CHAPTER - VII
Chest wall is covered from inside by parietal pleura and the surfaces of the lungs are covered by visceral pleura. The space between these two pleural coverings is the pleural space. Pleural space is filled with a thin layer of fluid called pleural fluid. Two forces have influence on the amount of fluid present;

(1) Hydrostatic and oncotic forces in the visceral and parietal pleural vessel and

(2) Extensive lymphatic drainage.

Pleural effusion result from disruption of this balance i.e. a pleural effusion may develop when there is excess pleural fluid formation (from the interstitial spaces of the lung, the parietal pleura, or the peritoneal cavity) or when there is decreased fluid removal by the lymphatics. Nature has provided a capacity in the human body that 20 times more fluid than is normally formed can be absorbed without resultant pleural effusion. But even than effusions are seen clinically and some time massive; under pathological conditions and patients complaints of fever, cough, sputum, breathlessness, chest pain, loss of appetite and night sweats.

The pleural effusion may be transudative or exudative. Transudative pleural effusions are caused by systemic factors that alter the balance of the formation and absorption of pleural fluid (eg. left ventricular failure, renal failure, hepatic failure and cirrhosis). Exudative pleural effusion occurs when local factors that influence the formation and absorption of pleural fluid are altered as seen in bacterial pneumonia, fungal infection, malignancy, viral infection, pulmonary embolism, sarcoidosis, transplant patients with graft rejection, trauma, cancer etc. due to a release of interleukins and lymphokines that alter the vascular permeability and lead to extravasation of proteins, inflammatory cells and certain enzymatic markers like ADA.
Several researchers have reported that estimation of pleural fluid ADA is useful in establishing the etiology of the effusion. Shibagaki T et al concluded that tuberculous pleural effusion had a much higher ADA activity than cancer effusion.\textsuperscript{74}

Sharma et al found that levels of pleural fluid ADA were significantly higher than serum ADA levels in both tuberculous and non-tuberculous pleural effusions.\textsuperscript{75} Porcel J M et al found that a high ADA level is characteristic not only of lymphocytic, but also of neutrophilic TB effusions. An extremely high ADA activity should raise suspicion of empyema.\textsuperscript{76} Gupta et al concluded that the pleural fluid ADA levels were significantly higher in tuberculous exudative pleural effusions when compared with non-tuberculous exudative pleural effusions.\textsuperscript{7}

Lymphocytosis is a medical condition characterized by elevated lymphocytes count. There are three main types of lymphocytes: natural killer cells, T cells, and B cells. Each is important when it comes to defending the body from disease. Lymphocytosis itself is not an illness, but is rather a condition caused by an illness. Causes of lymphocytosis are usually Infections (bacterial, viral, other), cancer of the blood or lymphatic system and an autoimmune disorder causing ongoing (chronic) inflammation. To be more specific, if we talk about pleural effusion the common causes include infections like tuberculosis, pneumonia & empyema; malignancies like acute & chronic lymphocytic leukemia and secondaries; autoimmune disorder like Collagen disease and pancreatitis and some other like, chronic renal and cardiac failures.

Lymphocyte rich pleural effusion shows neutrophil predominance in early stages and mononuclear cells later during the course of the disease. It is believed to be due to proliferation and differentiation of lymphocytes which release lymphokines, which in turn activate macrophages for an enhanced bactericidal activity.\textsuperscript{77}
Lee et al studied 106 nontuberculous pleural effusion samples of different etiologies, all with lymphocytic count >50% and observed that false positive test result rate is <3%; they concluded that the value of estimating ADA level not only helps in making the diagnosis in such patients, suspected of tuberculosis and also predicted that diagnosis cannot be based on total or differential leukocyte counts. We also selected a cutoff of >50% lymphocytosis in pleural effusion for the present study.

The incidence of tuberculosis is highest during late adolescence and early adulthood among infected persons; the reason is due to more exposure of adults to the outer environment. The incidence among women peaks at 25-34 years of age. In this age group incidence among women may be higher than those among men, while at older ages the opposite is true. The risk may increase in elderly possibly because of waning immunity and comorbidity.

