Review Article

Use of Adenosine Deaminase for the Diagnosis of Tuberculosis: A Review

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ABSTRACT

The current methods for the diagnosis of tuberculosis (TB) do not provide enough sensitivity and specificity. Adenosine deaminase (ADA) has been developed and widely used for the diagnosis of TB. This article reviews the characteristics, metabolism and clinical uses of ADA for the diagnosis of TB in clinical practices. ADA is an enzyme 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 may be used for early diagnosis of TB, especially in case of negative AFB smear from the body specimens. (*J Infect Dis Antimicrob Agents 2010;27:111-8.*)

INTRODUCTION

Tuberculosis (TB) remains one of the health problems in Thailand and worldwide. Although we have many methods for the diagnosis of TB, for example acid-fast bacilli (AFB) staining, polymerase chain reaction (PCR) and culture, these methods do not provide enough sensitivity and specificity. Adenosine deaminase (ADA) has been developed and widely used for the diagnosis of TB. Many studies have confirmed the high sensitivity and specificity of ADA for early diagnosis of extrapulmonary TB, such as tuberculous pleuritis, pericarditis and meningitis. The authors aimed to review the characteristics, its' metabolism and clinical uses of ADA for the diagnosis of TB.

Characteristics of adenosine deaminase

ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. It is also an essential enzyme of the purine catabolic pathway. ADA presents in all cell types, however, the amount of enzyme differs widely among tissues. The highest ADA levels in humans are found in lymphoid tissues.[1]

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. For example, deficiency in ADA in humans manifests primarily as severe lymphopenia and immunodeficiency.[2] Furthermore, ADA has been

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proposed to be a useful surrogate marker for TB because it can be detected in body fluids such as pleural, pericardial and peritoneal fluid.[3] The levels of ADA increase in TB because of the stimulation of T cells by mycobacterial antigens.

Metabolism of adenosine deaminase

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. ADA-2 is the major component of the activity of total ADA in the serum of healthy persons.[4] It increases in biological fluids in the course of infectious disease characterized by micro-organisms infecting the macrophages.

Total plasma ADA can be measured using many techniques which can be broadly categorized into 2 groups; Giusti and non-Giusti method.[5] The first method was described by Giusti and Galanti in 1974.[6] Diazyme® commercial kit that is used in Ramathibodi Hospital is one of the modified Giusti methods.[7] It uses the principles that the ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl) - 3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner.[8] The reaction of ADA assay uses colorimetric techniques. One unit of ADA is defined as the amount of ADA that generates 1 μmole of inosine from adenosine per minute at 37°C. The reaction is shown in Figure 1.

Use of adenosine deaminase in clinical practices stratified by body fluids

Pleural effusion

Ernam D et al.[10] did a retrospective study of 226 patients diagnosed with malignant, tuberculous and parapneumonic pleural effusions. The levels of pleural fluid ADA in tuberculous and parapneumonic effusions were significantly higher than those of malignant effusions. However, median value of pleural fluid ADA was not found to be statistically significant in comparison with tuberculous pleural effusion and empyema. As the results of this study, high ADA level can be found in both tuberculous pleural effusion and empyema. In fact, most pleural effusion and empyema can be differentiated by gross appearance. Thus, pleural fluid ADA is useful to diagnose tuberculosis in a non-empyema pleural effusion setting.

It is expected that ADA levels are lower in

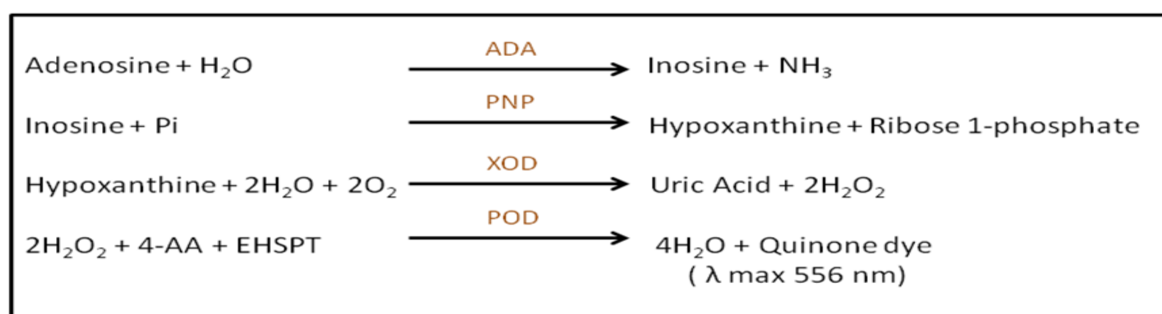

Figure 1. The reaction of adenosine deaminase assay using colorimetric techniques[9] (Adapted from Adenosine Deaminase Assay Kit. Catalog Number: BQ 014 - EALD).

