

Adenosine Deaminase in Diagnosis of Tuberculosis: A Review

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ABSTRACT

The diagnosis of tuberculosis (TB) continues to be a challenge in clinical practice. Traditional diagnostic methods are very useful but do not provide enough sensitivity and specificity. Adenosine deaminase (ADA) has been developed and widely used for the diagnosis of TB. ADA is an enzyme that increases in TB because of the stimulation of T-cell lymphocytes by mycobacterial antigens. This article reviews the characteristics, metabolism and clinical uses of ADA for the diagnosis of TB in clinical practices. There is sufficient data supporting yield of ADA in various body fluids for the diagnosis of TB. ADA may be used for early diagnosis of TB, especially in case of negative acid fast bacilli (AFB) smear from the body specimens.

Keywords: Adenosine deaminase, ADA, tuberculosis, diagnosis

Introduction

Tuberculosis (TB) is a bacterial disease caused by the tubercle bacilli which includes *Mycobacterium tuberculosis*. TB remains one of the major health problems in Bangladesh and worldwide. Globally 8.6 million people developed TB and 1.3 million died from the disease (including 320 000 deaths among HIV-positive people). Bangladesh ranks sixth among the world's 22 high-burden TB countries with estimated 350,000 new cases and 70,000 deaths per year¹. The lung tissue is involved in pulmonary TB and the tissue other than lung tissue like pleural fluid, ascitic fluid etc is involved in extrapulmonary TB (EPTB). Prompt diagnosis is essential for effective TB control programme. Although we have many methods for the diagnosis of pulmonary TB, for example Ziehl-Neelsen (Z-N) staining, polymerase chain reaction (PCR) and culture, these methods do not provide enough sensitivity and specificity. The sensitivities of ZN staining and culture are 10-40% and 8-49% respectively in the diagnosis of TB infection². The definitive diagnosis of EPTB depends on the demonstration of *Mycobacterium tuberculosis* in the specimens like pleural fluid, ascitic fluid, pericardial fluid,

cerebrospinal fluid (CSF) or pleural biopsy specimen, and can also be established with reasonable certainty by demonstration of granuloma in the parietal pleura, peritoneum, pericardium etc³. Although mycobacterial culture is the gold standard in diagnosing TB, *Mycobacterium* spp. grows very slowly and 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. Thoracoscopy in diagnosing tubercular serositis offers a near 100% positive diagnostic yield on histology and 76% positive on culture^{3,4}. However, historically, since pleural biopsy is more invasive and hazardous than thoracentesis, alternative diagnostic approaches have been extensively evaluated⁵. Adenosine deaminase (ADA) has been developed and widely used for the diagnosis of TB due to its simplicity, low cost, and quickly available results. Many studies have confirmed the high sensitivity and

specificity of ADA (sensitivity 92% and specificity 89%) for early diagnosis of EPTB, such as tuberculous pleuritis, pericarditis, ascites and meningitis⁶. The author aimed to review the characteristics, its metabolism and clinical uses of ADA for the diagnosis of TB.

Characteristics, metabolism and assay of adenosine deaminase (ADA)

ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. It is also an essential enzyme of the purine catabolic pathway. There are 2 isoforms of ADA, ADA-1 and ADA-2. ADA-1 is found in many tissues including red blood cells. ADA-2 is found only in macrophages and monocytes^{7,8}. ADA acts in proliferation and differentiation of lymphocyte, especially T lymphocyte. It also acts in maturation of monocytes transforming them to macrophage. ADA is a significant indicator of active cellular immunity. It increases in biological fluids in the course of infectious disease characterized by micro-organisms infecting the macrophages. For example, deficiency in ADA in humans manifests primarily as severe lymphopenia and immunodeficiency⁹. Furthermore, ADA has been proposed to be a useful surrogate marker for TB because it can be detected in body fluids such as pleural, pericardial and peritoneal fluid. The levels of ADA increase in TB because of the stimulation of T cells by mycobacterial antigens⁸.

Adenosine deaminase (ADA) activity assay

Total plasma ADA can be measured by a spectrophotometric method described by Guisti and Galanti in 1984. It uses the principles that the ADA assay is based on the enzymatic deamination of adenosine to inosine and formation of ammonia. Ammonia (NH₃) forms under conversion of adenosine 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 is used as the catalyst. The ammonia concentration is directly proportional to the absorbance of the indophenols measured at a wavelength of 620 nm. The reaction catalyzed by ADA is stopped at the end of one hour incubation at 37°C by the addition

of phenol nitroprusside solution. ADA activity is expressed in international units (IU) using the formula as follows: (Absorbance of sample/ Absorbance of standard) x 50 IU/l¹⁰.

