CHAPTER – 1

INTRODUCTION

Tuberculosis has been a serious problem since a long time. In 1880’s the disease caused more than 30% of all deaths in Europe (Goldrick 2004). With the advent of Anti-Tuberculous antibiotics in late 1940’s, the battle seemed to be won. The German physician called Robert Koch received a Nobel for his discovery on finding the cause for TB which being the TB bacilli (Bhansli S.K. 1977). According to medlexicon’s medical dictionary tuberculosis is a specific disease caused by infection with mycobacterium tuberculosis, the tubercle bacillus, which can affect almost any tissue or organ of the body and the most common site of the disease being the lungs. Because of inadequate public health resources, reduced immune responses due to AIDS, the development of drug resistance and extreme poverty in the many parts of the world, the disease continues to be deadly (WHO 2006). TB is an acute or chronic infection caused by Tubercle bacillus (WHO 2008).

Tuberculosis as pulmonary T.B is the most common form of T.B (Paul et al 2006). Extra pulmonary T.B is comparatively less common. T.B disease kills 2 million people each year (William 2008). It can be summed up as occurring in 3 distinct phases, such as exposure, infection and disease (Donald 2004). To become infected there has to be an exposure of a person for a relatively longer period to the environment where the air is contaminated by the person coughing & sneezing having a lot of mycobacterial load (Dubos et al 1952). On exposure, the infection can go unnoticed’ therefore, various diagnostic tests are used at the stage. A PPD is normally performed at this stage which is a purified protein derivative skin test, it detects the exposure to the disease and this
is where the commencement of treatment should take place. People who have been infected with TB may not have a positive skin test if their immune function is compromised by chronic medical conditions, cancer chemotherapy, or AIDS. Additionally, 10%-25% of people with newly diagnosed tuberculosis of the lungs will also have a negative result, possibly due to poor immune function, poor nutrition, accompanying viral infection, or steroid therapy. Over 50% of patients with widespread, disseminated TB (spread throughout the body, known as military TB) will also have a negative TB test. Hence, to confirm the diagnosis biochemical estimation too becomes a necessity.

TB continues to be like a deadly dragon engulfing many lives per year (Corbett et al 2003), despite many health awareness programmes initiated by WHO, UNICEF, Central Govt., State Govt., NGOs, Education and innovations in the field of medical science, it is still considered frightening and fatal disease (Collins et al 1993). Therefore, it needs to be properly diagnosed and treated. Researches on Tuberculosis are being carried out to find out as to how this fatal disease can be prevented, treated and eradicated and to ascertain whether certain biochemical parameters show variation in patients with pulmonary tuberculosis (Tracy 2004) or not. The researcher inspired by the nature of disease, its intensity and research interest conceptualized the configuration of various parameters, such as Adenosine Deaminase (ADA), (Ocana et al 1993) Vitamin E (Nwanjo 2007), Glycoproteins namely Protein bound Hexose, Protein bound Hexosamine and Protein bound Sialic acid (Pari et al 2006), Anti-Oxidants enzymes (Gambhir et al 1997) namely Catalase, Glutathione Peroxidase and Super Oxide Dismutase and Lipid Peroxidation (Vijayakumar et al 2006) to find out what remains the status of these parameters in Pulmonary Tuberculosis Patients classified as sputum +ve and sputum –ve pulmonary tuberculosis.
patients both before and after the treatment (Leonard et al 2005)\textsuperscript{17} Hence, the researcher formulated a topic for research that has been entitled as:

“STUDIES ON VARIATION OF GLYCO-PROTEINS, VITAMIN E, ANTI-OXIDANT ENZYMES, ADENOSINE DEAMINASE AND LIPID PEROXIDATION IN PATIENTS SUFFERING WITH PULMONARY TUBERCULOSIS”

Tuberculosis:

T.B. is an air borne infectious disease and is contagious, it is important to note that not everyone exposed to the T.B. bacteria develops an active infection and becomes sick. People with positive PPD are not sick and are said to be having latent TB infection or exposure. It is a disease of respiratory system, the history of TB is long and it's as old as mankind. New and modified methods to tackle the disease have been brought to practice. Hebrews, proscribed that eating flesh of TB animals and masses forbade hunchbacks from entering the inner sanctuary. Human remains and usual sources of information show evidence of TB throughout Egyptian history as also artistic representation in pre-Columbian Americans. In Greco Roman Period, Hippocrates, Celsus, Cealius, Aurelianus have written a lot on TB (Alderman et al 2007)\textsuperscript{18}. Many hymns in Atharveda refer to pathology and usefulness of the drugs as well. Only 10% of people infected with mycobacterium tuberculosis ever develop this disease. Many people suffer TB in first few years while under infection, but the bacillus lies dormant in the body for years and many are able to overcome the initial infections without any dominant symptoms except for fever, dry cough and abnormalities that are visible from chest x-ray that is indicative of primary pulmonary TB. When more symptoms surface up, it is called active or full fledged pulmonary TB. Pulmonary TB goes away by itself but in 50-60 percent of cases the
disease returns if the immunity is low. The active pulmonary TB disease has more prominent symptoms such as fatigue, weight loss, cough containing sputum laden with blood. Of the 1.8 million new TB cases occurring annually, around 0.8 million are sputum positive pulmonary TB. One patient tested sputum positive can infect 10-15 persons a year if left untreated. TB is the world’s leading cause of death in humans from a single infectious agent. TB is chronic bacterial infectious disease that gets spread through air, droplets and particularly affects the lungs. It’s one of the prevalent infectious diseases all over the world and is a matter of great concern.

1.1 Types of Tuberculosis: (Milosh Sakulich, (1907))

1.1.1 Tuberculosis is broadly divided into two types such as

- **Pulmonary TB**: It affects primarily the lungs
- **Extra Pulmonary TB**: It’s the type of Tuberculosis that affects organs other than the lungs. It includes TB Meningitis, Lymph nodes TB, Abdominal TB, Laryngeal TB, Cavitary TB, Miliary TB, TB Pleurisy and Serosal TB.

**Types of Extra Pulmonary TB:**

- **TB Meningitis**: Membranes around brain are affected, headache, stiff neck, drowsiness and vomiting are its symptoms. It’s fatal and needs immediate attention. It may lead to permanent brain damage. Fatal cases of TB meningitis reveal sub ependymal tubercle (rich focus) that has ruptured into the subarachnoid space.
- **Lymph nodes TB**: Painless swelling in lymph nodes occur in the neck area armpits and other areas.
- **Abdominal TB**: Abdominal swelling occurs, lymph nodes of abdomen swell up but it is generally seen that abdomen or stomach
are less commonly affected. In the terminal ileum or cecum the most common manifestations are pain which can be misdiagnosed as appendicitis and intestinal obstruction.