The present study included 108 patients; 48 were tubercular ranging (Figure-1) from 12-82 years while 60 were non-tubercular (Figure-2) from 12-77 years of age. In tubercular group Mean ± SD age was found to be 35.43 ± 16.5 while in non-tubercular group collectively it was 50.44±19.18. Mean ± SD was 25±1 in CHF; 58.56±17.7 in CRF; 50.13±20.9 in pneumonia; 31.4±22 in empyma and 58.17±8.76 in malignancy when calculated separately in different subgroups of nontubercular group.(Table-2)

L Valdes et al observed in his study of 254 patients of tuberculous pleural effusion that the mean±SD age was 34.1 ± 18.1 years, and 62.2% patients were younger than 35 years. These findings are similar to what we found in our study too. Epstein et al Seibert et al and Moudgil et al have reported that mean age of patients of tuberculous pleurisy gradually rises with the severity of disease. While P Riantawan reported the mean age as 43 ±1.5 years but his study included
HIV infected patients and Wipa Reehaipichitkul reported the mean age to be higher i.e., 52.2 ±16.3 years.83

In the present study of 108 patients overall male: female ratio is 2.5:1; in tubercular group this ratio is 4.3:1 while in non-tubercular it is 1.7:1. (Table-2)

Sudipta et al in his study of 72 patients found male: female ratio as 1.79: 1.83 While Wipa Reehaipichitkul in a study of 132 patients with symptomatic exudative lymphocytic pleural effusion reported the male to female ratio as 1.4:1.84 Gupta et al found this ratio as 3:1 in his study of 96 patients with tuberculous and non-tuberculous exudative pleural effusion.7

In the present study the average glucose (random) level in pleural fluid is 90.79± 15.30 in nondiabetic tubercular group (n=42), 129± 26.16 in diabetic tubercular group (n=6) and 95.20± 21.03 is found among all the patients of tubercular group (n=48) including diabetic and nondiabetic.

Serum glucose level is found 105± 18.23 in tubercular nondiabetic, 187.8± 44.39 in tubercular diabetic and 115.05± 35.48 mg/L in all the patients of tubercular group (including diabetic and nondiabetic). (Table-3)

Total protein in pleural fluid is found to be 4.7±0.97 in diabetic tubercular group (n=6), 4.46± 0.92 in non-diabetic tubercular group (n=42) and 4.54± 0.94, when calculated amongst all the patients of tubercular group (n=48) including diabetic and non-diabetic. Serum proteins is 3.8± 0.60 in diabetic tubercular group, 5.16± 1.11 in non-diabetic tuberculars and 4.99± 1.14 collectively in these two groups. (Table-3)
Protein levels are low in our study both in pleural fluid and serum as compared to the reference normal levels. Seibert FB et al observed that moderately advanced TB of questionable clinical significance shows a decrease in albumin. Damburam A. et al concluded that patients with PTB had lower serum total proteins and serum albumin but higher plasma gammaglobulin levels than controls.

Possibly bcoz of poor socioeconomic condition and malnutrition the low level of protein has been observed in patients in western UP in our study.

In Tubercular group the range of ADA was 5.1-271 (Figure 3) and mean ± SD was 86.17 ± 63.67 while in non-tubercular group collectively it was 3.4-48 U/L and 20.23 ± 12.23. In different subgroups of non-tubercular group, the range of ADA was 6.5-10.6 U/L (Figure 4) (mean ± SD 8.36 ± 2.07) in CHF subgroup; in CRF 3.4-17.0 U/L (Figure 5) (mean ± SD 8.54 ± 6.49), in Pneumonia 5-37.4 U/L (Figure 6) (mean ± SD 21.22 ± 8.44), in Empyema 8.6-48 U/L (Figure 7) (mean ± SD 21.89 ± 19.15), in malignancy 12.6-43.3 U/L (Figure 8) (mean ± SD 24.85 ± 10.10). (Table-4)

Figure 9 (Pi diagram) shows mean values of ADA in different study groups.