HIV/TB co-infected patients and/or other immunocompromised patients with low blood CD4 cell counts. This is because the production of the total ADA in pleural fluid reflects the presence of activated T lymphocytes and monocytes. Baba K et al.[11] evaluated the sensitivity of ADA in tuberculous pleuritis in HIV-infected patients comparing between patients with CD4 cell counts <50 cells/mm^3 and >50 cells/mm^3. The results showed that the ADA values among HIV-infected patients with confirmed tuberculous pleuritis were not affected by low CD4 cell counts. There was no significant difference in mean ADA values in patients with lower CD4 cell counts compared to those with higher CD4 cell counts. Furthermore, there was no correlation between the ADA values, blood CD4 cell counts, absolute lymphocyte count, red cell counts or lymphocyte counts, percentages, lactate dehydrogenase and total protein in pleural fluid.

Another study presented the diagnostic value of the ADA activity in the pleural fluid among renal transplant patients.[12] The ADA activity in the pleural effusion of 23 patients was compared with 23 immunocompetent patients, both with tuberculous pleural effusion. The mean ADA levels of these 2 groups were not different. The sensitivity for diagnosis of tuberculous pleuritis in renal transplanted patients was high (91.3%) and was the same as that of immunocompetent patients. Thus, ADA in the pleural fluid is a useful test in the diagnosis of tuberculous pleural effusion even in renal transplant patients.

In Thailand, Kawamatawong T et al.[13] performed a retrospective study in 178 patients with symptomatic exudative lymphocytic pleural effusion using Diazyme® commercial kit. A receiver operating characteristic curve (ROC) for identification of the best cut-off pleural fluid ADA level that provided the greatest sensitivity of 82 percent and specificity of 91 percent was 30 U/L.

Bronchoalveolar lavage fluid

Yield of ADA 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 [95% confidence interval (CI), 3.79-14.17], 7.63 (95% CI, 4.12-11.14) and 11.61 U/l (95% CI, 3.59-19.62), respectively. There was no difference of ADA levels in BAL fluid among these 3 groups of patients.[14]

Ascites

Ascites is the predominant finding and it is present in about 78 percent of patients with tuberculous peritonitis.[15] 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 peritonitis.[16] Because of low sensitivity of the current methods which are ascites total protein, serum-ascites albumin gradient, Ziehl-Neelsen staining and culture,[17] a better diagnosis test for diagnosis of tuberculous peritonitis is needed.

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 peritonitis.[18] Sensitivity and specificity levels over 90 percent have been reported[19] with the exception of a study by Hillebrand et al.[20] who reported a sensitivity of 59 percent. 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.[21] 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.[16]

In Thailand, a retrospective study regarding ascitic ADA testing by using Giusti's method for the diagnosis of tuberculous peritonitis was conducted.[22] The median (range) ADA activity in tuberculous peritonitis group was 71.2 (6.7-187) U/L and was significantly higher than in non-tuberculous peritonitis group (p <0.05). 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 percent and 82.6 percent, respectively. Positive predictive value was 40.6 percent, negative predictive value was 97.8 percent, false positive rate was 17 percent and false negative rate was 15 percent.

Another study was performed in HIV-seropositive patients compared to HIV-seronegative with tuberculous peritonitis and non-tuberculous peritonitis (cirrhosis and malignancy).[23] Mean ADA in patients with HIV-seropositive and HIV-seronegative tuberculous peritonitis were significantly higher than those in patients with cirrhosis and malignancy (109.5 U/l in HIV-seropositive, 109 U/l in HIV-seronegative with tuberculous peritonitis compared to 10.5 U/l in cirrhosis and 13 U/l in malignancy). When comparing between HIV-positive and HIV-negative serostatus, there was no significant difference in ADA levels. Thus, ADA can be used as a good, sensitive, noninvasive test in both HIV-seronegative and seropositive patients for the diagnosis of tuberculous peritonitis.

