pleural fluid ADA > 40 IU/L. Kalantri *et al.*, (2011) assessed 204 cases - 50 were confirmed pleural TB, 104 were probable pleural TB and 50 formed the non - TB group. For confirmed and probable pleural TB cases, ADA showed a sensitivity and specificity of 92% and 73%, respectively. Agarwal (2012) investigated 30 cases of TPE and showed sensitivity, specificity, PPV and NPV of 92%, 80%, 95.8% and 66.66%, respectively. Elevated levels of ADA in TPE have been noted by several authors. These observations were reproduced and further confirmed in this study.

This research clearly showed that ADA levels are significantly elevated in TPE cases as compared to transudates cases. The results showed a sensitivity of 100% and a specificity of 96% for the diagnosis of TPE with PPV and NPV of 97.22% and 100%, respectively. Its cost-effectiveness, rapidity (just 2 hours), ease of performance and a high diagnostic value makes it a useful complimentary test for the diagnosis of TPE. Further, the results validates that a simple test like ADA estimation should be included routinely in the diagnostic work - up for TPE in clinical practice.

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