In our study 48 patients have confirmed tuberculosis; out of which only 39 had ADA level >40 U/L (true positive) while 9 cases had ADA level <40 U/L (true negative) (Table-4)

In the subgroups of non-tuberculous group (n=60), viz. CHF, CRF, Pneumonia, Empyema, and Malignant group 54 cases had ADA levels <40 U/L (true negative) and 6 cases had ADA level >40 U/L (false negative). Out of those 6 cases 3 were of Empyema and 3 were of malignant pleural effusion. (Table-5)
False positivity could be due to empyema, lymphoma, malignancy, parapneumonic or collagen vascular disease.\textsuperscript{15,87}

We calculated Positive predictive value (ppv) and the negative predictive value (npv) and they were 86.6\% (at 95\% CI: 83.41-89.11), 85.7\% (at 95\% CI: 82.13-88.97) respectively. B K Gupta et al found diagnostic sensitivity as 92.80\%; 94.29\%; specificity as 90.00\% and 92.16\%; positive predictive value 92.86\% and 89.00\%; and negative predictive value 90.00\% and 95.92\% respectively for ADA estimation though his study included both cases of pulmonary and extra-pulmonary disease.\textsuperscript{70}

Wipa Reechaipichitkul compared a number of studies in which pleural fluid ADA cut off levels ranged from 40 to 70 U/L and found that the sensitivity and specificity was different in each of these, as summarized in the table below\textsuperscript{84}

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Cut-off ADAPF (U/l)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>De Oliveira et al</td>
<td>1994</td>
<td>40</td>
<td>91</td>
<td>96</td>
</tr>
<tr>
<td>Perez-Rodrigues et al</td>
<td>1995</td>
<td>40</td>
<td>88</td>
<td>97</td>
</tr>
<tr>
<td>Ocana et al</td>
<td>1983</td>
<td>45</td>
<td>100</td>
<td>97</td>
</tr>
<tr>
<td>Maartens et al</td>
<td>1991</td>
<td>45</td>
<td>77</td>
<td>83</td>
</tr>
<tr>
<td>Valdes et al</td>
<td>1993</td>
<td>47</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>W. Reechaipichitkul</td>
<td>2001</td>
<td>48</td>
<td>80</td>
<td>80.5</td>
</tr>
<tr>
<td>Burgess et al</td>
<td>1996</td>
<td>50</td>
<td>91</td>
<td>81</td>
</tr>
<tr>
<td>Riantawan et al</td>
<td>1999</td>
<td>60</td>
<td>95</td>
<td>96</td>
</tr>
<tr>
<td>Banales et al</td>
<td>1991</td>
<td>70</td>
<td>98</td>
<td>9</td>
</tr>
</tbody>
</table>
In the present study ADA level > 40 U/L is taken as cut-off and found the sensitivity and specificity of the test for the diagnosis of tuberculosis to be 81.25% and 90% respectively (Table-6); several other researchers have also taken the same cut off levels for their studies.\textsuperscript{19,23} Gupta et al had done a study on tubercular exudative serosal effusions taking same value as the cut off and found the sensitivity and specificity as 92.80 % and 90 % in pleuropulmonary disease and 94.29 % & 92.16 % in extra-pulmonary disease respectively.\textsuperscript{88}

A highly significant difference was observed for each pair of groups when compared with tubercular group at .1% level of significance by applying the unpaired t-test to find the significant difference of mean values of ADA between tubercular and different subgroups of non-tubercular (Table-7).

Strankinga W.F. in his study of 10 patients with tuberculosis pleurisy and 76 patients with pleural effusions of other etiology found a specificity of 87% and sensitivity of 100% when a reference limit of more than 53 U/L is taken. He also found that ADA activity in the tuberculous patients was significantly higher than in the other groups while the exception of those with empyema.\textsuperscript{89}

Baganha et al reported that with an ADA activity cut-off value of 54 IU/L, the sensitivity is 82% and specificity is 97% for the diagnosis of tuberculosis.\textsuperscript{47}

Gupta, D.K studied 53 cases of pleural effusion out of which 36 were of tuberculous etiology. The mean ADA level in tuberculous was 50.75 U/L while in malignant and parapneumonic effusion it was 14.47 U/L and 28.65 U/L respectively. The sensitivity and specificity for diagnosing tuberculosis were 100% and 94.1 % respectively.\textsuperscript{44}

