Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

J. Med. Sci., 3 (1): 30-45
January-February, 2003

Adenosine Deaminase: An Aid to Diagnose Tuberculosis

Salman Alrokayan

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

Department of Biochemistry, College of Sciences, King Saud University, P.O. Box 2455, Riyadh-11451, Kingdom of Saudi Arabia

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publish original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Salman Alrokayan
Department of Biochemistry
College of Sciences
King Saud University
P.O. Box 2455,
Riyadh-11451,
Kingdom of Saudi Arabia

Email:srokayan@ksu.edu.sa

ANSInet
Asian Network for Scientific Information

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 Mclement, 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. Non-viable 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, neurotuberculoma, abdominal and osteartícular 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 fuids

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 AID, there is renewed interest in tuberculosis all over the world. The cytochemical analysis of cerebrospinal fluid is the cornerstone for diagnosis but there are diagnostic difficulties in differentiating tuberculosis meningitis from non-tuberculous meningitis. 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; Gambbir ***et al.***, 1999 and Pettersson ***et al.***, 1992). False positive results have been reported with lymphomatous meningitis Petterson ***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 vales 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 crptococcal meningitis could be overcome by measuring the activity of ADA-2 isoenzymes. The proportion of ADA-2 isoenzyme of >80% seems to be 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 myobacterial 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 caries 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 Reechaipichitkul ***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).

Ascitic 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 bowl 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 paracenthesis 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; Voigt ***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

Aggarwal, A.N., D. Gupta and S.K. Jindal, 1999. Diagnosis of tuberculous pleural effusion. Indian J. Chest. Dis. Allied Sci., 41: 89-100.

Alvarez, S. and W.R. McCabe, 1984. Extrapulmonary tuberculosis revisited: A review of experience at Boston city and other hospitals. Medicine (Baltimore), 63: 25-55.

Berger, H.W. and E. Mejia, 1973. Tuberculous pleurisy. Chest, 63: 88-92.

Bueno, C.E., M.G. Clemente, B.C. Gastro, L.M. Martin, S.R. Ramos and A.G. Panizo, 1990. Cytologic and bacteriologic analysis of fluid and pleural biopsy specimens with cape's needle. Arch. Intern. Med., 150: 1190-1194.

Baganha, M.F., A. Pego, M.A. Lima, E.V. Gaspar and A.R. Cordeiro, 1990. Serum and pleural adenosine deaminase: correlation with lymphocyte population. Chest, 97: 605-610.

Bansal, S.K., R.P. Singh, R.K. Narang, L.D. Joshi, A. Bansal and A.K. Agrawal, 1991. Serum adenosine deaminase in pulmonary tuberculosis; malignancy and non-tubercular respiratory tuberculosis. Indian J. Chest. Dis. Allied Sci., 33: 189-193.

Bhargava, D.K., M. Gupta, S. Nijhawan, S. Dasarathy and A.K. Kushwaha, 1990. Adenosine deaminase (ADA) in peritoneal tuberculosis: Diagnostic value in ascitic fluid and serum. Tubercle., 71: 121-126.

Bothamley, G.H., 1995. Tuberculosis pleurisy and adenosine deaminase. Thorax, 50: 593-594.

Burgess, L.J., F.J. Maritz, I. Le-Roux and F. Taljoard, 1996. Combined use of pleural adenosine deaminase with lymphocyte/neutrophil Ratio. Increased specificity for the diagnosis of tuberculous pleuritis. Chest, 109: 414-19.

Burgess, L.J., F.J. Maritz, I. Le-Roux and J.J. Taljaard, 1995. Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax, 50: 672-674.

Burack, W.R. and R.M. Hollister, 1960. Tuberculosis peritonitis. A study of 47 proved cases encountered by general medicine unit in twenty-five years. Am. J. Med., 28: 510-523.

Binder, H.J., 1997. Tuberculous peritonitis: can ADA keep the laparoscope away? Gastroenterology, 113: 687-689.

Borhanmanesh, F., K. Hekmet, K. Vaezzadeh and H.R. Rezai, 1986. Tuberculous peritonitis. Prospective study of 32 cases in Iran. An. Inter. Med., 76: 567-572.

Collazos, J, P. Espana, J. Mayo, E. Martinez and F. Izquierdo, 1998. Sequential evaluation of serum adenosine deaminase in patients treated for tuberculosis. Clin. Invest., 114: 432-435.

