CHAPTER - II
Tuberculosis (TB) is a chronic bacterial infection caused by mycobacterium tuberculosis; first isolated in 1882 by a German physician “Robert Koch”. He received a Nobel Prize for this discovery. TB commonly affects lung but may involve any organ of the body. TB can remain in dormant state for years without causing symptoms. When the immune system of the patient with dormant TB is weakened, Mycobacterium may reactivate and can cause disease. The risk factors for TB include close-contact, excessive alcohol intake, IV drug abuse and certain diseases like diabetes, cancer & HIV and certain occupations like health-care providers. The common symptoms in such patients are cough, sputum, breathlessness, fatigue, evening rise of temperature, weight loss, and night sweats.

Overall one-third of the world’s population is currently infected with the Tuberculosis bacillus. TB kills one person every 90 sec in India and about 1000 people every day. According to WHO India account for over 20% of TB cases worldwide.¹

According to WHO, in 2010, there were 8.8 million (range, 8.5–9.2 million) incident cases, 1.1 million (range, 0.9–1.2 million) deaths among HIV-negative people and an additional 0.35 million (range, 0.32–0.39 million) deaths from HIV-associated tuberculosis. In 2010, there were 5.7 million notifications of new and recurrent cases, equivalent to 65% (range 63–68%) of the estimated number of incident cases in 2010 world over. India and China accounted for 40% of the world’s notified cases of TB in 2010.²

The development of tubercular lesion depends upon the immune response of the body to the tubercular infection, which in turn depends on the nutritional status of the individual, concurrent use of immunosuppressive drugs and immune depleting infections like the HIV. In persons with poor nutritional status the lesion develops within weeks after primary infection, and the organism lie dormant for many years before entering a phase of exponential multiplication leading to disease.
M. tuberculosis is a rod-shaped, non-spore-forming, thin aerobic bacterium measuring 0.5 µm by 3 µm. Mycobacteria, including M. tuberculosis, are often neutral on Gram's staining. However, once stained, the bacilli cannot be decolorized by acid alcohol, a characteristic justifying their classification as acid-fast bacilli. Acid fastness is due mainly to the organism’s high content of mycolic acids, long-chain cross-linked fatty acids, and other cell-wall lipids.  

When the inhaled mycobacterium enters the lung, it multiplies and causes a local lung infection (pneumonia). The tuberculous bacillus is non-motile but it can be transported intracellularly through the phagocytic cells. The disease can spread by direct extension, through lymphatics, through the blood stream or by natural passages through body cavities. The initial entry of the tubercle bacilli into a previously uninfected individual elicits a non specific acute inflammatory response. Tuberculosis that occurs after initial exposure to the bacteria is often referred to as primary TB and is common among children up to 4 years of age and among immunocompromised persons. Later on the bacilli are ingested by macrophages and transported to the regional lymph nodes. The local lymph nodes associated with the lungs may also become involved with the infection and usually become enlarged. The hilar lymph nodes (the lymph nodes adjacent to the heart in the central part of the chest) are often involved. If spread of the organism is not contained at the level of regional lymph nodes then tubercle bacilli reaches the blood stream and widespread dissemination ensues. Dissemination may result in milliary or meningeal TB, the illness with potential for major morbidity especially in infants and young children.

During initial 2 to 8 weeks after primary infections, while bacilli continue to multiply in their intracellular environment, cell-mediated hypersensitivity develops in the infected host. Immunologically competent lymphocytes enter areas of infection, where they release chemotactic factors like interleukins and lymphokines. In response, monocytes enter the area and undergo
transformation into macrophages and subsequently into specialized hystiocytic cells which are organized into granulomas. Mycobacteria may persist within macrophages for many years despite increased lysozyme production within these cells but their further multiplication and spread are usually confined to macrophages.