Cerebrospinal fluid

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 percent, specificity of 93 percent and area under the curve of 0.92.[24] 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 determined ADA levels in 417 CSF samples from HIV-infected patients with neurological symptoms.[25] 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 percent and specificity was 87 percent. A cut-off value of 10 IU/l gave a specificity of 90 percent but very low sensitivity (36%). False-positive results were found in patients with neurological cytomegaloviral disease, cryptococcal disease, lymphomatous and probable candidal meningitis. 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[26] suggests that the activity of ADA is raised in the CSF of patients with tuberculous meningitis and has been evaluated as a diagnostic assay. 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 Thailand is high and positive predictive value for ADA in diagnosis of tuberculous meningitis is much higher than that of European countries. The value of CSF ADA may have usefulness in Thailand.

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 percent, 80 percent, 91 percent, 66 percent and 83 percent, respectively. Mean ADA in tuberculous pericarditis among both HIV-seronegative and HIV-seropositive was 79 U/l which was higher than that of malignant pericardial effusion (39.3 U/l, p = 0.007) and uremic pericardial effusion (mean 21.1 U/l, p = 0.004). However, there was no difference from septic pericarditis (mean 102.3 U/l, p = 1.000) and connective tissue disease (mean 31.1 U/l, p = 0.06).[27] Therefore, in purulent pericardial effusion or suspected pericardial effusions from connective tissue diseases, ADA may not be useful. In this study, mean ADA level in pericardial effusion was not affected by HIV-serostatus. Nevertheless, the mean ADA level was lower in patients with anti-tuberculosis treatment compared to that in patients without anti-tuberculosis treatment.

Adenosine deaminase versus polymerase chain reaction

Nucleic acid identification by PCR is a rapid, sensitive and specific tool for the detection of *Mycobacterium tuberculosis*.[28] 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 IS*6110* and *hsp*65 kDa yield a sensitivity of 42-100 percent and a specificity of 85-100 percent.[29] Sensitivity of PCR was achieved when *devR* and IS*6110* test results were combined; the sensitivity and specificity values were 83 percent and 94 percent respectively in pleural fluid.[30]

A cross-sectional study was performed in a total of 179 body fluid samples.[29] All specimens were analyzed for AFB smear, ADA activity (by a method based on the Berthlot reaction) and multiplex PCR using amplicons such as IS*6110*, *dna*J gene and *hsp*65 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 fluids.

Another study collected samples of 67 consecutive patients with large pericardial effusions.[31] Sensitivity and specificity with a cut-off value of 40 U/L for diagnosis of tuberculous pericarditis were 83 percent and 78 percent, respectively, compared to PCR which sensitivity and specificity were 75 percent and 100 percent respectively. 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).

Comparison of the performance of adenosine deaminase assay using Diazyme® 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 time.[6] The modified Giusti method including the Berthelot reaction is developed in order to obtain better results. Despite being considered efficient, this method still has limitations; it requires reagent preparation; it is performed manually; readings are taken using a spectrophotometer and all steps are performed in-house, that is, by each laboratory individually. Therefore, there is a lack of standardization and it does not allow this assay to be used as a good diagnostic test.[32]

An automated method (Diazyme® 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 study.[33] 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® commercial kit), versus 9 U/L (for both methods) in CSF samples. Summary cut-off values of ADA from various fluids and references are shown in Table 1.

CONCLUSIONS

ADA is an enzyme 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 hospitals which need less equipment than PCR. In addition, price is inexpensive 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 Thailand, where it is a high burden TB area. Further research regarding ADA in unequivocal area is needed, such as how to improve specificity, minimize false positive and choose the suitable cut-off value.

Table 1. Summary of adenosine deaminase cut-off values by various fluids.

Body Fluid	Giusti and Modified Giusti method (U/l)	Diazyme® commercial kit (U/l)
Pleural effusion	40[12, 33]	3011, 13, 33
Ascites	30[16, 19]	No study
Pericardial effusion	40[27, 31, 34, 35]	No study (may use 30 which apply from pleural effusion)
Cerebrospinal fluid	6[376], 9[33], 8.5[25], 15.5[24]	9[33]

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