Laryngeal TB—Affects throat, vocal chord area and it’s contagious.

Cavitary TB—It forms cavities in the lungs and is highly contagious.

Military TB—It affects the young or any one with weak immune system. It leads to a dangerous fever and nearly all organs are affected. Many small granules are formed in lungs and seen through chest X-ray.

TB Pleurisy—It develops shortly after catching the infection characterized by shortness of breath chest pain and fluid in the lungs.

Serosal TB—The clinical manifestations of serosal TB can reflect either or both of two process: 1. Multiplication tuberculosis within the space 2. Host inflammatory response to mycobacterium TB antigen hypersensitivity reaction. It is the latter of these processes that often predominates. It is a convenient term for appreciating the pathogenesis presenting the diagnosis of four syndromes: 1. Tuberculous pleurisy 2. Meningitis 3. Pericarditis, 4. Peritonitis

Pleural, subarachnoid, pericardial, peritoneal and synovial membranes, define spaces that under normal circumstances contain small amount of sterile fluid that serves to lubricate the under tissues or to allow freedom of movement, when this fluid is affected by the bacterium it can lead to impairment of the organ these spaces surround.
1.1.2 Common types of tuberculosis include:
- Pulmonary TB:
- Skeletal TB
- TB Pleurisy
- Laryngeal TB

1.1.3 Rare types of Tuberculosis
- Tuberculosis related to pelvic inflammatory disease.
- Genitourinary tuberculosis
- Cutaneous TB
- Miliary TB
- Cavitary TB
- Serosal TB
- Intramyocardial tuberculosis

Types of TB Mentioned in Various Sources: (Hatfield) Include
- Active TB-Fully active TB disease
- Latent TB infections – An inactive form also just called TB infection about 10-15 million people in the United States.
- Multi drug resistant TB newer TB strain those are resistant to many drugs.
- Miliary Tuberculosis causing multiple abscesses in various body parts.

TB as divided into 3 clinically important categories
1. Primary TB – It refers to infection development that eliminates the pathogen or results in a stalemate between mycobacteria and immune system, the immune system contains and prevents the spread so effective immunity is must.
2. Secondary reactivated TB – The infection is reactivated if mycobacteria rupture the tubercle and spread out.

3. Disseminated TB – It spreads within the body and is caused by floating macrophages through blood and lymph. Once infected symptoms of disease correspond to local infections, the antiquated term consumption arose from various systems associated with disseminated TB because those infected slowly just waste away.

1.2 Classification of Tuberculosis (George Schiffman 2010)\textsuperscript{22}

The TB is classified into three forms:

1. Minimal (Exudative or Proliferative)
2. Moderately Advanced (Exudative or Proliferative)
3. Advanced

Tuberculosis can be clinically classified by signs and symptoms (Jacobs, Kenneth 2005)\textsuperscript{23-24}

- Tuberculous pneumonia: Tuberculous pneumonia is highly contagious.
- Tuberculous Pleurisy: It appears in young people and is acute, the main sign is exudates.
- Extra pulmonary: It may occur in the context of 1. Miliary TB, 2. Reactivation of a pulmonary focus, 3. In the absence of clinical pulmonary disease.
- Tuberculous Meningitis: It is caused by mycobacterium tuberculosis Or bovis, the body shows neurological deficit.
- Ophthalmic Tuberculosis: leads to infection in the eye especially iris, ciliary body and choroids.
- Cardio Vascular Tuberculosis: It affects the heart, blood and pericardium, corticosteroids are used in the treatment.
Central Nervous System Tuberculosis: Affects the central nervous system.

Genitourinary Tuberculosis: causes piurea which is the cause of access to genitourinary infection is usually via blood.

Miliary Tuberculosis: is caused due to dissemination of bacilli in blood affecting different organs, symptoms are fever and other constitutional ones. If cultures are negative then liver biopsy must be performed and the mantoux test too is negative the disease is not contagious at this point.

Classification on the Basis of Extent of Infection (Thomas et al 2009)

1. Latent TB Infection (Bergamini et al 2009) – TB germs are present in the patient but no symptoms are revealed and is not contagious.

2. Active TB Disease- When TB bacteria is there in the lungs it multiplies with in weeks, months or even years after infection and leads to active state of the disease.

1.3 Pulmonary Tuberculosis

Pulmonary TB is one of the types of TB that affects the lungs, just because TB affects lungs primarily pulmonary TB is the another name for general TB. Even the extra pulmonary TB has its roots in the lungs but is non-contagious. Pulmonary TB is marked by the formation of granuloma in infected lung tissues, by cell mediated hypersensitivity that can potentially cause inflammation and fibro-cavitary destruction in the lungs, leading to chronic respiratory illness and deteriorates the quality of life.
This study has traced the variation of above mentioned parameters in the serum of sputum positive and sputum negative pulmonary tubercular patients before and after 9 months of intensive treatment. Analysis of Sputum has been the most elementary and common diagnostic test, where sputum being not a nasopharyngeal discharge or saliva but a material brought up from lungs after a productive cough.

1.3.1 Types of Pulmonary TB

If we talk of types or forms of pulmonary TB it is very difficult to sort them out from that of general TB as both have the same chief manifestations.

Primary and secondary TB are two distinct types of the disease with an entirely different natural history, onset, character and localization of the lesions course and termination. The late results of collapse theory and early results of chemotherapy contributed new facts regarding the process of regression of the disease and regarding arrest of the process of progression at an earlier stage than had been previously seen. From the facts thus collected, it became clear that all four forms of the disease exhibited are similar but reversed course during these two phases. It then seemed to be reasonable to describe this process as general law called as law of evolution in tuberculosis.

Types of Pulmonary TB (Stanley 2007) is as follows:

- **Primary pulmonary TB**: It's a form of pneumonia and affects people with low immunity such as children, elderly etc, gets manifested as pneumonia and is contagious.
- **Laryngeal TB**: Affects throat, vocal chord area and is contagious.
- **Cavitary TB**: Forms cavities in the lungs, it's highly contagious.
Miliary TB: - Affects the young or anyone with weak immune system leads to a dangerous fever, small granules are formed in lungs and can be seen through chest X-ray.

