CHAPTER - IV
Tuberculosis is a disease still killing good number of people especially in developing and under developed countries. In human beings it is caused by Mycobacterium tuberculosis. Quick modes of transport across five continents has increased and spread this problem worldwide. It can effect almost every part of human body but commonest being lungs. It can cause effusions when it affects the serous cavities.

Pleural effusion is regarded as a delayed hypersensitivity reaction rather than infection of the pleura. In spite of a good history, thorough clinico-radiological examination, biochemical and microbiological analysis and even biopsy, the etiology of the disease still eludes the clinician very often.

Inability to diagnose the disease in early stages not only delays initiation of treatment but also increases the morbidity and mortality. Conventional methods like clinico-radiological and bacteriological analysis have proven to be insufficient for confirming the diagnosis of disease.

Various workers have evaluated several parameters to overcome this increasing problem and designed various tests with varying accuracy. Some researchers have investigated the diagnostic potential of ADA estimation in correctly establishing the diagnosis of tuberculosis.

Tung et al (1976) described ADA as a polymorphic enzyme. Its distribution in the human organism is ubiquitous but its physiologic role is especially important in lymphoid tissue. It is widely distributed and exists especially in tissues with a high content of lymphoid cells such as lymph nodes, spleen, Payer’s patches and thymus. Hereditary ADA deficiency causes severe combined immunodeficiency diseases and red cell ADA over production causes hereditary haemolytic anaemia.
Piras et al (1978) were first to report high ADA in tubercular pleural effusion. Subsequently several workers explored its efficacy in the diagnosis of tuberculosis and determined that pleural fluid ADA level < 40 U/L virtually excludes the diagnosis of tuberculosis. Metaanalysis of studies conducted between 1966 and 1999 concluded that the test performance was reasonably good (sensitivity range 47.1 to 100 % and specificity 0 to 100 %) in diagnosing tuberculosis etiology in pleural effusion.

Barton et al (1979) determined ADA activity in young adult rats lymphocyte populations. The ADA specific activity was 3-10 fold higher in thymocytes than in lymphocytes from thoracic duct, lymph nodes, spleen and bone marrow. The high ADA activity in thymocytes appear to be preferentially associated with cortical thymocytes.

Other cause of ADA activity includes; bacterial infections, rheumatic disease and lymphoproliferative disorders. This article reviews; ADA estimation as an effective diagnostic criterion for tuberculous and non-tuberculous disease in pleural, ascetic, synovial fluids and CSF.

Mann et al (1982) found that Adenosine deaminase specifically reacts with adenosine and adenine nucleoside analogues. It is widely distributed in animal tissues. Increased levels have been found in C.S.F, serum and cell lysates in variety of diseases but the reports are not totally consistent. This may be due to differences in ADA production by different types of cells during the growth cycle of the cells.

Ocana et al (1983) studied ADA activity in 221 patients with pleura-peritoneal effusions in six groups: 48 cases of tuberculosis; 46 with malignancies; 36 post pneumonic effusions; 19 cases of miscellaneous diseases; 18 patients with pleural effusion of unknown origin; 60 with acellular transudate.
They concluded that specificity and sensitivity of the test with a cut-off value of more than 45 U/L for the detection of tuberculosis is very high. In patients with pleural tuberculosis, T-lymphocyte predominate in the fluid but their number did not correlate with ADA activity. Assessment of ADA in pathologic fluids is of great value in the diagnosis of tuberculosis of the pleura but they gave a note of caution because of a high ADA level in one patient with pleural effusion due to rheumatoid arthritis.

Its main biological role is related to proliferation and differentiation of lymphocytes. Specific activity of this enzyme is higher in T-lymphocytes than in B-lymphocytes; being inversely correlated to degree of T-cell differentiation.

Ocana (1986)\textsuperscript{36} studied the activity of adenosine deaminase in 74 lymphocytic pleural effusions which were divided into four groups according to the aetiology: tuberculous (38 cases), neoplastic (17), lymphomatous (7) and miscellaneous (12). The mean enzyme value was significantly higher in the tuberculous cases (93.81 ± 29.56 U/L) than for the other three groups and significantly higher in pleural effusions of lymphomatous origin than in the neoplastic and miscellaneous groups. Based on the lowest value of enzyme activity found in the tuberculous group (50 U/L), the test had a sensitivity of 1 and a specificity of 0.97.