The body's immune (defense) system, however, can fight off the infection and limit its spread. If the body is able to form scar tissue (fibrosis) around the TB bacteria, then the infection is contained in an inactive state. Such an individual typically has no symptoms and cannot spread TB to other people. The scar tissue and lymph nodes may eventually harden, like stone, due to the process of calcification of the scars (deposition of calcium from the bloodstream in the scar tissue). These scars often appear on X-rays and imaging studies like round marbles. The body's immune system becomes weakened, the TB bacteria break through the scar tissue and can result in a recurrence of the pneumonia and cause active disease, referred to as secondary TB. Secondary TB spread to other parts of the body like kidneys, bone, joints, peritoneum, pericardium and lining of the brain and spinal cord (meninges).

PLEURAL EFFUSION:

The pleura is a thin serous layer, which covers the lungs (visceral pleura) and is reflected, by way of the lung hila (the region where the vessels and air passage enter the lung), on to the chest wall and pericardium (parietal pleura). The pleural space lies between the lung and chest wall and contain a very thin layer of fluid, which serves as a coupling system. Only a thin layer of pleural fluid separates the parietal and visceral pleura. The parietal layer secretes 2400 ml of fluid daily, which is reabsorbed by the visceral layer. Maintenance of negative intrapleural pressure is necessary for respiration.
A pleural effusion is present when there is an excess quantity of fluid in the pleural space. Pleural fluid accumulates when pleural fluid formation exceeds pleural fluid absorption. Normally, fluid enters the pleural space from the capillaries in the parietal pleura and is removed via the lymphatics situated in the parietal pleura. Fluid can also enter the pleural space from the interstitial spaces of the lung via the visceral pleura or from the peritoneal cavity via small holes in the diaphragm. The lymphatics have the capacity to absorb 20 times more fluid than is normally formed. Accordingly, 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.

When a patient is found to have a pleural effusion, an effort should be made to determine the cause. The effusion can be of transudative or exudative in nature. Transudative pleural effusions are caused by systemic factors that alter the balance of the formation and absorption of pleural fluid. Exudative pleural effusion is caused by alterations in local factors 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. Other causes of pleural effusion include tuberculosis (though pleural fluid smears are rarely positive for AFB, this is the most common cause of pleural effusion in some developing countries).

Transudative and exudative pleural effusions are distinguished by measuring the lactate dehydrogenase and protein levels in the pleural fluid. Exudative pleural effusions meet at least one of the following criteria:

1. pleural fluid protein/serum protein >0.5
2. pleural fluid LDH/ serum LDH >0.6
3. pleural fluid LDH more than two thirds normal upper limit for serum

Differential Diagnosis of Pleural Effusions:

**Transudative Pleural Effusions**

1. Congestive heart failure
2. Cirrhosis
3. Pulmonary embolization
4. Nephrotic syndrome
5. Peritoneal dialysis
6. Superior vena cava obstruction
7. Myxedema
8. Urinothorax

**Exudative Pleural Effusion**

1. Neoplastic diseases
   a. Metastatic disease
   b. Mesothelioma
2. Infectious diseases
   a. Bacterial infections
   b. Tuberculosis
   c. Fungal infections
   d. Viral infections
   e. Parasitic infections
3. Pulmonary embolization
4. Gastrointestinal disease
a. Esophageal perforation
b. Pancreatic disease
c. Intraabdominal abscesses
d. Diaphragmatic hernia
e. After abdominal surgery
f. Endoscopic variceal sclerotherapy
g. After liver transplant

5. Collagen-vascular diseases
   a. Rheumatoid diseases
   b. Systemic lupus erythematosus
c. Drug-induced lupus
d. Immunoblastic lymphadenopathy
e. Sjogren’s syndrome
f. Wegener’s granulomatosis
g. Churg-Strauss syndrome

6. Post-coronary artery bypass surgery

7. Asbestos exposure

8. Sarcoidosis

9. Uremia

10. Meigs’ syndrome

11. Yellow nail syndrome

12. Drug-induced pleural disease
   a. Nitrofurantoin
   b. Dantrolene
c. Methysergide
d. Bromocriptine

e. Procarbazine

f. Amiodarone

13. Trapped lung
14. Radiation therapy
15. Post-cardiac injury syndrome
16. Hemothorax
17. Iatrogenic injury
18. Ovarian hyperstimulation syndrome
19. Pericardial disease
20. Chylothorax