Comparison of the performance of adenosine deaminase assay using Diazyme^R commercial kit and Giusti including modified Giusti method

The determination of ADA levels has been performed using the method proposed by Giusti which has undergone certain modifications over time¹⁰. The modified Giusti method including the Berthelot reaction is developed in order to obtain better results. But there is a lack of standardization and it does not allow this assay to be used as a good diagnostic test¹¹. An automated method (Diazyme^R commercial kit, Diazyme Laboratories, San Diego, CA, USA) with automation (ADVIA 1650 analyzer; Bayer Diagnostics, Tarrytown, NY, USA) was developed for the determination of ADA activity in pleural fluid and CSF samples. A study had compared the results with those obtained from the modified Giusti method, which is considered a reference test for biochemical study¹². The values found for the pleural fluid and CSF confirmed the strong correlation between these 2 methods. Pleural fluid with cut-off values of 40 U/L (conventional method) and 30 U/L (automated method), had the concordance of 96.8 percent. For the detection in CSF samples, the cut-off value was 9 U/L (for both methods) and the concordance was 100 percent. Therefore, the reference values for the diagnosis of TB in pleural fluid samples are 40 U/L (modified Giusti method) and 30 U/L (automated method: Diazyme R commercial kit), versus 9 U/L (for both methods) in CSF samples.

Use of adenosine deaminase in clinical practices stratified by body fluids

Pleural effusion

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. Gupta et al. showed sensitivity

and specificity for diagnosing tuberculous pleural effusion of 100% and 94.1% respectively¹³, which was almost similar to the study done by Burgess et al. (sensitivity 90% and specificity 89% at cut-off value of 50 U/L)¹⁴, Strankinga et al. (sensitivity 100% and specificity 87% at cut-off value of 53 U/L)¹⁵ and Farhana et al. (sensitivity 95% and specificity 83.3% at cut-off value of 40 U/L)¹⁶.

Bronchoalveolar lavage fluid

The role of ADA estimation in bronchoalveolar lavage (BAL) fluid for diagnostic of smear-negative pulmonary TB is not clearly useful. A prospective study was conducted in Thailand to determine the diagnostic value of ADA activity in BAL fluid for diagnosis of pulmonary TB among 148 patients with abnormal chest X-ray and unknown definite diagnosis. All patients were either sputum-smear negative for AFB or failed to produce sputum. The mean ADA activity in the BAL for pulmonary TB, malignancy and miscellaneous causes groups was 8.98, 7.63 and 11.61 U/l respectively. There was no difference of ADA levels in BAL fluid among these 3 groups of patients¹⁷.

Ascites

Ascites is the predominant finding and it is present in about 78% of patients with tuberculous peritonitis¹⁸. A systematic review from 35 studies of patients with tuberculous peritonitis was conducted and the authors aimed to determine the performance of the available tests for diagnosis of tuberculous peritonitis¹⁹. Because of low sensitivity of the current methods like ascites total protein, serum-ascites albumin gradient, Ziehl-Neelsen staining and culture²⁰, ADA may be a better diagnostics for tuberculous peritonitis. Ascites ADA is increased in tuberculous ascitic fluid because of the stimulation of T cells by the mycobacterial antigens. ADA activity in the peritoneal fluid has been proved to be a simple and reliable method for early diagnosis of tuberculous peritonitis²¹. Sensitivity and specificity levels over 90 percent have been reported²² with the

exception of a study by Hillebrand et al.²³, who reported a sensitivity of 59%. Lower sensitivity may have been related to the higher incidence of cirrhosis in the study group of patients. These observations were countered by Burgess et al.²⁴, when they evaluated cirrhotic patients with tuberculous peritonitis and reported a sensitivity of 94 percent. At present, an ascites ADA activity of > 30 U/L is generally accepted as the cut-off level expected to yield the best results. Hortiwakul et al. studied ADA testing among the patients with tuberculous peritonitis. Using ROC curves, a cut-off value of 22 U/L for the diagnosis was found to yield the best results; corresponding sensitivity and specificity were 84.8% and 82.6%, respectively²⁵. In the study done by Voight et al. the mean ADA level for tubercular etiology was 99.8 U/L with sensitivity and specificity for diagnosis tubercular ascites was 95% and 98% respectively²⁶, which was similar to the study done by Dwivedi et al. (sensitivity 100% and specificity 96.6% at an ADA level >33 U/L)²⁷ and Gupta et al. (sensitivity 95% and specificity 94.1% at an ADA level >30 U/L)²⁸.