TB pleurisy:-- It develops shortly after catching the infection in many cases, it's characterized by shortness of breath, chest pain and fluid in the lungs.

Lymph node disease: Lymph nodes contain bacteria causing enlargement.

Tuberculosis peritonitis: M. Tuberculosis involves the outer linings of the intestines and the lining of abdominal wall, increasing fluid content.

Tuberculosis pericarditis: Pericardium is affected, space between and the heart is filled with fluid.

Osteal tuberculosis: Infection in bone may occur causing deformity.

Renal tuberculosis: Asymptomatic pyuria and can spread to reproductive organs.

Adrenal tuberculosis: Leads to adrenal insufficiency causing weakness.

TB meningitis: M. tuberculosis affects the meninges. This can be devastating leading to permanent damage.

### 1.3.2 Classification of Pulmonary TB

Albrecht 1907, gave the first pathological classification based on description of pathological lesions. The lesions were divided into three groups:

1. Indurating, Cirrhotic, heading
2. Nodular (Productive)
3. Caseous pneumonic (Exudative)
First Clinico-pathological classification was given by Bard 1927\textsuperscript{29}. He classified pulmonary TB into 4 forms:

1. Parenchymatus
2. Interstitial
3. Bronchial
4. Post pleuritic

Based on the Pathogenetic classification pulmonary tuberculosis is classified into two forms or types:

**(I) Primary Pulmonary TB:**

1. **Benign primary Tuberculosis:** It consists of a benign primary complex with its associated lesions, the benign primary complex consists of a primary focus and its tuberculous regional lymphatic node.
   
   (a) **Active benign primary Tuberculosis** - This includes persons who are radio-logically tested.

   (b) **Quiescent benign primary Tuberculosis** - It is manifested as a calcified nodule or round focus or incomplete residual lesion absorption

2. **Malignant primary Tuberculosis:** It is characterized by caseation in the original lesion and its regional node as well as in the associated foci. It is divided into two forms namely:
   
   (a) **Active malignant primary tuberculosis** - This has senous sub forms, lobar caseous pneumonia bronchopneumonia or acute miliary TB.
(b) Quiescent malignant primary tuberculosis: Characterized by calcified nodules or round foci or again no residual lesion

(II) Secondary Pulmonary T.B:
Secondary Pulmonary T.B is the advanced stage of Disease and is characterized bi-lateral fibro caseous disease followed by the presence of an associated foci in the opposite lung to that of initial lesion.

1. Fibrous Form–
The initial lesion of the fibrous form is characterized by multiple small tubercles or small areas of inflammation, they arise not by inhalation but by reactivation and haemic spread to produce following types of lesions:-
- Disseminated chronic miliary TB
- Localized fibrous TB with emphysema
- Diffuse tuberculosis with emphysema
- Fibro cavitaria –where cavitation occurs typically within a caseous focus. This occurs in both active and latent disease.

(a) Active forms-
- Benign primary
- Malignant primary
- Minimal secondary
- Moderately advanced secondary
- Advanced secondary
- Fibrous secondary
- Fibro cavitaria secondary
- Non pulmonary
(b) **Quiescent forms - residual lesions of any of the above.**

2. **Fibrocaseous Form:**

   It is characterized by Minimal lesion every case of minimal lesion should be treated. It seems that the most rapid method of treating is chemotherapy which major may not be followed by collapse therapy.

   Major surgery is even less justifiable.

   (a) **Active Fibrocaseous Form:** This includes unilateral and bilateral minimal moderately and advanced disease as well as abortive tuberculosis. The central focus of lamellated hyaline fibrous tissue is totally consistent with old TB

   (b) **Quiescent Fibrocaseous Form:** Includes residual lesions, calcified or round foci and no visible residual lesion (Complete Absorption).

   Pulmonary TB is also further divided or classified into two categories on the basis of development of infection:–

<table>
<thead>
<tr>
<th>Primary TB</th>
<th>Reinfection of Exogenous or Endogenous TB</th>
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<tr>
<td>It is one of the form of pulmonary TB and is also classified as A) perihilar in filtrate, B) fissular infiltrate. With or without interlobar exudation.</td>
<td>It is based on the morphological elements interpreted from chest plates ego Nodular, Parenchymal, Inflammatory and mixed forms. Nodular is further classified as- A) The form of early infiltrate B). Nodular dissemination, C) Generalized Nodular (Miliary) Parenchymal can be classified as- A) Form showing homogenous path. B) Showing non-homogenous path. Mixed Form IS classified as - A) One with prevailing nodular alteration B) Prevailing patchy infiltrations.</td>
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A logical and broader classification of acute pulmonary TB as obtained from many sources is:

- Acute general miliary TB
- Acute pulmonary miliary TB
- Acute pneumonic TB
- Acute bronchopneumonic TB.

Both acute general miliary and pulmonary miliary are hematogenous infection. Acute bronchopneumonic TB is like bronchopneumonia but the former is practically a bronchopneumonia in which tubercle bacillus is the specific factor.

According to Papanicolaou (1951), Ten selected findings for each of four categories have been arranged in a pattern. Each finding is assigned a digit form, from 0 to 9 and as the order of the digit increases, the finding increases in seriousness. The code then is a four digit number, with the digit positioned so that the first digit always relates to the first category. (T) tubercle bacilli, the second to (C) cavities, the third to (L) lesions and the fourth to (F) principal symptoms.

Comparison of four digit code for the same individual examined at different times, readily reveals progress of the disease, when the latest code contains digits that are higher than before. A patient's improvement is reflected by a code with digits of a lower order.

1.3.3 Symptoms

Symptoms of pulmonary TB and general TB are almost same as the complexity of tuberculous lesions and other clinical features such as fever, night sweats occur in both of them (Beers. et al (1999)). In some patients symptoms are pronounced but lesions are slight, whereas in some patients lesions are extensive, easily discernible and slight clinical manifestations occur.
The physical examination (Iseman 2007)\textsuperscript{32} such as:
- Swollen liver
- Swollen lymph nodes
- Swollen spleen

In early stages pulmonary tuberculosis is silent but as it advances following symptoms (Kenneth 2005)\textsuperscript{33} get manifested:
- Coughing out blood
- Cough producing phlegm
- Sudden weight loss
- Night sweating which is a severe problem with patients of pulmonary TB and is called as Hyperhidrosis.

