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Adenosine Deaminase: An Aid to Diagnose Tuberculosis

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The present study was carried out to determination of serum Adenosine deaminase (ADA) activity in tuberculosis. ADA has two main isoenzymes, ADA-1 and ADA-2 with different optimal pH, Michaelis constants and relative substrate specificity pattern. ADA-1 isoenzyme is found in all cells, with the highest concentration found in lymphocytes and monocytes, where as ADA-2 isoenzyme appears to be found only in monocytes. ADA-2 is the predominant isoforms in tuberculous pleural effusion, counting 88% of total ADA activity, where as ADA-1 is elevated in empyema, accounting for 70% of total activity. Determination of individual ADA isoenzymes and their ratio could increase the overall diagnostic value of ADA determination in tuberculosis.

Key words: Adenosine deaminase, tuberculosis, ADA-1 and ADA-2 isoenzyme, serum, pleural fluid, cerebrospinal fluid
Introduction

Tuberculosis as a chronic granulomatous disease caused by *Mycobacterium tuberculosis* with protean manifestations, involving most commonly the lung but all other systems as well. It is a re-emergent killer, threatening to assume serious population all over the world. The World Health Organization recently estimated that during the decade 1990 to 1999, approximately 90 million new cases of tuberculosis would occur worldwide (Raviglione et al., 1995).

The problem in diagnosing tuberculosis is that no symptom or sign is exactly typical of it. The presence of infection in the body does not necessarily mean disease. From the disease point of view, recovering the bacilli from patient’s specimen (by smear/culture) is specific but not sensitive. The AIDS epidemic has reminded us of importance of identifying tuberculosis and treating it (Berger and Mejia, 1973). It has been proved that the patients with tuberculosis can have a negative tuberculin skin test (Berger and Mejia, 1973) and plural fluid culture results can be positive in <25% cases (Scharer and W Clement, 1968). The traditional answer to this clinical problem is to perform needle pleural biopsy for both histological study and culture, which can lead to diagnosis of tuberculosis 86% of the time (Bueno et al., 1990). The needle aspiration cytology of lymph nodes and intrathoracic lesions reportedly have false positive of 7% and false negative of 23% in identifying the disease and more over is invasive technique (Lau et al., 1990; Parsad et al., 1993). Routine culture of mycobacterium in solid Lowenstein Jensen medium may take 6-8 weeks but time can be reduced to 2 weeks by using radiometric technique (Gupta et al., 1995; Schluger and Rom, 1994; Jimenez et al., 2002). These procedures, combined with cultures of body fluid and sputum provide the microbiological conformation of *Mycobacterium tuberculosis* as often as 90% of the time (Seibert et al., 1991). Hence the significant minority of the patients (10-20%) will not have positive results or granulomas on biopsy specimen.

Four relatively new techniques have been reported to help make the diagnosis of tuberculosis: adenosine deaminase (ADA), lysozyme, interferon gamma and polymerase chain reaction. Polymerase chain reaction has relatively low sensitivity in body fluids (42 to 81%) (de Lassence et al., 1992; Querol et al., 1995; Villena et al., 1998) and is fairly expensive. Moreover it gives positive result for mycobacterium in patients with history of treated tuberculosis. Nontuberculous organisms are detected and negative PCR makes the diagnosis very unlikely. The sensitivity of an elevated interferon level appears better (89-99%) (Kataria et al., 2001 and Villena et al., 1996) but relatively only few studies of its use have been reported and the assay is expensive and can not be done in routine laboratory. Lysozyme pleural fluids to serum ratios have been reported to improve the sensitivity of body fluid ADA (Villena et al., 1996). The test with most data to support its use is the serum/body fluid total ADA and its isoenzymes.

In 1970, serum ADA activity was first used as a serological diagnostic marker for lung cancer (Nishihara et al., 1970). It was not until 1978, when ADA was found to be useful in diagnosing tuberculous pleurisy (Piras et al., 1978; Ocana et al., 1983; Pettersson et al., 1984; Fontan-Bueso
et al., 1988; Porcel and Vives, 2002). Adenosine deaminase is an enzyme involved in purine catabolism and is responsible for the conversion of adenosine to inosine and ammonia. It is also involved in proliferation and differentiation of lymphocytes, particularly T subtypes. There are several isoforms of ADA, but the prominent ones are ADA-1 and ADA-2, which are located on different gene loci (Hirschhorn et al., 1980) and have different optimal pH, Michaelis constants and relative substrate specificity pattern. ADA-1 isoenzyme is found in all cells, with highest concentration in lymphocytes and monocytes, whereas ADA-2 isoenzyme appears to be found only in monocyte (Ungerer et al., 1992).