**Aetiologies of lymphocytic pleural effusions**

1. Malignant pleural effusions
2. Lung
3. Lymphoma
4. Mesothelioma
5. Idiopathic
6. Parapneumonic
7. Post-CABG (coronary artery bypass grafting)
8. Trauma
9. Rheumatoid Arthritis
10. Chylothorax
11. Post-transplantation
12. Pancreatitis
The pleural fluid of tuberculous pleuritis (TP) is usually predominantly lymphocytic. Some studies suggest that pleural fluid lymphocyte percentage of more than 85% are very suggestive of tuberculosis\(^6\), while the other series of patients with TP indicates that only 10% patients had less than 50% lymphocyte in pleural fluid. Acute TP may show an increase in neutrophils.\(^7\)

**Adenosine Deaminase (ADA):**

Adenosine deaminase (ADA) is an enzyme in the purine nucleoside salvage pathway that converts adenosine to inosine. This enzyme is widely distributed in various tissues and plays an important role in the metabolism of purine nucleotides. This enzyme is distributed in human tissues in three forms:

1) Monomeric 35 kd enzyme (ADA-1),

2) 280 kd enzyme containing 35kd catalytic units combined with a non enzymatic 200kd combining protein (ADA-1+CP)

3) 100kd enzyme (ADA-2).\(^8\)

ADA-1 is distributed in solid organ such as liver, kidney and intestine in human beings as a major component of ADA activity.\(^9\) ADA-2 is found only in macrophages and monocytes.\(^10\) ADA-2 is the major component of the activity of total ADA in the serum of healthy person (Gakis.C). It
increases in biological fluids in the course of infectious disease characterized by micro-organisms infecting the macrophages.\textsuperscript{11}

Adenosine deaminase (ADA) plays an important role in differentiating lymphoid cells and is present in abundance in active T-lymphocytes whose concentration is inversely proportional to the degree of differentiation. Levels of ADA are 10 times higher in T-lymphocytes than in erythrocytes.\textsuperscript{12} The enzyme activity increases during mitogenic and antigenic responses of lymphocytes and T-lymphocyte blastogenesis can be inhibited by inhibitors of ADA.

ADA deficiency leads to accumulation of adenosine and dATP; this would inhibit further production of precursors for DNA synthesis especially dCTP. The disease is caused by a mutation in a gene on chromosome 20, the gene codes for the enzyme adenosine deaminase (ADA).\textsuperscript{10} Without this enzyme, the body is unable to break down a toxic substance called deoxyadenosine. This toxin builds up and destroys infection-fighting immune cells called T and B lymphocytes. The discovery that the genetic absence of ADA results in severe combined immunodeficiency, an inherited autosomal recessive condition, has stimulated investigations on the association of ADA with the immune system.\textsuperscript{13,14} Thus ADA deficiency is rare, but very dangerous, because a malfunctioning immune system leaves the body open to infection from bacteria and viruses.

Lymphocytes are known to contain high quantity of ADA. Therefore a congenital deficiency of ADA affects the normal Lymphocytic activity, reducing their count. This leads to impaired cellular and humoral immunity. Hypouricemia is due to defective breakdown of purine nucleotides.

ADA levels in nontubercular exudative pleural effusion 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.\textsuperscript{15}
Cell mediated immune response result in effusion in tuberculous and is characterized by the accumulation of activated T-lymphocytes and macrophages. ADA is now recognized as a marker of cell mediated immunity particularly as a marker of T-lymphocyte activation. Thus, ADA activity may help differentiate tubercular etiology from non-tubercular.\textsuperscript{16}

ADA is frequently considered as diagnostic aid in tubercular effusion cases with a sensitivity of 90\% to 100\% and specificity of 89\% to 100\%. Adenosine deaminase levels have also been considered by several researchers to differentiate tubercular diseases from non-tubercular diseases.\textsuperscript{17, 18}