Some additional symptoms (Dixon et al 1999)\textsuperscript{34} in the advanced disease can be:
- Wheezing
- Excess of sweating
- Chest pain
- Breathing difficulty

Normally, the primary stage of the disease which is the Inactive Pulmonary tuberculosis doesn't cause any apparent symptoms but some of the problems that the patient may encounter are:
- Loss of appetite
- Vaguely unwell, lethargy
- Intermittent cough blamed on smoking or cold
- Greenish or yellow sputum coughed out when the person gets up in the morning with the advancement of the disease the blood content increases.

If the infection is allowed to escape from lungs into chest cavity or if the fluid gets collected in the pleural space as pleural effusion, the patient faces difficulty in breathing. As the infection spreads to lymph
nodes in the sides and the back of the neck the infection breaks out through skin and the pus comes out.

**1.3.4 Etiology of Pulmonary Tuberculosis**

T.B. is caused by the intracellular pathogen (Russel)\(^{35}\) Mycobacterium tuberculosis (MTB). Since, MTB has a cell wall but lacks phospholipid outer membrane (Me Neil et al 1991)\(^{36}\) it is classified as gram positive bacterium. The Mycobacterium complex includes for other TB causing Mycobacteria M.bovis M. africanum M. canetti and M.microtti.(Osgood 2009)\(^{37}\)

Other known pathogenic Mycobacteria include mycobacterium leprae, mycobacterium aviam and M.kansasii (Kent PT et al 1985)\(^{38}\). It can withstand weak disinfectants and survive in a dry state for many days. The main single causative agent is the mycobacterium TB which is a highly successful pathogen that parasitizes the macrophages of the host (Collins 1996)\(^{39}\) Four types (Stewart 1983)\(^{40}\) of mycobacterium tuberculosis are recognized such as human, bovine, murine and avian. The human and bovine are of clinical importance. Avian mycobacteria causes rare infections. The mycobacterium TB causes TB and is a member of a large mycobacterium TB complex, this complex constitutes of M. bovis, M. Africanum, M.Microti (Niraj Shende et al 2007)\(^{41}\)

Mycobacterium TB is classified as a prokaryotic bacteria that has cell wall and no nuclei (Barsdale et al 1977)\(^{42}\). The phylum Actinobacteria belonging to the order Actinomycetales, consists of bacteria that are widely same in there morphological, physiological and genomic characteristics. Actinobacteria are coccoid to rod shaped to filamentous, they are gram positive with a high guanine and cytosine content in their DNA. When the order of actionmycetales is taken we see that actinobacteria takes the form of a rigid bacilli or filamentous cells
that have a tendency to branch. Thus, mycobacterium TB belongs to actinomycetales because the bacteria is rigid and rod shaped.

1.3.5 **Those at greater risk (Fisher et al 2000)**

Risk factors (Bass 2008) for pulmonary tuberculosis can be categorized as follows:

**Category A:**
- HIV
- Fibrotic lesion CXR
- Recent contacts with TB cases
- Organ transplant or other immuno suppressed patients

**Category B:**
- Newly positive tuberculin reaction.
- Positive tuberculin test in persons with inactive TB on Chest X-ray without any previous diagnosis of active TB.
- Those using corticosteroids and some of the new prescription anti-inflammatory drugs such as Adalimumab, Humira, Enbrel etc.
- Medical conditions such as diabetes silicosis chronic renal failure.
- Immuno-suppressed people with high oxidative stress and being Iowan antioxidant status are quite susceptible.
- If any patient suffering with latent TB contracts HIV then the chances of developing Active disease is more (Wong 2008) than in the one who is HIV negative and suffering with latent TB.
- Poor medication and carelessness observed in seeking treatment just because the patient starts getting a little better leads to drug resistant TB.
- Smokers, passive smokers, alcoholics and the one’s doing drugs at much greater risk.
- Hyponatremia/hypokalaemia, older patients, who may have co-existent chronic bronchitis can be missed upon unless a chest x-ray is taken and they are at higher risk.
- HIV people doing drugs
- The fetus has the chances of developing congenital TB from the infected mother.
- Increase susceptibility to opportunistic infections in carcinoma can lead to reactivation of TB.
- Patients with tumors, at the edge of pre existing scars, parenchymal scars stimulate a typical epithelial cell proliferation and metaplasia involving terminal air space, are at higher risk.
- Coexistence of bronchogenic cancer with TB is an atypical cause of the latter.
- Sudden appearance of new lesions, Atelectasis, unilateral hilar enlargement, coexisting lung carcinoma indicates presence of bacteria and increased susceptibility towards TB.
- Individuals who have prolonged exposure to re-circulation of air containing infectious droplet nuclei.
- Individuals that are exposed to inadequate ventilation that result in either insufficient dilution or removal of infectious droplet nuclei.
- TB is stigmatized and many people are reluctant to believe that they have any latent infection.
1.3.6 Transmission of Pulmonary TB (Bloom\textsuperscript{48} 1992 and Valway et al 1998)\textsuperscript{49}

(a) Transmission of Infection from Person to Person (Cantrell S.A et al (2008))\textsuperscript{50}:

Pulmonary TB IS transmitted by the inhalation of air borne organisms. Infectious particles are generated when individuals with pulmonary TB cough, sneeze or speak. Once the infectious particles are aerolized, they spread throughout the room or building by air currents and are inhaled by another individual (Anderson et al 2006)\textsuperscript{51}. Initially, the particles are sufficiently large and they can't be trapped by respiratory system of the person who is inhaling but when the exhaled particles remain air borne they become dehydrated and decrease in size until they are 1 to 5 microns in size (Mandell 2000)\textsuperscript{52}, particles of this size are called as droplet nuclei. The droplet nuclei easily avoids the defense system of the upper airway, the bronchi and can reach the alveoli (Friedman 1997\textsuperscript{53}) where the infection occurs fast.

Mycobacterium bovis can live in animals and it's an exception. In developing countries children become infected by drinking unpasteurized milk from infected cattle. The bacterium survives in air for several hours if another person breathes in the air he/she may catch infection. People with treated TB and extra pulmonary TB do not expel bacteria.

(b) Transmission with in the Body (Pelczar et al 2008)\textsuperscript{54}

-TB infection some times spreads in the blood stream and lymphatic system (Kong. Y et al 2007)\textsuperscript{55} and causes infection in other parts of the body such as:

-Lymphglands
-Gut and abdomen
-Bones and joints
- Heart
- Kidneys & Bladder
- Brain
- Skin

- Depending upon which part of the body is affected various symptoms may occur.