Conde, M.B., S.R. Marinho, F. Pereira-Mde, J.R. Lapae-Silva, M.H. Saad, C.L. Sales, J.K. Ho and A.L. Kritski, 2002. The usefulness of serum adenosine deaminase 2 (ADA-2) activity in adults for the diagnosis of pulmonary tuberculosis. Respir. Med., 96: 607-610.

Chaturvedi, P., J. Vaidya, B.C. Harinath and B. Pramanick, 2000. Adenosine deaminase levels in cerebrospinal fluid and serum in diagnosis of tuberculosis of tubercular meningitis. J. Trop. Pediatr., 46: 378-379.

Carstens, M.E., L.J. Burgess, F.j. Maritz and J.J. Taljaard, 1998. Isoenzymes of adenosine deaminase in pleural effusions: a diagnostic tool. Int. J. Tuberc. Lung Dis., 2: 831-835.

Canbolat, O., S. Ulusdoyurian, G. Ozgen, I. Ceythan, F. Gumuslu and A. Akbay, 1999. The comparison of adenosine deaminase activity values with polymerase chain reaction results in patients with tuberculosis. J Clin Lab Anal., 13: 209-212.

de Lassence, A., D. Lecossier, C. Pierre, J. Cadranel, M. Stern and A.J. Hance, 1992. Detection of mycobacterial DNA in pleural fluid from patients with tuberculous pleurisy by means of the polymerase chain reaction: comparison of two protocols. Thorax, 47: 265-269.

Desi, H.N., 1979. Tuberculous pericarditis: a review of 100 cases. S. Afr. Med. J. 55: 877-880.

Dogan, R., M. Demircin, A. Sarigul, G. Ciliv and A.Y. Bozer, 1999. Diagnostic value of adenosine deaminase activity in pericardial fluids. J. Cardiovasc. Surg., 40: 501-4.

Donal, P., C. Malan, A. van-der-Walt and J.F. Schoeman, 1986. The simultaneous determination of cerebrospinal fluid and plasma adenosine deaminase activity as a diagnostic aid in tuberculous meningitis. S. Afr. Med. J., 69: 505-507.

Dwividi, M., S.P. Misra, V. Misra and R. Kumar, 1990. Value of adenosine deaminase estimation in the diagnosis of tuberculous ascites. Am. J. Gastroenterol., 85: 1123-1125.

Demir, K., A. Okten, S. Kaymakuglu, D. Dincer, F. Besisik, U. Cevikbas, S. Ozdil, G. Bostas, Z. Mungan and Y. Cakaloglu, 2001. Tuberculous peritonitis- report of 26 cases, detailing diagnostic and therapeutic problems. Eur. J. Gastroentrol. Hepatol., 13: 581-585.

Eintracht, S., E. Silber, P. Sonnenberg, H.J. Koornhof and D. Saffer, 2000. Analysis of adenosine deaminase isoenzyme-2 (ADA-2) in cerebrospinal fluid in the diagnosis of TB meningitis. J, Neurol. Neurosurg. Psychiatry., 69: 137-138.

Epstein, H.W., L.R. Kline, S.M. Albelda and W.T. Miller, 1987. Tuberculous pleural effusions. Chest, 91: 106-109.

Fontan-Bueso, J., H. Verea-Hernando, J.P. Garcia-Buela, L. Dominguez-Juncal, M.T. Martin-Egana and M.C. Montero-Martinez, 1988. Diagnostic value of simultaneous determination of pleural adenosine deaminase and pleural lysozyme/serum lysozyme ratio in pleural effusions. Chest, 93: 303-307.

Fowler, N.O., 1995. Tuberculous pericarditis. JAMA., 266: 99-103.

Fernandez, E., S. Garcia, F. Gutierrez, G. Ocio, L. Rodrigo and S. Riestra. Diagnostic value of adenosine deaminase Isoenzymes in ascitic fluid. Am. J. Gastroentrol., 94: 3658-3659.

Frostad, S., 1944. Tuberculosis in cipiens: a clinical roentgenological investigation of earliest forms of pulmonary tuberculosis with special view to its relation to primary infection. Acta Tuberc. Scand., 1: 269-280.

Ferrer, J.S., X.G. Munoz, R.M. Orriols, R.W. Light and F.B. Morell, 1996. Evolution of idiopathic pleural effusion: a prospective, long term-follow up study. Chest, 109: 1508-1513.

Fernandez-Rodriguez, C.M., B.S. Perez-Arguelles and L. Ledo, 1991. Ascites adenosine deaminase activity is decreased in tuberculous ascites with low protein content. Am. J. Gastroenterol., 86: 1500-1503.