ADA analysis is simple and inexpensive colorimetric test that can be performed on body fluids. Increased ADA activity may be found in effusions due to a number of causes, including tuberculosis, bacterial infections, rheumatological disease and lymphoproliferative disorders (Pettersson et al., 1984). Determination of the individual ADA isoenzymes could help in distinguishing between the increased activity in effusions especially between tuberculosis and parainfective causes (Ungerer et al., 1992).

Numerous studies have suggested the diagnostic significance of increased adenosine deaminase (ADA) levels in different body fluids i.e. serum, pericardial fluid, cerebrospinal fluid, pleural fluid and ascitic fluid for different conditions of tuberculosis.

**Serum**

Several reports have suggested the used of serum ADA levels for the diagnosis of pulmonary tuberculosis (Collazos et al., 1998; Baganah et al., 1990; Bansal et al., 1991; Segura et al., 1989; Shibagaki et al., 1996; Bhargava et al., 1990; Conde et al., 2002). The serum ADA levels decrease to normal levels after one month of the initiation of effective treatment (Ida et al., 1990; Ishii et al., 1997). Highest usefulness of serum ADA is expected in those patients who have higher serum ADA values at presentation. The decrease in serum ADA levels could be due to the normalization of altered lymphocytes turnover induced by tuberculosis. The measurement of serum ADA activity was useful diagnostic tool in childhood pulmonary tuberculosis. The significantly (P<0.05) elevated serum ADA values (74.06±18.5 U/L) were found in children with pulmonary tuberculosis than the healthy children (40.36±12.0 U/L) (Kuyucu et al., 1999). Children with different forms of tuberculosis like pulmonary, military, neurotuberculosis, abdominal and ostearticular tuberculosis have significantly (P<0.05) serum ADA values than the healthy individuals (Mishra et al., 2000). Abnormally high levels of serum ADA activity in all the patients with pulmonary tuberculosis indicated the serum ADA is good diagnostic tool for tuberculosis (Ida et al., 1990). Serum ADA was found a selective marker of Immune stimulation in tuberculosis but not in cancer when compared the serum ADA activity of pulmonary tuberculosis patients with the patients of lung cancer pre-treatment and healthy Individuals (Kelbel et al., 1995). With cutoff values of 30 U/L the specificity, sensitivity, positive predictive values and negative
predictive value of the serum ADA was found to be 90, 87, 90 and 66.5%, respectively (Lakshmi et al., 1992).

ADA isoenzymes have been suggested to increase the overall diagnostic value of ADA determination (Ungerer et al., 1994; Gorguner et al., 2000; Gakis, 1995; Gakis, 1996; Gakis et al., 1991; Ungerer et al., 1988 and Kurata et al., 1992). ADA isoenzyme pattern appears to be a reflection of the difference in immune response and of the corresponding predominant cell population in the body fluid. One study reported significantly (P<0.05) higher activity of total ADA, ADA-1 and ADA-2 in the sera of patients with pulmonary tuberculosis than those of healthy persons. Close correlation between the activity of ADA-2 and lymphocyte subpopulation in pulmonary tuberculosis was found and levels of the activity of total ADA, ADA-1 and ADA-2 decreased significantly (P<0.05) in the sera of the patients after three months of effective treatment (Ishii et al., 1997). The work in our lab supports above findings that the levels of serum ADA and ADA-2 were higher in the pulmonary tuberculosis patients and decreased to its normal levels after 4 weeks of effective treatment (unpublished data). The strong relationship between the ADA-2 and neutrophil/lymphocyte ratio (r=0.98) enhances the specificity and sensitivity of the test.