Transmission within the body is called as progression or dissemination of disease. Once inhaled tubercle bacilli reach the alveoli, the macrophages take up the bacilli, these bacilli multiply there and then spread through lymph vessels to nearby lymphnodes (Goldman 2000). In advanced cases the bacilli may move through blood vessels to distant organs as well. As, TB tries to spread to other parts of the body, it is interrupted by body's immune system. The immune system forms a scar tissue or fibrosis around TB bacteria and this helps fight the infection and prevents the spread of the disease.

The route by which the disease spreads from lungs to stomach, pancreas, liver spleen is however not clear but three routes are possible (NicasM et al 2005).

1. Haematogenous
2. Direct extension from neighboring organs particularly the caseating lymphnodes.
3. Retrograde spread from lymphatics.

Haematogenously disease spreads from lungs to stomach, spleen, pancreas and liver with some involvement of lymphnodes. Hematogenous dissemination of mycobacterium tuberculosis occurs into the membrane of a localized lesion. Disease may spread through lymphatics from hilar and tracheobronchial lymphnodes to pancreateo splenic lymphnodes with
subsequent affliction of stomach, spleen and pancreas, this however fails to explain tuberculous lesions in the liver to some extent.

Granules are seen in lungs indicating military tuberculosis revealing dissemination of the disease,

1.3.7 Pathogenesis (Smith 2003)\textsuperscript{58}

The step by step development of a disease is called as Pathogenesis (Maartens G. 2002)\textsuperscript{59}

Development of the disease takes place via known and unknown pool of infections (Vinay et al 2007)\textsuperscript{60}

(1) Known pool of infection: It has diagnosed +ve sputum patients and it consists of potential infectors

(2) Unknown pool of infection: It has undiagnosed patients and it is dealt with in two ways:-

(A) Environmental hygiene;
- Avoidance of over crowding
- Ventilation
- Spitting

Environmental hygiene is required to reduce the chance of infection by unknown infectious cases,

(B) Case findings: to reduce size of unknown pool to minimum.

Stages of Pathogenesis: (WHO 2007)\textsuperscript{61}

Rich's five fundamental factors affecting the pathogenesis (milosh seulich):

1. Quantity of Pathogenetic tubercle bacilli
2. Virulence of the infecting bacilli
3. Natural resistance
4. Acquired resistance
5. Hypersensitivity
**Stage No.1** Droplet nuclei are generated by talking, coughing and sneezing. Talking for five minutes generates 3000 droplet nuclei (Nancy 2009). Sneezing generates the most droplet nuclei by far that can spread to individuals up to 10 feet away.

**Stage No.2** Begins shortly after infection MTB multiplies virtually unrestricted within inactivated macrophages until the macrophages burst (David.H. Hail et al 2008). Other macrophages phagocytose MTB, but they are also inactivated and cannot destroy the bacteria (Van Crevel et al 2002).

**Stage No.3** At this stage lymphocytes begin to infiltrate, lymphocytes specially t-cells recognize processed and presented MTB antigen in context of MHC molecules.

**Stage No.4** Though many activated macrophages can be found surrounding the tubercles (Porth 2002), many other macrophages present remain un-activated and poorly activated MTB use these macrophages to replicate and hence, the tubercle grows.

**Stage No.5** For unknown reasons the caseous centers of the tubercles liquefy (Riley 2008), this liquid is very conducive to mycobacterium tuberculosis growth and the organism begins to multiply extra cellularly.

According to Crofton and Douglas" Factors that lead to the development of full blown disease (Active disease):

- Genetic: It includes Racial susceptibility and genetic difference.
- Physiological: Tall, thin men are more liable to tuberculous disease.
- Environmental: It includes Nutrition, Housing Conditions and Occupation.
- Toxic: The relative high incidence of TB In alcoholic IS well known.
- Immunological: Those with low immunity are highly susceptible towards deficiency diseases.
- Psychological: Emotional factors contribute to development of clinical TB.

1.3.8 Prognosis of Pulmonary TB

With treatment almost everyone makes a full recovery, TB bacteria multiply quite slowly compared to most other bacteria, therefore active TB tends to cause an illness that gets worse. Some people survive without treatment and may even fully recover, outlooks tends to be worsened where the TB is more difficult to treat. If some one has TB along with HIV then antiviral treatment has to be started for HIV patients(Small 1991). It has been generally seen that symptoms may improve in 2-3 weeks but chest x-ray doesn't show any improvement so soon, x-ray to give clear report takes time. Resection of the organ affected is not required (Barnes et al 1928). It has been observed that those at greater risk can develop drug resistant and potentially incurable forms of TB. Many side effects (John 2010) such as:
- Liver problems
- Vision changes
- Neuropathy
- Orange coloured tears occur if doctor is not seen urgently or the medication is discontinued breaking apart the norms of full fledged treatment:

The recovery from TB is good for most patients. If early, certain and clear diagnosis is made then it can prevent the disease from taking it's toll on the patients. Only along with proper and regular medication as directed by the physicians can the diagnosis become a success (Lawrason Brown et al). Hospitalized patients with active TB should be monitored
for relapse by having tuberculin skin test and sputum AFB smear examination every two weeks. The success of treatment relies heavily on patient's compliance and direct supervision should be the aim of any treatment programme. Compliance is important in preventing drug resistance.

Miliary TB, which is one of the fatal forms of TB, even this can be checked if treatment is given at the right time keeping in mind the drug susceptibility of the patient.

Extensively drug resistant TB (EDR- TB) (Cowley. D et al 2008) and multiple drug resistant TB (MDR- TB) (Fauci et al 2008) are also treatable and may require a longer time span.

By the use of prompt drug therapy and empiric therapy, negative chest x-rays and sputum smears can be achieved within 4-8 weeks.

Reliability of code system and it's proper application helps in providing the right diagnosis and good knowledge of prognosis.

Once the latent TB is identified the treatment must begin otherwise there are chances that infection may progress towards active disease (Roper 1955). Latent TB gets cured by the standard 9 months therapy in 96% of cases. Many a times long time intake of few medicines results in disorders of mineral metabolism that worsens the prognosis.

**1.3.9 Epidemiology of Pulmonary Tuberculosis (Comstock 1982)**

A person with active TB but untreated can infect 10-15 other people per year (Murray A.B. et al 1996).