Gupta, S., R. Bhatia and K.R. Datta, 1995. Serodiagnosis of tuberculosis. J. Commun. Dis., 27: 208-214.

Gorguner, M., M. Cerci and I. Gorguner, 2000. Determination of adenosine deaminase activity and its isoenzymes for diagnosis of pleural effusions. Respirology, 5: 321-324.

Gakis, C., 1995. Adenosine deaminase in pleural effusions. Chest, 107: 1773.

Gakis, C., 1996. Adenosine deaminase (ADA) isoenzymes ADA1 and ADA2 diagnostic and biological role. Eur. Respir. J., 9: 632-633.

Gakis, C., G.M. Calia, A.G.V. Naitana, A.R. Ortu and A. Contu, 1991. Serum and pleural adenosine deaminase activity. Correct interpretation of the findings. Chest, 99: 1555-1556.

Gupta, V.K., S. Mukherjee, S.K. Dutta and P. Mukherjee, 1992. Diagnostic evaluation of ascitic adenosine deaminase tubercular peritonitis. J. Assoc. Physicians., 40: 387-9.

Gimenez-Roca, A., X. Xiol, J. Castellote, M. Sanchez, C. Iglesias, J.M. Ramon and L. Casais, 1992. The value of ADA in peritoneal tuberculosis. Rev. ESP. Enferm. Dig., 82:32-4.

Gelani, D.R., F.S. Parikh, A.S. Hakim and J.V. Pai-Dhungat, 1999. Diagnostic significance of immunoglobulins and adenosine deaminase in pleural effusion. J. Assoc. Physicians India., 47: 787-790.

Gambbir, I.S., M. Mehta, D.S. Singh and H.D. Khanna, 1999. Evaluation of CSF-adenosine deaminase activity in tubercular meningitis. J. Assoc. Physician India., 47: 192-4.

Gilinsky, N.H., I.N. Marks and R.E. Kottler and S.K. Price, 1983. Abdominal tuberculosis. A 10 years review. S. Afr. Med. J., 64: 849-857.

Hirschhorn, R. and H. Ratcch, 1980. Isozymes of adenosine deaminase: In M.C. Rattazzi, J.G. Scandalios and G.S. Witt. Isozymes: current topics in biological and medical research (vol 4), New York, NY: Liss, pp: 130-157.

Hamada, T., M. Sanaka, E. Hata and T. Hasegawa, 1998. Pleural adenosine deaminase levels in tuberculous pleurisy-its diagnostic performance under different prevalences in different age of population. Jpn. J. Thorac. Cardiovasc. Surg., 46: 51-57.

Hillebrand, D.J., B.A. Runyon, W.G. Yasmineh and G.P. Rynders, 1996. Ascitic fluid adenosine deaminase insensitivity in detecting tuberculous peritonitis in the united states. Hepatology, 24:1408-412.

Ida, T., S. Taniai, M. Nitta, J. Shimase, K. Makiguchi, I. Miyasato, M. Chida, H. Shinada, Y. Shinohara and H. Tachi, 1990. Serum adenosine deaminase activity in patients with active pulmonary tuberculosis. Kekkaku, 65: 477-481.

Ishii, S., H. Nagasawa, H. Tai, Y. Noda, K. Akiyama, H. Takada, N. Okajima and H. Okano, 1997. Relationship between activity of serum adenosine deaminase including its isozymes and lymphocyte subpopulation in patients with pulmonary tuberculosis. Kekkaku, 72: 153-159.

Jimenez, D., E. Perez-Rodriguez, G. Diaz, L. Fogue and R.W. Light, 2002. Determining the optimal number of specimens to obtain the needle biopsy of the pleura. Respir. Med., 96: 14-17.

Jimenez, D., G. Diaz and E. Perez-Rodriguez, 2002. Diagnosis of pleural tuberculosis. Chest, 121: 1005-1015.

Jadvar, H., R.E. Mindelzun,, E.W. Olcott and D.B. Levitt, 1997. Still the great mimicker: abdominal tuberculosis. AJR. Am. J. Roentgenol., 168: 1455-1460.

Kataria Y.P. and I. Khurshid, 2001. Adenosine deaminase in the diagnosis of tuberculous pleural effusion. Chest, 120: 334-336.