Morisaki et al (1985)\textsuperscript{37} defined one unit of ADA activity as the amount of enzyme required to hydrolyze 1 micro mole of adenosine per minute.

Sinha et al (1985)\textsuperscript{38} found significantly high pleural fluid ADA activity in patients with tuberculosis pleural effusion as compared to those with metapneumonic effusion and malignant effusion. They also compared the results of other prevalent laboratory procedures for diagnosing tuberculosis pleural effusion like Mycobacterial culture and histopathology and found that the estimation of ADA activity proved more sensitive, simple, rapid and least invasive.
Jhamaria JP et al (1988) found Serum ADA levels were significantly higher in all the three groups of patients, viz. pulmonary tuberculosis, non-tuberculous suppurative lung diseases and malignancy as compared to healthy controls. However, the rise was much more in pulmonary tuberculosis.

Segura et al (1989) concluded that Adenosine deaminase–ADA (E.C 3.5.4.4. adenosine aminohydrolase) is an enzyme of the purine salvage pathway which catalyses the irreversible deamination of adenosine into inosine. Also found that its main role is related to proliferation and differentiation of lymphocytes. Specific activity of this enzyme is higher in T-lymphocytes than in B-lymphocytes, being inversely correlated to the degree of T-cell differentiation.

Moriwaki et al (1989) found that tuberculosis pleural effusion had significantly higher levels of ADA and lysozyme than did carcinomatous effusion and thus ADA could be used to discriminate tuberculous from carcinomatous pleural effusion.

Jibiki et al (1989) determined the clinical evaluation of various tumor markers in pleural effusion and in the serum and found that levels of ADA were significantly higher in tuberculous pleural effusions than in carcinomatous effusions.

Luo et al (1989) found that zinc may be an important agent to activate the ADA or to prompt synthesis of ADA.

Gupta (1990) studied 53 cases of pleural effusion out of which 36 were of tuberculous etiology and found that the mean ADA level of tuberculous effusion was 50.75 U/L, while in malignant and parapneumonic effusion it was 14.47 U/L and 28.65 U/L with the sensitivity and specificity 100% and 94.1% respectively.
Muraoka et al (1990) developed a simple rapid and automated method simultaneous measurement of ADA isoenzyme in human serums, based on their apparent difference in Km values for erythro-9(2-hydroxy-2-nonyl) adenine (EHNA) as inhibitor and found it suitable for analysis of large number of samples in clinical laboratories for routine monitoring of the activities of ADA isoenzymes in serum.

Ito et al (1990) observed a positive correlation between soluble IL-2R (Interleukin-2 receptors) levels and adenosine deaminase levels in tuberculous pleural fluid.

Baganha et al (1990) correlated levels of ADA in TB and neoplastic pleural exudates with the different immunologic cellular expressions that follow these clinical situations. They determined the activity of ADA and study of lymphocytic populations was made through the use of monoclonal antibodies. The data obtained showed that the levels of ADA were significantly higher in the pleural fluid and the serum of tuberculous effusions compared neoplastic effusions. The pathogenic implications of these results suggest the possibility that ADA could be a new marker of cell-mediated immune activity.

Akaike et al (1990) evaluated various biochemical parameters in influenza virus-infected mice and focussed on adenosine catabolism in the supernatant of bronchoalveolar lavage fluid (S-BALF) lung tissue and serum of plasma. The activities of adenosine deaminase and Xanthine oxidase (XO), which generates O₂ were evaluated in the S-BALF, lung tissue homogenate, and serum of plasma. The elevations were most remarkable in S-BALF and in lung tissue.

Bansal et al (1991) concluded that ADA activity can be of diagnostic value as it has been found to increase in pleural effusions associated with tuberculosis and rheumatoid arthritis. Significantly high levels of ADA were observed in pleural fluids and serum of patients with
tuberculous effusions, compare to neoplastic effusions. Thus ADA activity in serum could be helpful in the differential diagnosis of pulmonary tuberculosis, malignancy and non-tubercular respiratory disease.

Da Cunha JG (1991)\textsuperscript{50} stated that ADA catalyses deoxy-adenosine into deoxyinosine with production of ammonia.

Matoo et al (1991)\textsuperscript{51} stated that adenosine deaminase is considered to originate mainly from the liver.