**Pericardial effusion**

It is difficult to establish a definitive bacteriological diagnosis of tuberculous pericarditis because of several factors. The most difficult diagnostic case involves the patient with pericarditis is whom the PPD (purified protein derivative) test is positive but tubercle bacilli are demonstrated in pericardial fluid by histological examination of pericardium or elsewhere in the body in the absence of another cause of pericarditis (Sagrista-Sauleda et al., 1988; Martinez-Vasquez et al., 1986; Koh et al., 1993; Desi, 1979 and Fowler, 1991). Because of the difficulty in isolating the causative organism, pericardial tuberculosis is hardly diagnosed (Komsuo and Galidela, 1995). A few reports about the use of ADA in the diagnosis of tuberculous pericarditis have been reported (Martinez-Vasquez et al., 1986; Koh et al., 1993 and Desi, 1979). The concentration of adenosine deaminase in T-lymphocytes is inversely proportional to the their degree of differentiation. Since mycobacterium tuberculosis invades the pericardial cavity chiefly through rupture of subpericardial caseous lesions. Bacillus antigens stimulate lymphocytes, which in turn release certain lymphokines that activate macrophages against the mycobacterium and influence the formation of granulomas (Kuralay and Comlecki, 1998).

Recent reports in patients with TB pericarditis have shown that ADA levels in pericardial fluid are diagnostically useful in early diagnosis of TB pericarditis, particularly when the results of other clinical and laboratory tests are negative (Dogan et al., 1999 and Koh et al., 1994). Using a cutoff value of ADA activity of 40U/L, the sensitivity and specificity of ADA testing in one series of nine proven patients and five patients with suspected TB pericarditis were 93 and 97%,
respectively (Koh et al., 1994). In another series, there was a positive correlation between high pericardial adenosine deaminase levels and the subsequent development of constrictive pericarditis. Therefore, the ADA value is a significant prognostic indicator for the development of constrictive pericarditis in tuberculous pericarditis (Komsuo and Galidela, 1995). Another study reported 100% sensitivity and 91% specificity with 60 U/L cutoff value of ADA activity in pericardial fluid (Chaturvedi et al., 2000).

Cerebrospinal fluids

Meningeal tuberculosis, the most fearsome manifestation of tuberculosis, presents with fever, headache and altered consciousness. It can easily be missed in insidious forms, particularly in HIV infected patients without evidence of tuberculosis outside the central nervous system (Fernandez et al., 1999).

Tuberculosis meningitis is a common cause of morbidity and mortality. With the emergence of AIDS, there is renewed interest in tuberculosis all over the world. The cytological analysis of cerebrospinal fluid is the cornerstone for diagnosis but there are diagnostic difficulties in differentiating tuberculosis meningitis from non-tuberculous meningititis. Acid-fast bacilli are seen in less than a quarter of patients and mycobacterium culture is positive in 45-90% of cases. Polymerase chain reaction (PCR), though highly sensitive to identify mycobacterial DNA, however it is costly, not widely available and problem with its specificity have been encountered (Eintracht et al., 2000).

The determination of ADA activity in cerebrospinal fluid is a reliable and valuable adjunct in differentiating tuberculous from non-tuberculous meningitis (Eintracht et al., 2000). The study consisted of 11 patients with tuberculous meningitis, 9 with cryptococcal meningitis, 13 with acute bacterial meningitis, 9 with aseptic meningitis and 19 with normal lumber puncture. Using a cut off value of total CSF adenosine deaminase activity of >6 U/L they found the 91% sensitivity and 94% specificity in all the patients by detecting total ADA in tuberculous meningitis and 77.3% compared with those with cryptococcal meningitis or acute meningitis. Results indicated that ADA of cerebrospinal fluid could differentiate patients with tuberculous meningitis from those with aseptic meningitis or a normal lumber puncture. However, there was overlap for values of ADA between patients with tuberculous meningitis and those with cryptococcal meningitis or acute bacterial meningitis.

Using cutoff values of total cerebrospinal fluid ADA activity of 8-20 U/L, various studies have shown the sensitivities of 44-100% and specificities of 75-99% (Eintracht et al., 2000; Gambhir et al., 1999 and Pettersson et al., 1992). False positive results have been reported with lymphomatous meningitis (Pettersson et al., 1992). These different findings on sensitivity and specificity of ADA in cerebrospinal fluid may be explained by different disease profiles, time of presentation and ages of patients, as lower values of ADA have been reported from the children with tuberculous meningitis (Donal et al., 1996).
The problem of overlapping values of ADA from tuberculous meningitis and cryptococcal meningitis could be overcome by measuring the activity of ADA-2 isoenzymes. The proportion of ADA-2 isoenzyme of >80% seems to be a reliable marker of tuberculous meningitis yielding a sensitivity of 100% and specificity of 86.4%. Among all types of meningitis only other category with more than >80% ADA-2 was cryptococcal meningitis, which can be easily diagnosed on Indian Ink staining and serology (Eintracht et al., 2000).