Kuyucu, K., C. Karakurt, E. Bialaloglu, T. Karacan and T. Tezic, 1999. Adenosine deaminase in childhood pulmonary tuberculosis: diagnostic value in serum. J. Trop. Paediatr., 45: 245-247.

Kelbel, C., B. Stumpf, W. Schmidt, E. Wetzel, J. Lorenz, 1995. Role of serum adenosine deaminase as a parameter of tuberculosis. Pneumologie, 49:684-688.

Kurata, N., M. Nihara, K. Matsubayashi, K. Kase and M. Haneda, 1992. Activities and isozymes of adenosine deaminase and lactate dehydrogenase in tuberculous effusion with special reference to ***Mycobacterium Tuberculosis***. Rinsho. Byori., 40: 670-672.

Komsuo, A. and B. Galidela, 1995. The diagnostic and prognostic value of adenosine deaminase in tuberculous pericarditis. Eur, Heart J., 16: 1126-30.

Kuralay, F. and A. Comlecki, 1998. Adenosine deaminase activity: to be a useful marker in distinguishing pleural effusion due malignancy from tuberculosis. Biochem. Soc. Trans., 26: S163.

Koh, K.K., E.J. Kim, C.H. Cho, M.J. Choi, S.K. Cho, S.S. Kim, Kim, M.H.C.J. Lee, S.H. Jin and J.M. Kim, 1994. Adenosine deaminase and carcinoembryonic antigen in pericardial effusion diagnosis, especially in suspected tuberculous pericarditis. Circulation, 89: 2728-35.

Khan, M.A., D.M. Kovnat, B. Bachus, M.E. Whitcomb, J.S. Brod and G.L. Snider, 1977. Clinical and roentgenological spectrum of pulmonary tuberculosis in adults. JAMA, 62: 31-38.

Koh, K.K., M.J. Choi, C.H. Cho, C.J. Lee, J.M. Kim, S.K. Cho and S.S. Kim, 1993. Adenosine deaminase in pericardial aspiration: diagnostic significance in tuberculous pericarditis. J. Am. Coll. Cardiol., 21: 198A.

Karmy, W.W., J.M. O'Donoghue and J.H. Ostrow, 1977. The spectrum of tuberculous peritonitis. Chest, 72: 310-315.

Lau, S.K., W.I. Wei, C. Hsu and U.C. Engzell, 1990. Efficacy of fine needle aspiration cytology in diagnosis of tuberculous cervical lymphadenopathy. J. Laryngol. Octol., 104: 24-27.

Lakshmi, V., P.R. Rao, N. Joshi, P.N. Rao, 1992. Serum adenosine deaminase activity in bacillary or paucibacillary pulmonary tuberculosis. Indian J. Pathol. Microbiol., 35: 48-52.

Leibowitz, S., L. Kennedy and M.H. Lessof, 1973. The tuberculin reaction in pleural cavity and its suppression by antilymphocyte serum. Br. J. Exp. Pathol., 54:152-162.

Levine, H., P.B. Szanto and D.W. Cugell, 1968. Tuberculous pleurisy. Arch. Inter. Med. 122: 329-332.

Lado-Lado, F.L., B. Cabana-Gonzalez, M.J. Ferreiro-Regueiro, A. Cabacros- Ortiz-de-Barron and E. Donado-Budino, 2002. Tuberculous peritonitis. Ann. Med. Intern., 19: 296-298.

Martinez-Vazquez, J.M., I. Ocana, E. Ribera, R.M. Segura and C. Pascual, 1986. Adenosine deaminase activity in diagnosis of tuberculous peritonitis. Gut, 27: 1049-1053.

Mishra, O.P., S. Yusaf, Z. Ali, G. Nath and B.K. Das, 2000. Adenosine deaminase activity and lysozyme levels in children with tuberculosis. J. Trop. Paediatr., 46: 175-178.

Martinez-Vasquez, J.M., E. Ribera, I. Ocana, R.M. Segura, R. Serrat and J. Sagrista, 1986. Adenosine deaminase activity in tuberculous pericarditis. Thorax, 41: 888-889.

Marshall, J.B., 1993. Tuberculosis of gastrointestinal tract and peritoneum. Am. J. Gastroenterol., 88: 989-999.

Nishihara, H., H. Akedo, H. Okada and S. Hattori, 1970. Multiple enzyme pattern of serum adenosine deaminase by agar gel electrophoresis: an evaluation of diagnostic value in lung cancer. Clin. Chem. Acta, 30: 251-258.