Incidence (cases arising in a given time period) indicates the burden of tuberculosis and size of the task faced by national TB control
programme. In 2007 the estimated global incidence of active TB disease was 9.27 million cases. The five countries with the highest burden of TB in terms of total number of incident cases are— India (1.96 million) (Chakraborty2004), China (1.31 million), Indonesia (0.53 million), Nigeria (0.46 million) and South Africa (0.46 million).

2007 Tuberculosis Data:
   1. Estimated global incidence 9.27 million cases
   2. Incidence rate 139/100,000
   3. Prevalence: 13.7 million cases
   4. Prevalence Rate: 206/1,00,000
   5. Sputum smear- Positive incident cases 44%
   6. HIV coinfection: Incident cases 15%,Prevalent cases 5%

WHO (1997) recommends:
   (a) Intensified case finding for TB: interventions among patients with active TB:
      1. Testing for HIV
      2. If HIV infected:
         (a) Cotrimoxazole Prophylaxis
         (b) CD4+ cell count measurement to assess need for HAART.
      (b) Interventions among HIV infected patients the three I's–
         1. Intensified case finding for TB
         2. Isoniazid preventive therapy
         3. Infection control for TB
      (c) Incidence changes: changes in the transmission or changes in rate at which people infected with Mycobacterium can develop TB disease. Effect of TB control is less than prevalence or mortality.
      (d) Millennium development: Target 6C is to “have halted by 2015 and begun to reverse the incidence of malaria and other major diseases”.
Incidence in United States and other countries that are developed is low (Heymann 2004)\textsuperscript{79}. 1/3 of the world's population about 2 billion people are infected with TB. 200 million people will become active in any given year (Sudre 1992)\textsuperscript{80}. MDR -TB is rare in many countries but is becoming a serious problem in many parts of the world where incomplete treatment and empiric therapy (Richeldi et al 2007)\textsuperscript{81} are prevalent.

- Every second, someone in the world is newly infected with TB.
- Nearly 1% of world's population is newly infected with TB each year.
- 200 million people world wide or 10% of those infected will develop active TB and be in a position to infect others for 3 decades.
- Six – Eight million new cases of TB are diagnosed each year. In the last 100 years 200 million people have died of TB.
- It kills 8000 people a day 2-3 million people each year.
- It kills more people than AIDS or malaria
- 10% of infected people (who don't have HIV or AIDS) develop active TB at some point in life.

1.3.10 Prevention:

Prevention of pulmonary tuberculosis takes place in the following ways:

1. Stopping the spread of Infection:
   a) Right diagnosis with prompt treatment (Sree Ram Reddy et al 2009, WHO 2008)\textsuperscript{82} needs to be given.
   b) Immunity compromising lifestyle must be avoided at best.
   c) Application of STOP strategy under DOTS programme is implemented in the Global plan to stop TB, 2006-2015 (Piot et al2009)\textsuperscript{83}
d) Vaccination (Bretscher 1992)\textsuperscript{84}: BCG vaccination is done, and BCG immune therapy works best in bladder cancer. Prevention through vole bacillus vaccination is also provided, if high dose of bacilli is given there is a lupoid reaction, when a lower dose is used the proportion of individuals whose tuberculin test converts from negative to positive is small.

2. Chemoprophylaxis: It is the process of giving of chemo therapy to prevent development of tuberculous disease and it is divided into two:

a) Primary chemoprophylaxis- Use of isoniazid in preventing the disease.

b) Secondary chemoprophylaxis- Use of chemo therapy on individuals whose only evidence of disease is their positive tuberculin skin test. (Sandler et al 2009)\textsuperscript{85}

1.3.11 Treatment:

1. Drug management (Grant et al 2008)\textsuperscript{86} Treatment or the chemoprophylaxis or the chemotherapy must be started while the disease is in inactive state to prevent it from becoming active and must not be stopped at random .The era of chemotherapy (David et al 2009)\textsuperscript{87} began with the isolation of streptomycin from 1. Streptomycyes griseaus. 2. Isonicotinic acid hydra-azide synthesis 3. Para-amino salicyclic acid. 4. Isonicotinic acid derivatives. Ethionamide and prothionamide chemical complexes used in drugs are derivatives of isonicotinic hydrazide acid 5. Rifampicin-Group of antibiotics produced by streptomycyes meditteraene (McNerney2000)\textsuperscript{88} It is a semisynthetic derivative of Ethambutol, it was discovered in 1961 and is a second line drug and is now one of the standard Anti-TB drugs but the drug has toxic effect (Menzies et al 2008)\textsuperscript{89} on eyes or cases resistant to streptomycin (Parsons et al 1997)\textsuperscript{90}.
chemotherapy has it's own credits such as patients don't die but are cured, period of infectivity is reduced and relapses are avoided.

The goal of treatment is to cure the infection with drugs and remove the bacteria. The most commonly used drugs (Toth et al 2004, Zhang2007)\(^1\) are: 1. Rifampicin 600 mg OD to be given 30 minutes before food. 2. Isoniazid 300 mg OD to be given 30 minutes before food. 3. Pyrazinamide 2 mg (1.5 mg if weight is less than 50 kg) to be given for 2 months.4. Ethambutol 25 mg/kg given for 2 months, Streptomycin can be substituted for Ethambutol. 5. Pyridoxine for patients at higher risk of isoniazided induced peripheral neuropathy, Diabetes mellitus, Chronic renalfailure, malnutrition, alcohol abused and HIV positive.

Steroid treatment (prednisolone) is recommended as an additional form of treatment for some forms of TB e.g. T.B of brain or of heart. Health care providers watch over patients that are found negligent towards taking the treatment that's called as DOT (Direct observe therapy).The chemotherapy must be an empiric therapy based on drug susceptibility (Lobato et al 2008)\(^2\) High quality drugs must be available in the market.

Good diagnosis is needed when dual disease ie lung cancer and in active pulmonary tuberculosis occur together in the body (Van Beurden et al 2002)\(^3\) So, to prevent inactive pulmonary tuberculosis from becoming active accurate diagnosis is required.

2. **Appropriate Education and Counseling** (Jensen et al 2005)\(^4\)

Appropriate Education and Counseling require awareness programmes.

3. Isolation (Flores 2008, Willeke et al 1998, Decker 1993)\(^5\): Patients of pulmonary tuberculosis are advised to live in isolation, either
hospitalized or kept in a place where there is no danger that others get infected.