Pleural effusion

Tuberculous pleural effusion is thought to result from delayed hypersensitivity reaction that occurs in response to the presence of mycobacterial antigens in pleural space (Leibowitz et al., 1973). These myobacterial antigens may gain access to pleural space from the rupture of a small, subpleural caseous focus (Stead et al., 1995). Tuberculous pleural effusion has been described as an acute granulomatous pleuritis occurring as a sequel to recent tuberculous infection in young adults and children who usually do not have roentgenographically apparent parenchymal tuberculosis (Levine et al., 1968; Stead et al., 1968; Khan et al., 1977; Sibley, 1950; Frostad, 1944). However it is now known that tuberculous pleural effusion may occur in older adults and patients with classic reactivation tuberculosis (Epstein et al., 1987).

Diagnosis of tuberculous pleural effusion is difficult and remains a common clinical challenge because all the classic findings of a lymphocytic exudative pleural effusion, pleural granulomata, and cutaneous sensitivity to pleural protein derivative (PPD) may not be present or available to the clinician. Pleural fluid and pleural biopsy, which grow Mycobacterium Tuberculosis, have the highest specificity, but their diagnostic utility is limited by their sensitivity. As a result, pleural biopsy and pleural fluid culture findings are negative (Bothamley, 1995; Roth, 1999).

Adenosine deaminase (ADA) has gained increasing popularity as a diagnostic test for tuberculous pleuritis since 1973, especially in countries where the prevalence of tuberculosis is high. It carries 90-100% sensitivity (Raintawan et al., 1999; Valdes et al., 1996; Burgess et al., 1996; Aggarwal et al., 1999) and is inexpensive (Roth, 1999). The ADA measurement is used commonly in European and Asian countries where there is a higher incidence of tuberculosis (Ferrer et al., 1996). In regions with a high prevalence of tuberculosis and in patient groups with a low risk of other causes of pleurisy, especially among patients with a low probability of neoplasia who may also have high ADA level, the positive predictive value of this marker (ADA) is increased (Burgess et al., 1995). The problem with using the ADA assay in a population with a lower incidence of tuberculosis is that the positive predictive value decreases, so there is a higher likelihood that a test would give a false-positive results (San Jose et al., 1999; Sharma et al., 2001). One study showed that ADA level, especially when combined with differential cell counts and lymphocyte/neutrophil ratios, remains a useful test in the diagnosis of tuberculous pleuritis. When the lymphocytes to neutrophils ratio (L/N) >0.75 was considered
together with the ADA activity >50 U/L, the result improved considerably for the diagnosis of tuberculous pleuritis. The pleural fluid ADA values can be used in conjunction with cell counts: 1; A lymphocyte exudate (L/N ratio >0.75) with a high ADA value (>50 U/L) is highly suggestive of TB pleurisy, 2; A lymphocyte exudate with low ADA value (<50 U/L) is suggestive of non hematologic malignancies and 3; A neutrophilic exudate (L/N <0.75) with a high ADA concentration (>50 U/L) is suggestive of parainfective effusions (Valdes et al., 1995).

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 Giusti method is used (Gelani et al., 1999; Jimenez et al., 2002; Hamada et al., 1998). The reported cutoff value for ADA (total) varies from 47 to 60 U/L (Perez-Rodriguez et al., 1999; Valdes et al., 1993 and Reechalpichitkul et al., 2001).

A study on 350 patients with pleural effusion revealed that the isoenzyme ADA-2 is elevated significantly in pleural fluid with activated lymphocytes, such as tuberculosis. Levels of ADA-2 above 40 U/L indicate probable tuberculosis. ADA-2 is a more efficient diagnostic marker of tuberculous pleurisy than total ADA activity (Perez-Rodriguez et al., 2000). With diagnostic threshold of 40 U/L, ADA-2 has 100% sensitivity and 96% specificity for early diagnosis of tuberculous pleurisy. False positive results can occur with lymphoma, rheumatoid arthritis and rarely with adenocarcinoma.

The isoenzyme ADA-1 is elevated in the presence of empyema and parapneumonic effusions (Titarenko et al., 2002; Carstens et al., 1998). In cases of suspected false negative or positive ADA levels, (frequent in empyema, lymphoma and other malignant effusions and in areas of low tuberculosis prevalence), level of ADA-1/ADA (total) ratio is a good parameter (Valdes et al., 1993). A proportion of ADA-1/ADA (total) <0.42 is a good indicator of tuberculosis, with an accuracy of 99%, a sensitivity of 100% and a specificity of 98.6% (Valdes et al., 1993) but high ADA activity with ADA-1/ADA (total) ratio >0.45 is indicative of malignancy or emphysema.