The levels of adenosine deaminase(ADA) in pleural fluid offers high performance in its discriminating capacity to identify TP (sensitivity 87 to 100\%, specificity 81 to 97\%). Adenosine deaminase expresses the sum of two isoenzymes (ADA-1 and ADA-2). ADA-1 is ubiquitous in all cells, including lymphocytes and monocytes, whereas ADA-2 is found only in monocytes.\textsuperscript{19} Analysis and determination of these isoenzymes have shown that ADA in TP increases particularly at the expense of ADA-2 and that the ADA\textsubscript{1}/ADA\textsubscript{2} activity ratio improves performance in terms of sensitivity, specificity, and efficacy (100\%, 92 to 97\%, and 98\%, respectively) in correcting all false-negative and false-positive results except 1 to 9\% of nonlymphoproliferative malignancies. Only the high performance of ADA in the identification of TP allows it to be assumed that pleural biopsy can be obviated, especially in patients aged less than 35 years of age or having a lymphocyte-to-neutrophil proportion of more than 0.75 in regions of high prevalence. Quick determination and low cost justify its routine use in exudates. The ADA\textsubscript{1}/ADA\textsubscript{2} activity ratio improves performance even more and could be used in cases with uncertain diagnoses or in regions with low prevalence of tuberculosis.\textsuperscript{20}

Diagnosing tuberculosis early is very important to control the disease progression and prevent spread. Delay in diagnosis and in initiating treatment results in poor prognosis and sequelae in up to
Demonstration of Acid fast bacilli (AFB), tuberculin skin test, radiography, bacterial culture, interferon-Gamma and bacterial nucleic acid amplification are the various means to confirm the etiology. Though the diagnosis of TB using acid fast staining of sputum smear or standard culture is considered as’ Gold standard” however the sensitivity of the detection has been shown to be only 40-70%, but visualization of AFB in direct smears by Zeihl-Neelsen staining requires bacillary densities of 10,000/ml and therefore detects AFB only in open cases of TB. Detection of AFB in cultures also has a low sensitivity of 30-40% with the inclusion of BACTEC rapid culture technique, a sensitivity of 90% and specificity of 95% has been achieved; but this technique needs expertise and very expensive equipments. AFB culture is expensive and requires long incubation period and is positive in less than 25% cases.

ESR is not very sensitive test for the presence of disease and is usually found raised in chronic infection but significant numbers of patients with malignancy, chronic infection, or inflammatory disorders may have normal ESR.

Chest X-rays are valuable for detecting pulmonary lesions of tuberculosis but it is not suitable for field studies in developing countries and the activity of the disease cannot be judge with certainly. Moreover the findings can be due to other pathology, and pulmonary tuberculosis can have many other non-classic presentations with broad differential diagnoses.

Nucleic acid amplification test had a sensitivity of 79.3% a specificity of 80.3%. The test sensitivity in clinical trials was 95.5% and specificity was 100%. But this technique is very expensive, time consuming requires expertise and equipments.

Tuberculin skin test (Mantoux test) has limited sensitivity and specificity. In addition, the sensitivity of this test is reduced in HIV positive patients. The lack of a reliable and rapid test for smear-negative TB in HIV-positive patients means that the correct diagnosis is often missed and/or
patients are erroneously started on anti-tuberculosis therapy on the basis of clinical presentation alone.\textsuperscript{17}

Interferon gamma release assay is an innovative tool for detection of tuberculosis infection. Conflicting results have been reported in patients as the assay is more specific but less sensitive among immune-compromised individuals, and more so in a low prevalence country.\textsuperscript{31}

Incidence of Tuberculosis is not declining in the developed world including India even after 130 years from the first isolation of Mycobacterium tuberculosis by Robert Koch. In India Government is also understanding the seriousness and trying on warfooting to tackle the problem and has devised "Revised National Tuberculosis Control Program".

Efforts need to come from all directions to control this burning problem. The role of a biochemist can be to device a test for its early diagnosis. The review of literature indicates that different researchers are trying different means to devise some test for its early diagnosis. ADA estimation either in blood, CSF, synovial, ascitic or pleural fluid has shown promising results.

We thought of doing some work in this direction and decided to perform a study for the diagnostic role of ADA in lymphocyte rich pleural effusion.