Voight studied 82 cases out of which 41 cases have bacteriologically confirmed tuberculosis and other 41 cases with other causes. He found the mean ADA level for tubercular etiology was 99.8
U/L with sensitivity and specificity for diagnosis tubercular ascites was 95% and 98% respectively.\textsuperscript{90}

Dwivedi M studied 49 patients with ascites of which 19 were of tubercular etiology with mean ADA level of 98.8 U/L. At an ADA level > 33 U/L the sensitivity, specificity, positive and negative predictive values were 100%, 96.6%, 95% and 100% respectively.\textsuperscript{91} Gupta D.K. in his an other study of 24 ascites case found 7 cases of tubercular etiology with an ADA level of >30 U/L and sensitivity and specificity of 100% and 94.1% respectively.\textsuperscript{44} The sensitivity and specificity for tubercular ascites on the basis of ADA level were 100% and 97% respectively as per the study of Bhargava DK.\textsuperscript{92}

Krenke R found that ADA activity and IFN-gamma concentration were significantly higher in tubercular pleural effusion than in non-tubercular pleural effusion (P<0.0001). The diagnostic sensitivity and specificity of IFN-gamma measurement 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). He concluded that pleural fluid ADA activity and IFN-gamma concentration are highly sensitive and specific markers of tuberculous pleurisy. Hence, the role of ADA and IFN-gamma in the differential diagnosis of tuberculous pleurisy is pivotal.\textsuperscript{65}

Although a few studies have shown IFN-\(\gamma\) levels to be more sensitive and specific than ADA, but it is less preferred in resource-limited settings as it is more expensive and less readily available compared to ADA.\textsuperscript{61,65,93}

In cases of tuberculous pleurisy, the infection is characterized by the accumulation of activated T-lymphocytes and macrophages in pleural space. The infection being localized and restricted to a certain area results in T-lymphocyte rich pleural effusion, leading to high ADA in pleural effusions.
It was also observed that level of ADA in pleural fluid was significantly higher than those observed in serum in both tuberculous and non-tuberculous patients, suggesting a localized intrapleural production of ADA.\textsuperscript{75}

Some researchers has found that the level of pleural fluid ADA was three times then the serum ADA in tuberculous disease and two times in non-tuberculous disease.\textsuperscript{94}

In a few cases with pulmonary tuberculosis a low ADA activity was obtained in spite of positive sputum for TB. The most likely mechanisms could be suppressed or immature cell mediated immunity especially affecting the differentiation of the T-lymphocyte population.\textsuperscript{95}

Value of ADA activity in pleural effusion was studied by Shibagaki T et al He concluded that tuberculous pleural effusion had a much higher ADA activity than cancer effusion and total ADA activity in tuberculous pleural effusion decreases after anti tuberculosis therapy.\textsuperscript{74} We have also found that ADA values are less in malignant patients (24.85± 10.10) than in tubercular patients (86.17± 63.67).

Pratheep Riantawan observed that Tuberculous pleuritis is more common in patients coinfected with HIV and TB than in patients without HIV infection.\textsuperscript{57} Pleural fluid adenosine deaminase has been shown to be a useful biochemical marker of TBpleuritis and provides a reliable basis for a treatment decision, particularly in areas where TB is prevalent.\textsuperscript{96,97} However Hsu et al suggested that ADAPF may be less diagnostically useful for immunocompromised patients with TBpl and the diagnostic value of ADAPF is independent of HIV serologic status.\textsuperscript{98}

The presence of a large / massive pleural effusion enables the clinician to narrow the differential diagnosis, since most effusions are secondary to malignancy or infectious (either bacterial or
mycobacterial). Bloody pleural fluid with low ADA content favors a malignant condition.\textsuperscript{76} These findings are nearly identical to those reported by Lee et al and it was concluded that measurement of the pleural fluid ADA level is an excellent test both for ruling out and ruling in a suspected diagnosis of tuberculous effusion due to its high sensitivity and specificity, at least in areas with a high prevalence of tuberculosis.\textsuperscript{58}

Thus ADA can be used for ruling out suspected cases of tuberculosis and can be a very effective screening test.