The ADA value in the HIV positive patients did not differ significantly from those in the HIV-negative patients, contrary to what has been previously suggested by findings in a much smaller group of patients (Canbolat et al., 1999).

Asctic fluid

Peritoneal tuberculosis results from reactivation of latent tuberculosis foci in the peritoneum, seeded previously from haematogenous spread from primary infection in the lungs. Tuberculosis peritonitis is associated with active tuberculosis in 4 to 21% of cases (Marshall, 1993). Tuberculosis peritonitis is divided into three types (Dwividi et al., 1990) the wet type is the most common type and is characterized by large amounts of free or loculated viscous fluid; second the fibrotic-fixed type is less common and has large omental masses, matted and tethered bowel loops and mesentery and occasionally loculated ascities; third the dry or plastic type is characterized by caseous nodules, fibrous peritoneal reaction and dense adhesion (Jadvar et al., 1997).
Tuberculous peritonitis is a significant problem in the countries with high incidence of tuberculosis. Peritoneal involvement in tuberculous infection is frequently associated with cirrhosis and immunodeficient states (Alvarez and McCabe, 1984; Burack et al., 1960; Karmy et al., 1977; Borhanmanesh et al., 1986 and Gilinsky et al., 1983). The available diagnostic tests for tuberculous peritonitis include paracentheses with acid-fast smears and culture, laparoscopy with directed biopsy, blind percutaneous peritoneal biopsy and diagnostic laparotomy. However, laparoscopy is invasive, expensive and associated with complications. It may also be used up to 3% of patients (Lado-Lado et al., 2002). A new test measuring adenosine deaminase activity in the ascitic fluid has been used with promising results (Dwividi et al., 1990). Ascitic fluid ADA activity has been proposed as a useful diagnostic test for diagnosis of tuberculous peritonitis. Various reports have suggested 100% sensitivity for diagnosis of peritoneal tuberculosis with specificities in the range of 92-100% (Dwividi et al., 1990; Martinez-Vazquez et al., 1986; Fernandez-Rodriguez et al., 1991; Ribera et al., 1991; Volg et al., 1997, Binder, 1997 and Gupta et al., 1992). A cutoff value of >33 U/L eliminates false positive tests resulting from cirrhosis or malignancy (Dwividi et al., 1990; Fernandez-Rodriguez et al., 1991).

In countries with high incidence of tuberculosis and in high-risk patients, measurement of ADA in ascitic fluid, should be used as a useful screening test for tuberculosis (Binder, 1997 and Gimenez et al., 1992) but populations with low prevalence of tuberculosis and high prevalence of cirrhosis, ascitic fluid ADA activity has good accuracy but poor sensitivity and imperfect specificity (Hildebrand et al., 1996).

ADA-2 activity in ascitic fluid presents similar features to that of total ADA and can provide additional information with good sensitivity (83.3%) and specificity (97.3%) in diagnosis of tuberculous peritonitis (Demir et al., 2001). Higher values of ADA-2 were reported for the peritoneal tuberculosis than in peritoneal carcinomatosis (56.8 vs. 19.6 U/L, respectively). In the peritoneal tuberculosis ADA-2 activity similar to total ADA correlates with ascitic fluid total protein; so ADA-2 should be used with caution when cirrhosis is associated with the TB peritonitis.

In the regions with high incidence of tuberculosis and diagnostic procedures are expensive, ADA appears to a useful marker for early diagnosis of tuberculosis. The sensitivity and specificity of ADA depends on the prevalence of tuberculosis in the population. The differences between the reported ADA levels are due to the different methods of ADA measurement. The isoforms of ADA, especially the ADA-1 and ADA-2 are found in all the cells with highest concentration in monocytes and lymphocytes but ADA-2 found to be the predominant isoforms in tuberculosis accounting for 80-90% of the activity. Where as ADA-1 elevates in empyema, accounting for 70% of the activity. Determination of individual ADA isoenzymes and ratio of the isoenzymes could help to differentiate the various causes of increased ADA activity. The ADA isoenzymes increase the overall diagnostic value of ADA determination in tuberculosis.
References