Cerebrospinal fluid

Chotmongkol et al. conducted a study comparing the ADA activity in cerebrospinal fluid (CSF) between patients with tuberculous and non-tuberculous meningitis was conducted. The ROC curve identified a CSF ADA level of 15.5 U/l as the best cut-off value to differentiate between the 2 groups, with a sensitivity of 75%, specificity of 93%²⁹. In bacterial meningitis, mean ADA is quite high when compared with non-tuberculous and non-bacterial meningitis group. The yield of ADA may be low in setting to differentiate bacterial from tuberculosis meningitis. The possible explanation may be from ADA value in most assays detected total ADA which includes ADA-1 and ADA-2. Thus, fluid with high cell counts (e.g. bacterial meningitis) can have high total ADA and may be undifferentiated from tuberculous meningitis. ADA activity in the CSF of HIV-infected patients had limited value for diagnosis of tuberculous meningitis. A retrospective study

was conducted to determine ADA levels in 417 CSF samples from HIV-infected patients with neurological symptoms³⁰. HIV-associated neurological disorders and progressive multifocal leukoencephalopathy were not associated with elevated ADA in CSF. When using a cut-off point of 8.5 IU/l for the diagnosis of tuberculous meningitis, sensitivity was only 57% and specificity was 87%. A cut-off value of 10 IU/l gave a specificity of 90% but very low sensitivity (36%). The results of this study indicated that ADA determination in CSF has limited utility for the diagnosis of tuberculous meningitis in HIV infected patients. Recommendation from British Infection Society for the diagnosis and treatment of TB of the central nervous system in adults and children suggests that the activity of ADA is raised in the CSF of patients with tuberculous meningitis and has been evaluated as a diagnostic assay³¹. The major problem was lacking of specificity. High CSF ADA activity has been reported from patients with lymphomas, malaria, brucellosis and pyogenic meningitis. Thus, CSF ADA activity is not recommended as a routine diagnostic test for TB of the central nervous system. However, prevalence of tuberculous meningitis in Bangladesh is high and positive predictive value for ADA in diagnosis of tuberculous meningitis is much higher than that of western countries. The value of CSF ADA may have usefulness in Bangladesh.

Pericardial effusion

A prospective study in South Africa showed that an ADA cut-off level of 40 U/l resulted in a test sensitivity, specificity, positive predictive value, negative predictive value and diagnostic efficiency of 84%, 80%, 91%, 66% and 83%, respectively³². Kwan et al. studied pericardial fluid ADA level along with histopathology of pericardial biopsy and found a cut off ADA level of 40 U/L in pericardial fluid which has sensitivity of 93% and specificity of 97% in diagnosis of tubercular pericardial effusion³³, which is similar to the study by Mathur et al. showed 100% sensitivity and 83.3% specificity³⁴.

Adenosine deaminase (ADA) versus polymerase chain reaction (PCR)

Nucleic acid identification by PCR is a rapid, sensitive and specific tool for the detection of *Mycobacterium tuberculosis*³⁵. It permits direct identification of the *M. tuberculosis* complex and results are available in a day or two. However, sensitivity depends on a target site. PCR targets such as IS6110 and hsp65 kDa yield a sensitivity of 42-100% and a specificity of 85-100%³⁶. Sensitivity of PCR was achieved when devR and IS6110 test results were combined; the sensitivity and specificity values were 83% and 94% respectively in pleural fluid³⁷. A cross-sectional study was performed in a total of 179 body fluid samples. All specimens were analyzed for AFB smear, ADA activity (by a method based on the Berthlot reaction) and multiplex PCR using amplicons such as IS6110, dnaJ gene and hsp65 genes. On comparing AFB and ADA results with PCR, the PCR is clearly more effective than AFB smear ($p < 0.001$) and ADA estimation ($p < 0.02$) in all types of body fluids³⁶. Another study collected samples of 67 consecutive patients with large pericardial effusions. Sensitivity and specificity with a cut-off value of 40 U/L for diagnosis of tuberculous pericarditis were 83% and 78%, respectively, compared to PCR which sensitivity and specificity were 75% and 100% respectively³⁸. In this study, PCR had better specificity and positive predictive value than ADA for diagnosis of tuberculous pericarditis, but the sensitivity was not different from ADA. Disadvantages for PCR are: it needs more resources and sophisticated equipments than ADA, price is higher, needs longer time for test results and not every hospitals can set PCR lab (especially small to medium sizes hospitals).

Conclusion

ADA is an essential enzyme of the purine catabolic pathway catalyzing the deamination reaction from adenosine to inosine that increases in TB because of the stimulation of T-cell lymphocytes by mycobacterial antigens. There is sufficient data supporting yield of ADA in various

body fluids for the diagnosis of TB. ADA assays can be performed in many health care centres with limited diagnostic facilities other than mycobacterial culture, PCR etc. In addition, it is cheap and it has good sensitivity. ADA may be used for early diagnosis of TB, especially in case of negative AFB smear from the body specimens. However, culture is still the gold standard and mandatory for the confirmatory diagnosis. Prompt treatment of TB is crucial, especially in Bangladesh, where it is a high burden TB area. Further research regarding ADA is necessary to improve specificity, minimize false positive and choose the suitable cut-off value.

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