Ocana, I., J.M. Martinez-Vazquez, R.M. Segura, T. Fernandez-De-Sevilla and J.A. Capdevila, 1983. Adenosine deaminase in pleural fluids: Test for diagnosis of tuberculous pleural effusion. Chest, 84: 51-53.

Parsad. R., S.K. Garg, P.K. Mukherji and P.K. Agarwal, 1993. Role of fine needle aspiration cytology in diagnosis of lymphoadenopathy. Indian J. Chest Dis. Allied Sci., 35: 27-29.

Piras, M.A., C. Gakis, M. Budroni and G. Andreoni, 1978. Adenosine deaminase activity in pleural effusions: an aid to differential diagnosis. Br. Med. J., 2: 1751-1752.

Pettersson, T., K. Ojala and T.H. Weber, 1984. Adenosine deaminase in the diagnosis of pleural effusion. Acta Med. Scand., 251: 299-304.

Porcel, J.M. and M. Vives, 2002. Adenosine deaminase levels in nontuberculous pleural effusions. Chest, 121: 1379-1380.

Petterson, T., M. Klockars, T.H. Weber, H. Somer, 1992. Diagnostic value of cerebrospinal fluid adenosine deaminase determination. Scand. J. Infect. Dis., 24: 121-122.

Perez-Rodriguez, E., I.J. Perez-Walton, J.J. Sanchez Hernandez, E. Pallares, J. Rubi, D. Jimenez-Castro and G. Diaz-Nuevo, 1999. ADA1/ADAp ratio in pleural tuberculosis: an excellent diagnostic parameter in pleural fluid. Respir. Med., 93: 816-821.

Perez-Rodriguez, E. and D. Jimenez-Castro, 2000. The use of adenosine deaminase and adenosine deaminase isoenzymes in diagnosis of tuberculous pleuritis. Curr. Opin. Pulm. Med., 6: 269-266.

Querol, J.M., J. Minguez, E. Garcia-Sanchez, M.A. Farga, C. Gimeno and J. Garcia-de-Lomas, 1995. Rapid diagnosis of pleural tuberculosis by polymerase chain reaction. Am. J. Respir. Crit. Care. Med., 152: 1977-1981.

Raviglione, M.C., D.E. Snider and A. Kochi, 1995. Global Epidemiology of Tuberculosis. JAMA, 273: 220-226.

Roth, B.J., 1999. Searching for tuberculosis in the pleural space. Chest, 116: 3-5.

Raintawan, P., P. Chaowalit, M. Wongsangiem, P. Rojanaraweewong, 1999. Diagnostic value of pleural fluid adenosine deaminase in tuberculous pleuritis with reference to HIV co-infection and a Bayesian analysis. Chest, 116: 97-103.

Reechaipichitkul, W., T. Kawamatawong, Y. Teerajetgul and B. Patjanasoontorn, 2001. Diagnostic role of pleural fluid adenosine deaminase in tuberculous pleural effusion. Southeast Asian J. Trop. Med. Public Health, 32: 383-389.

Ribera, E., J.M. Martinez-Vazquez, I. Ocana, I. Ruiz, J.G. Jiminez, G. Encabo, R.M. Segura and C. Pascual, 1991. Diagnostic value of ascites gamma interferon levels in tuberculous peritonitis: comparison with adenosine deaminase activity. Tubercle., 72: 193-197.

Stead, W.W., G.R. Kerby, D.P. Schleuter and and C.W. Jordahl, 1968. The clinical spectrum of primary tuberculosis in adults. Ann. Inter. Med., 68: 731-745.

Scharer, L. and J.H. Mclement, 1968. Isolation of tubercle bacilli from needle biopsy specimens of parietal pleura. Am. Rev. Respir. Dis., 97: 466-468.

Schluger, N.W. and W.N. Rom, 194. Current approaches to the diagnosis of active pulmonary tuberculosis. Am. J. Respir. Crit. Care. Med., 149: 264-267.

Seibert, A.F., J. Haynes, R. Middleton and B.B. John, 1991. Tuberculous pleural effusion: twenty-year experience. Chest, 99: 883-886.

Segura, R.M., C. Pascual, I. Ocana, J.M. Martinez-Vazquez, E. Ribera, I. Ruiz and M.D. Pelegri, 1989. Adenosine deaminase in body fluids: a useful diagnostic tool in tuberculosis. Clin. Biochem., 22: 141-148.

Shibagaki, T., Y. Hasegawa, H. Saito, S. Yamori and K. Shimokata, 1996. Adenosine deaminase isozymes in tuberculous pleural effusion. J. Lab. Clin. Med., 127: 349-352.