Gakis et al (1991)\textsuperscript{51} according to him it is of utmost importance to establish whether high ADA activity in biologic fluid is due mainly to the presence of the isozyme ADA-1 or to the presence of ADA-2. The knowledge that ADA activity may be related to two isoenzymes that have different pH, Km and relative substrate specificity patterns is of utmost importance in correctly interpreting the results of ADA activity in biologic fluids.

Yoshida et al (1991)\textsuperscript{52} they performed studies on the mode of progression of alcoholic liver disease. They concluded that the amount of ethanol was considered to be the most important factor to affect on a progression of alcoholic liver diseases. Assessment of laboratory data such as IgA and ADA on hospitalization and change in GGTP after hospitalization were also thought to be useful in foreseeing the prognosis of alcoholic liver disease.

Kaur et al (1992)\textsuperscript{53} they estimated adenosine deaminase in pleural, peritoneal and CSF to study its diagnostic usefulness as a routine test for tuberculosis. The differences in mean ADA levels between tuberculous and non-tuberculous, peritoneal and CSF fluids although statistically significant were of no practical clinical value. A wide scatter in ADA values was seen in both tuberculous and non-tuberculous fluids. ADA estimation in plasma, lymphocytes
and cell fractions of fluids was also not diagnostically useful nor did it throw light on the source of elevated ADA in fluids.

**Thiel and Bardenheuer (1992)** revealed that in polymorphonuclear leukocytes (PMNL) adenosine is a potent inhibitor of stimulus/response coupling, as demonstrated by its adverse action on phagocytosis, the granulation oxygen radical production. In their study, all the Ca$^{2+}$ dependent stimuli were concentration dependently inhibited by adenosine. In contrast, leucocyte-stimulation by the Ca$^{2+}$ independent activator was not affected by adenosine. In the presence of intracellular Ca$^{2+}$, adenosine exerted a strong inhibition on the latex-induced cell activation but failed to inhibit in the Ca$^{2+}$ depleted state.

**Ungerer et al (1992)** developed an electrophoretic technique between ADA$_1$, ADA$_1$ + CP and ADA$_2$ (three isoenzymes) the isoenzyme pattern was studied in tissue and cell homogenates as well as serum from normal subjects and from patients with increased ADA who had either hepatitis, infectious mononucleosis, tuberculosis, pneumonia, rheumatoid arthritis or acute lymphoblastic leukaemia (ALL). The highest ADA activity was found in lymphocytes and monocytes (18% of total ADA activity). It was also the predominant isoenzyme in the sera of controls and all disease groups, except for ALL- the only condition evaluated that is not of an inflammatory nature. They concluded that serum ADA reflects monocytes/macrophage activity or turnour in most diseases studied. The exception is ALL, where serum ADA most probably originates from lymphocyte precursors.

**Ungerer et al (1994)** In this study, the composition of the ADA enzymes in tuberculous effusions was investigated. The effusions were mostly of pleural origin, but a few cases of ascites were included. A small number of parainfective and empyemic effusions, characterized by an increased ADA activity, also were examined. Two techniques, one a
spectrophotometric and the other an electrophoretic method, were used to determine the activity of the isoenzymes. The correlation between the two methods also is discussed.

Kwan et al (1994) studied that diagnostic role of ADA in pericardial fluid and found that at 40 U/L cut-off; the sensitivity is 93% and specificity is 97% in the diagnosis of tubercular pericardial effusion.

Pratheep Riantawan et al (1998) concluded that pleural fluid ADA was diagnostically useful across the various prevalence of tubercular pleuritis, and its diagnostic utility is in areas of intermediate prevalence of the disease. Diagnostic value of pleural fluid ADA is independent of HIV serologic status.

Lee Y C et al (2001) observed that ADA levels in nontuberculous lymphocytic effusions seldom exceeded the diagnostic cutoff for TB. Effusion ADA levels cannot be predicted from total or differential leukocyte counts. Post-CABG pleural fluids had ADA levels similar to other nontuberculous lymphocytic effusions. ADA is stable in effusion fluids, and its measurement is reproducible.