In contrast to other studies Rafael concluded that the ADA assay should be considered as a screening test to guide further diagnostic procedures in cases of exudative pleural effusion; He further concluded that false positive diagnosis of tuberculous pleural effusion (TPE) by ADA measurement is limited to lymphocytic pleural fluids. However other lymphocyte-rich pleural effusions could also have elevated ADA pleural fluid levels including malignant conditions (eg. Adenocarcinomas, leukemias, and lymphomas) and collagen vascular diseases (eg. Rheumatoid pleuritis and systemic lupus erythematosus),\textsuperscript{20,57} which make the test less useful in countries with a low prevalence of Tuberculosis.\textsuperscript{58} 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.

Gupta et al (2010) concluded that ADA levels in nontuberculous exudative pleural effusions rarely exceeded the cut-off; set for tuberculous disease. The pleural fluid ADA levels were significantly higher in tuberculous exudative pleural effusions when compared with non-tuberculous exudative pleural effusions. They have further observed that ADA 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 Extrapulmonary 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 sequelae.  

To establish the diagnostic role of ADA we further subjected our findings to statistical analysis & observed that the diagnostic accuracy to be 86% (at 95% CI: 83.97-89.98), positive likelihood ratio 8.13(at 95% CI: 6.98-9.98), negative likelihood ratio 0.21 (at 95% CI: 0.17-0.26) and Diagnostic odd ratio 39 (at 95% CI: 36.51-41.12). (Table-8)

The sensitivity of the ADA as a serological marker for tuberculous exudative pleural effusion depends on the prevalence of the disease in the population and India being a high prevalence area the sensitivity and specificity here for this test should be high. In the present we found that disease prevalence is 48% (at 95% CI: 45.76-49.98) (Table -8).

Liang et al (2008) conducted a meta-analysis which summarized the result of 63 studied to determine the accuracy of ADA measurements in the diagnosis of tuberculous pleurisy and he found that ADA determination is a relatively sensitive and specific test for the diagnosis of tuberculous pleurisy. Measurement of ADA in pleural effusion is likely to be a useful diagnostic tool for tuberculous pleurisy and to avoid pleural biopsy in young patients from areas with high prevalence. He suggested that the results of ADA assays should be interpreted in parallel with clinical findings and the results of conventional tests.  

While Kataria and Imtiaz found that with the decline in the prevalence of TPE, the positive predictive value of pleural fluid ADA also declines, but the negative predictive value actually increases. Therefore they concluded that ADA testing can be used to rule out a tuberculous etiology of lymphocyte pleural effusions both in countries with high and low prevalence of tuberculosis.
We included patients with pleural effusion having above 50% lymphocytes count for the present study but simultaneously we noticed that two patients who having lymphocytes count below 50%; were sputum smear positive; having favorable radiological findings; gave history of being habitual defaulter in anti tubercular therapy previously and were having periods of sputum smear changing to negative; probably cases of relapse. This generated our academic interest and we tested their pleural fluid for ADA and found that the level was below the cutoff value. Probably such cases may be having immunosuppression because of previous intermittent treatment which resulted in their low pleural fluid ADA levels. The same were the findings of Collazos et al; who in their prospective study of 25 patients of pulmonary/pleural tuberculosis observed a significant decrease in the serum ADA values during the first two months followed by stabilization of the serum ADA activity for a period of 6-months after initiation of treatment.\textsuperscript{100} Ishii et al in their study tried to found the relationship between ADA activity and lymphocyte subpopulation in pulmonary TB and found similar results. Future research is required to confirm such a hypothesis on relapse TB.\textsuperscript{101}

Several researchers have reported that the estimation of ADA activity in body fluid serves as a reasonable tool in the diagnosis of TB; especially when there was a dilemma in the diagnosis of etiology. ADA estimation by the colorimetric method as was described by Guisti and Galanti has an advantage over other tools available because of its low cost, simplicity of technique and rapid turn-around time.\textsuperscript{102} Therefore ADA estimation being a rapid and non-invasive test, should become an integral part of the diagnostic work up of exudative pleural effusions in suspected cases of tuberculosis.

Finally we can conclude from the present study that ADA estimation in the pleural fluid is a powerful tool in the differentiation of etiology of pleural effusion and so ADA estimation has a definite diagnostic role in lymphocyte rich pleural effusion & may be used as a routine investigation for the diagnosis of such patients.