Sagrista-Sauleda, J., H.Y. Sohn, G. Permanyer-Miralda and T. Soler-Soler, 1988. Tuberculous pericarditis: ten-years experience with prospective protocol for diagnosis and treatment. J. Am. Coll. Cardiol., 11: 724-72.

Stead, W.W., A. Eichenholz and H.K. Strauss, 1955. Operative and pathologic findings in twenty-four patients with syndrome of idiopathic pleurisy with effusion, presumably tuberculosis. Am. Rev. Tuberc., 71: 473-502.

Sibley, J.C., 1950. A study of 200 cases of tuberculosis pleurisy with effusion. Am. Rev. Tuberc., 62: 314-323.

San-Jose, M.E, L. Valdes, M.J. Saavedra, J.M. De-Vega, D. Alvarez, J. Vinuela, P. Penela, J.M. Valle and R. Seoane, 1999. Lymphocyte population in tuberculosis pleural effusion. Ann. Clin. Biochem, 36: 492-500.

Sharma, S.K, V. Suresh, A. Mohan, P. Kaur, P. Saha, A. Kumar and J.N. Pande, 2001. A prospective study of sensitivity and specificity of adenosine deaminase estimation in the diagnosis of tuberculosis pleural effusion. Indian, J. Chest, Dis. Allied, Sci., 43: 149-155.

Titarenko, O.T., M.E.D'iakova, T.L. Perova, A.I. D'iakova and M.L.U. Popov, 2002. Informative value of adenosine deaminase and 2-deoxyadenosine deaminase in the diagnosis of tuberculous pleurisy. Klin. Lab. Diagn., 5: 11-14.

Ungerer, J.P.J. and S.M. Grobler, 1988. Molecular forms of adenosine deaminase in pleural fluids. Enzyme, 40: 7-13.

Ungerer, J.P.J., H.M. Oosthuizen, S.H. Bissbort and W.J. Vermaak, 1992. Serum adenosine deaminase: isoenzymes and diagnostic application. Clin. Chem., 38: 1322-1326.

Ungerer, J.P.J., H.M. Oosthuizen, J.H. Retief and S.H. Bissbort, 1994. Significance of adenosine deaminase activity and its isoenzymes in tuberculous effusions. Chest, 106: 33-37.

Villena, V., M.J. Rebollo, J.M. Aguado, A. Galan, A. Lopez-Encuentra, E. Palenque, 1998. Polymerase chain reaction for the diagnosis of pleural tuberculosis in immunocompromised and immunocompetent patients. Clin, Infect, Dis., 26: 212-214.

Villena, V., A. Lopez-Encuentra, J. Echave-Sustaeta, P. Martin-Escribano, B. Ortuno-de-Sol and J. Estenoz-Altro, 1996. Interferon-gamma in 388 immunocompromised and immunocompetent patients for diagnosing pleural tuberculosis. Eur. Respir. J., 9: 2635-2639.

Villena, V., J.A. Navarro-Gonzalvez, C. Garcia-Benayas, J.A. Manzanos, J. Echave, A. Lopez-Encuentra and J. Arenas-Barbero, 1996. Rapid automated determination of adenosine deaminase and lysozyme for differentiating tuberculous and non-tuberculous pleural effusions. Clin. Chem., 42: 218-221.

Valdes, L., E. San Jose, D. Alvarez and J.M. Valle, 1996. Adenosine deaminase isoenzyme analysis in pleural effusions: diagnostic role and prelevance to the origin of increased ADA in tuberculous pleurisy. Eur. Respir. J., 9: 797-751.

Valdes, L., D. Alvarez, E. San-Jose, J.R. Juanatey, A. Pose, J.M. Valle, M. Salguerio and J.R. Suarez, 1995. Tuberculous pleuritis: a study of 254 patients. Arch. Intern. Med., 158: 2017-2021.

Valdes, L., E. San-Jose, D. Alvarez, A. Sarandeses, A. Pose, B. Chomon, J.M. Alvarez-Dobano, M. Salgueiro and J.R. Rodriguez-Suarez, 1993. Diagnosis of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme and interferon gamma. Chest, 103: 458-465.

Voigt, M.D., I. Kalvaria, C. Tery, P. Berman, C. Lombard and R.E. Kirsch, 1989. Diagnostic value of ascites adenosine deaminase in TB peritonitis. Lancet, 8: 751-754.