Kayacan O (2001) in their prospective clinical trial determined the diagnostic value of ADA activity in bronchoalveolar lavage (BAL) in sputum smear-negative subjects highly suggestive for pulmonary Tb. Sputum smear-negative patients highly suggestive for pulmonary Tb constituted Group I. Non-tuberculous pulmonary diseases constituted Group II. Twelve subjects who constituted the controls (Group III) undergoing fiberoptic bronchoscopy (FOB) for various indications and the lungs were found to be normal eventually. Albumin and ADA activity levels were measured in plasma and BAL in all the subjects. \( \text{Local}_{\text{ADA}} \) was calculated. \( \text{Plasma}_{\text{ADA}} \) and \( \text{BAL}_{\text{ADA}} \) of Group I was significantly higher (P<0.001) than that of the other groups. \( \text{Local}_{\text{ADA}} \) was also the highest in Group I when
compared with the others (P<0.001) but that of Group II was also higher (P<0.01) when compared with controls. With a cut-off value derived from the control subjects, sensitivity of BAL$_{\text{ADA}}$ was 100% and specificity 85.3%.

Jimenez Castro D (2003)\textsuperscript{60} concluded that adenosine deaminase levels in nontuberculous lymphocytic pleural effusions seldom exceed the cut-off set for tuberculous effusions. The pleural fluid adenosine deaminase levels were significantly higher in different types of exudative effusions than in transudates. An adenosine deaminase level < 40 IU x L(-1) virtually excluded a diagnosis of tuberculosis in lymphocytic pleural effusions. Adenosine deaminase1/adenosine deaminase(p) correctly classified all nontuberculous lymphocytic pleural effusions with high adenosine deaminase levels.

Greco et al (2003)\textsuperscript{61} in the study he included 121 patients, 54 with TPE and 67 with malignant effusion. He concluded that in low and intermediate TB incidence settings, the negative predictive value was sufficient that a negative ADA activity result would preclude the need for pleural biopsy. However, in the same settings, the positive predictive value was poor. In contrast, in high prevalence settings, a positive ADA would provide a 99% post-test probability of TB.

Rafael Laniado-Laboria (2005)\textsuperscript{27} concluded that high levels of ADA also have been reported in noninfectious conditions associated with pleural fluid lymphocytosis, including malignant conditions (eg. adenocarcinomas, leukemias, and lymphomas) and collagen vascular diseases (eg. rheumatoid pleuritis and systemic lupus erythematosus), which make the test less useful in countries with a low prevalence of tuberculosis. Therefore, an increased ADA level should not be considered as an equivalent to the presence of mycobacteria in the pleural fluid or pleural biopsy specimens. A higher rate of false-positive test results can lead
to the unnecessary administration of antituberculous therapy or a delay in making an alternative diagnosis

Daniil et al (2007)\(^6\) concluded that the combination of pleural ADA and CRP levels might be sufficient for discriminating between the different groups of Lymphocyte rich pleural effusion.

Jadhav and Bardapurkar (2007)\(^4\) concluded that ADA is a useful biochemical marker to evaluate Lymphocyte rich pleural effusions.

Bojan Zaric et al (2007)\(^6\) ADA is specific and sensitive test for diagnosis of pleural tuberculosis even in a region with average incidence of pulmonary tuberculosis. When cost benefit relation is concerned low price of this test when compared to invasive surgical procedures makes it excellent diagnostic tool for TPE.

Krenke et al (2008)\(^6\) The ADA activity and IFN-gamma concentration were significantly higher in TPE than in non-TPE (\(P<0.0001\)). The diagnostic sensitivity and specificity of IFN-gamma measurement (cut-off value of75.0 pg/ml) were 100% and 98.5% respectively and were similar to those of ADA (100% and 93.9% at the cut-off value of 40.3 U/L). They conclude that pleural fluid ADA activity and IFN-gamma concentration are highly sensitive and specific markers of tuberculous pleurisy.

Titarenko OT et al (2008)\(^6\) compared the characteristics of the informative value of tests determining the activity of adenosine deaminase (ADA) activity and the level of gamma-interferon (gamma-IFN) for the differential diagnosis of tuberculous pleurisy (n = 35) and non-tuberculous pleural effusion (n = 53). Both tests were ascertained to have the similar differentially diagnostic capacities with their threshold values of 35 U/l and 180 pg/ ml, respectively and found that the sensitivity and specificity of the ADA test were 98.4% and of
the gamma-IFN test were 94.3 and 96.2%, respectively, with the positive and negative predictive value being 91.3 and 98.3% and 94.3 and 96.2%, respectively, and with the diagnostic value of 94.5% for the ADA test and 96.4% for the gamma-IFN test. There was a high unidirectionality of changes in both values: it was higher than the cut-off points in 94.1% of the patients with tuberculous pleurisy and below them in 88.7% of those with other pleural effusions.