The variation of the parameters has been studied in reference with sputum positive and sputum negative tubercular patients. The detailed description of the parameters considered in the study is being given below:

1.4 Glycoproteins

These are complexes in which carbohydrates are attached covalently to asparagines (N-Glycans) or serine/threonine (o-glycans) residues of peptides. Most proteins synthesized in mammalian cells are glycosylated. The Nand O-glycans contain different core structures but biologically active Oligosaccharides are often found on outer chains attached to these cores. In addition, Glycoproteins with N-acetyl glucosaminylated or mannosylated serine/threonine residues are found in various cellular compartments and tissues. Attachment of oligosaccharides to peptides increases solubility, cover the antigenic proteases like polysialic acid attached to neural cell adhesion molecules, and (N-CAM). The carbohydrates often modulate protein functions, in contrast the carbohydrate moieties of serum glycoproteins and pituitary glycoprotein hormones are involved in their clearance from the circulation by targeting of hormones to respective organs. N-Glycosylation occurs in most animal species. Sialic acid moiety of carbohydrate epitope is important for biological interactions including cell adhesion to selectin and lectins(Olden et al 1982)\textsuperscript{96}. Sialic acid is an important constituent for the characteristic changes of transformed cells.
GLYCOPROTEINS

Glycoproteins found in a variety of tissues including the arterial wall are very similar in structure and composition to those in circulation (Latha and Pari 2003). Therefore, vascular complications that involve complex protein carbohydrate molecules could contribute to an increase in serum glycoproteins.

1.5 Vitamin-E

Vitamin E is the generic descriptor for any of a group of several related fat soluble organic compounds tocopherols and tocotrienols that act as vitamins with antioxidant properties (Walker 1992). In particular vitamin E is associated with a tocopherol, also written as \( \alpha \) tocopherol.

Generally 8 basic forms of vitamin E are recognized that is 4 tocopherols and 4 tocotrienols (Herrera and Barbas 2001). Antioxidant vitamins such as E, C and betacarotene have health promoting properties (Matthew H 2006). They protect cells, it’s added to others and is a collective name for a group of fat soluble compounds with distinctive antioxidant activities. The free radicals damage cells (Kumar et al 1993) and contribute to the development of the cardiovascular disease and cancer. Unshared electrons are very energetic and react with oxygen to form reactive oxygen species ROS (Fujita 2003) the intake of free
radicals by the body from environmental exposures and endogenous production of ROS when food is converted into energy builds up the reservoir of reactive oxygen species.

Vitamin E

The air pollution, cigarette smoke and ultra violet radiation from the sun cause an increase in the level of free radicals. Vitamin-E is a fat soluble antioxidant that halts the production of ROS formed when fat undergoes oxidation (Christopher et al 2008)\(^{103}\). Vitamin-E is involved in immune function (Van Acker et al 1993)\(^{104}\), cell signaling regulation of gene expression and other metabolic processes. \(\alpha\)-tocopheral inhibits the activity of protein kinase C, it's an enzyme involved in cell proliferation and differentiation in smooth muscle cell, platelets and monocytes (Shephard et al 1974)\(^{105}\). Vitamin increases the expression of two enzymes that suppress arachidonic acid metabolism causing an increase in the release of prostacyclin \(\alpha\) from the endothelium which causes dilation of blood vessels and inhibits platelet aggregation (Burton 1994)\(^{106}\). It is one of the four fat soluble vitamins.

The vitamin is synthesized by plants and has eight different iso forms called vitamers divided into two classes of 4 each (Traber2007)\(^{107}\). Each vitamer is comprised of 6 chromanol ring and an isoprenoid side chain. The groups attached to R1, R2 and R3 positions on 6 chromanol ring determine whether the vitamer is \(\alpha\), \(\beta\), \(\gamma\) and \(\Delta\).

It is an integral part of cell membranes that defends the cell against oxidation. Within cells and organelles vitamin-E is the first line of defense against lipid peroxidation and it plays an important role in lending RBCs flexibility as they make their way to the arterial network.
Absorption of vitamin-E is highly dependent upon the processes that are utilized during fatty acid digestion and metabolism (Brigelius-Flohe2009)\textsuperscript{108}. Vitamin-E absorption takes place through micelle and chylomicron formation. Bile acid is essential for vitamin-E absorption and micelle formation. Once formed the micelle is able to cross the unstirred water layer and releases it's content into enterocyte upon reaching the basolateral surface of the enterocyte (Azzi2007)\textsuperscript{109}.

Vitamin-E is surrounded by chylomicrons and then transported throughout the body. After 5 minutes the chylomicrons are broken down by lipase and contents are dispersed through a variety of paths. It is a lipid soluble vitamin and 90% of vitamin-E is found in adipose tissue, in plasma the concentration of vitamin-E is approx. 27 \( \mu \text{mol/l} \). It is excreted viabile, urine, feces and skin. It is oxidized to form hydro quinone and is then conjugated to form glucuronate to be excreted into bile or degraded in the kidneys and excreted in urine (Rakel 2007)\textsuperscript{110}. Fecal excretion is the main route of vitamin E elimination, it is considered as the main chain breaking antioxidant in membranes and helps to reduce production of prostaglandins such as thromboxane that cause platelet clumping. It stabilizes the membranes by increasing the orderliness of membrane lipid packaging.

\( \alpha \) tocopherol is the only form that meets human requirements (Baker H , Hamrick et al 2008)\textsuperscript{111} Serum concentrations of vitamin-E depend on the liver that takes up the nutrient after it has been absorbed from the small intestine. Liver preferentially resecretes (Ausiello 2007)\textsuperscript{112} only \( \alpha \)-tocopherol via the hepatic \( \alpha \) tocopherol transfer protein, the liver metabolizes and excretes the other vitamin-E forms therefore, blood and cellular concentrations of other forms of vitamin-E is usually less than \( \alpha \) tocopherol therefore, less research is conducted on them.
1.6 Anti-Oxidant Enzymes

Antioxidant enzymes are compounds that may reduce the energy of free radicals from forming in the first place (Utoila et al 1993, Dormandy 1978)\textsuperscript{113}, or interrupt an oxidizing chain reaction to minimize the damage caused by free radicals (Laguerre et al 2007, Crapo 2003, Karlsson 1997)\textsuperscript{114}. Free radicals are believed to play a major role in more than 60 health conditions including the ageing process, cancer, artheroscleroris (Parkeet al 1996)\textsuperscript{115}. By reducing exposure to free radicals and increasing intake of antioxidant nutrients that have the potential to reduce the risk of free radicals-related health problems (Ray et al 2002)\textsuperscript{116}, anyone can lead an healthy life.