Verma S K (2008) found Pleural fluid adenosine deaminase was more than 36 IU/L (36 to 229.7 IU/L) in tubercular pleural effusion. In case of malignancy it was more than 18.5 IU/L (18.5 to 87.6 IU/L). While in one case of hypoprotememia pleural fluid adenosine deaminase was 8.21 IU/L. If 36 IU/L is taken as cut of limit the sensitivity and specificity of ADA for tuberculosis is 100 % and 77.7 %. More than 100 IU/L was exclusively seen in tubercular pleural effusion.

Zay Soe et al (2010) concluded that analysis of pleural fluid can have an important contribution for investigation of patients with pleural effusion. Although highly specific, percentage positivity of microbiological examination on pleural fluid does not reach the degree required for a single diagnostic investigation for tuberculosis. The Light’s criteria are fulfilled in all cases. Pleural biopsy will be useful as an ultimate procedure in cases with diagnostic problem as it is a procedure which can give a definitive tissue diagnosis.

Kamaaldeen Baba et al (2008) concluded that ADA analysis is a sensitive marker of tuberculous pleuritis even in HIV patients with very low CD4 counts in a high TB endemic region. The ADA assay is inexpensive, rapid and simple to perform and is of great value for the immediate diagnosis of tuberculous pleuritis while waiting for culture result and this has a positive impact on patient outcome.
Porcel JM (2009)\textsuperscript{69} concluded that in areas with high TB prevalence, pleural fluid adenosine deaminase (ADA) levels greater than 40 U/l argue strongly for TB; in contrast, low levels of pleural ADA have high negative predictive value in low-prevalence countries. The specificity of this enzyme increases if only lymphocytic exudates are considered. The shortcoming of the ADA test is its inability to provide culture and drug sensitivity information, which is paramount in countries with a high degree of resistance to anti-TB drugs.

Bharat Kumar Gupta et al (2010)\textsuperscript{7} concluded that, ADA levels in non-tuberculous exudative pleural effusions rarely exceeded 40 U/L cut-off; set for tubercular disease and the pleural fluid ADA levels were significantly higher in tuberculous exudative pleural effusions when compared with non-tuberculous exudative pleural effusions.

Bharat Kumar Gupta et al (2010)\textsuperscript{70} concluded that ADA estimation in CSF is not only simple, inexpensive and rapid but also fairly specific method for making a diagnosis of tuberculous etiology in TBM, especially when there is a dilemma of differentiating the tuberculous etiology from non-tuberculous ones. For this reason ADA estimation in TBM may find a place as a routine investigation.

Bharat Kumar Gupta et al (2010)\textsuperscript{71} Studied three hundred and thirty patients with pleural, ascitic, meningeal and synovial effusion. In cases of pulmonary and extra-pulmonary disease they found the sensitivity was 92.80\% and 94.29\%; specificity 90.00\% and 92.16\%; positive predictive value 92.86\% and 89.00\%; and negative predictive value 90.00\% and 95.92\% respectively. They concluded that Adenosine deaminase estimation is not only a fairly sensitive and specific test (more than 90\%), helpful in differentiating tubercular from non-tubercular etiology both in pulmonary and extra-pulmonary disease, but is also simple, inexpensive and rapid. For this reason this test may help in early diagnosis, improve the prognosis and reduce spread of disease and sequlae.
Khalid Hassanain (2010) found Adenosine deaminase (ADA) in pulmonary tuberculosis patients revealed significantly increased level in both serum and BALF for all patients with pulmonary tuberculosis compared to ADA level of cancer, pneumonia and normal persons.

Arunabha Datta Chouduri (2011) found that sputum was bacteriologically (smear and/or culture) positive for tuberculosis in 10 out of 30 cases (33.33%) in which tuberculous etiology was confirmed by histology and/or bacteriology (definite tuberculosis). No sputum AFB (smear and culture) was found in 15 cases of probable tuberculosis where tuberculous etiology was established by indirect methods like Adenosine deaminase level more than 40 unit/l and other relevant investigations. Over all, sputum was bacteriologically smear and/or culture positive in 10 out of 45 cases (22.22%). They concluded that careful and thorough sputum examination in cases of tuberculous pleural effusion may help as a diagnostic tool and it has therapeutic and epidemiological implications.