The body produces several antioxidants enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) that neutralize many types of free radicals (Mayne 2003, Heffner 1989)\textsuperscript{117}. Supplements of these enzymes are available for oral administration. However, their absorption is probably best by supplementing with the building blocks the body requires to make SOD, CAT and GPx (Halliwell.B 1994, Yu 1994)\textsuperscript{118}. These building block nutrients include the Minerals, Manganese, Zinc, Copper for SOD, and Selenium or Glutathione Peroxidase. In addition to enzymes many vitamins and minerals act as antioxidants in their own right such as vitamin C, vitamin-E, Beta-carotene, lutein, lycopene, vitamin B\textsubscript{12}, coenzyme Q-10, cystiene (an Amino Acid) (Noguchi et al 2000)\textsuperscript{119}.

1.6.1 Catalase

Catalase is an antioxidant enzyme which is like SOD and GPX. Catalase is one of those antioxidant enzymes found in nearly all living
organisms that are exposed to oxygen where it catalyzes the
decomposition of hydrogen peroxide to water and oxygen (Lurie
1964). It has one of the highest turn over numbers of all enzymes one
molecule of Catalase converts millions of molecule of hydrogen peroxide
to water and oxygen per second. It's a tetramer of 4 polypeptide chain
each over 500 amino acid long. Catalytic activity is present in nearly all
animal cells and organs and in micro organisms. (Clarkson 1995)
Study on it's biosynthesis have been reported by (Higashi et al 1974, Sakamoto
and Yasukochiet al 1974) like peroxidase, it's a hemoprotein and forms
intermediate addition compounds with H₂O₂. The stepwise mechanism of
it's activity was given by (Nicholls and Schonbaum 1963) have
reviewed catalase. Catalase uses H₂O₂ as a substrate as well as a
hydrogen acceptor. (Lanir and Schejter 1975) have reported on the difference in the reactivity of 2 enzymes. Catalase is of interest when
ever hydrogen peroxide is used as a germicide example in the food
industry. Microencapsulated catalase is also of interest. Catalase, helps
the body to convert hydrogen peroxide into water and oxygen thus
preventing the formation of carbon dioxide bubbles in the blood. It uses
hydrogen peroxide to breakdown potentially harmful toxins in the body
including alcohol, phenol and formaldehyde. Our body produces free
radical that damages our cells, cell membranes, proteins and DNA.
Antioxidants are agents that scavenge ROS (Kinnula et al 1995, Mameett et al 2002) prevent their formation and fix the damage they cause, (Halliwell 1991) The complex system of antioxidant enzymes consists of superoxide dismutase, catalase, and glutathione peroxidase, glutathione ancillary enzymes (such as glutathione reductase, glutathione-S-transferase, glucose-6-phosphate dehydrogenase), metal binding proteins (transferrin, ceruloplasmin, albumin) alpha tocopherol, ascorbate β-Carotene, flavonoids and urate (Sies 1991, Halliwell 1994). Of all the antioxidant enzymes found Catalase and Glutathione peroxidase mainly eliminate free radicals. Catalase is detected in the cytoplasm and mitochondria (Radi et al 1991, Kinnula et al 1995, Fridovich 1998). The Catalase gene is located in chromosome II. It has a relatively minor role in catabolism of $H_2O_2$ at low rates of $H_2O_2$ generation but becomes important as the rate is enhanced (Jones et al 1981). Catalase can be detected in human type II Pneumocytes, it's low expression is seen in endothelial cells, smooth muscle cells, bronchial epithelial cells and Clara cells (Coursin et al 1996, Kinnula et al 1992). Catalase activity has been detected in the epithelial lining fluid of normal human lower respiratory tract (Cantin et al 1990).

1.6.2 Superoxide Dismutase

It was discovered by Irwin Fridovich and Joe Mccord these enzymes were previously thought to be possessing several metallo proteins with unknown functions, several common forms of SOD exist. They are proteins co factored with copper and zinc or manganese iron or nickel. Brewer 1967 identified a protein, which became known as superoxide dismutase, as an indophenol oxidase by protein analysis of starch gels using the phenazine tetrazolium technique. There are three
major families of SOD depending on the metal cofactor. Copper/Zinc which binds both Cu and Zn, Fe and Mn types which bind either Fe or Mn and finally the Ni type which binds nickel.

**Super oxide Dismutase**

Super oxide dismutase is an enzyme that catalyzes the dismutation of superoxide anion into hydrogen peroxide (luo et al 1997)\textsuperscript{134}. The most popular of the enzyme forms are copper, zinc, super oxide dismutase. Superoxide dismutase (SOD) (EC1.15.1.1) is important in antioxidant defense in nearly all cells exposed to oxygen (Gregory et al 1974)\textsuperscript{135} It is widespread in nature and is present in all oxygen metabolizing cells. One of the exceedingly rare exceptions is lactobacillus plantarum and related lactobacilli which use a different mechanism. It protects oxygen metabolizing cells against harmful effects of superoxide free radicals (Petkau et al 1975, Fridovich 1972, 1973; Lavelle et al 1973; Pashen and Weser1973)\textsuperscript{136}.

Mc.Cord (1974, 1969)\textsuperscript{137} found that SOD protects hyaluronate against depolymerization by free radicals and indicated that exogenous SOD might have an anti inflammatory effect (Salin and Mc.Cord 1975)\textsuperscript{138}.The $O_2^{-}$ which has been considered important in ageing ,lipid peroxidation and the peroxidative hemolysis of red blood cells (Fee and Teitelbaum1972)\textsuperscript{139} is formed by the univalent reduction of $O_2$ during
various enzymatic reactions or by ionizing radiation. A superoxide radical formation occurs during leukocyte phagocytosis (Allen et al 1974). Hewitt and Morris 1975 found SOD in anaerobic bacteria, it has been purified from diverse sources such as fungi (Rapp et al 1973) green pea (Sawda et al 1972); Streptococcus Mutans. The super oxide dismutases are characterized by different metal content. A blue green Cu (II)-Zn (II) enzyme comes from human and bovine erythrocytes, a wine red Mn (II) is found in Ecoli. A yellow Fe III enzyme from Ecoli sacchromyces cerevisiae and neurospora.

Bovine erythrocyte SOD, has been studied and is identical to the enzyme from human erythrocyte. Whenever the body is under high oxidative stress the activity of the SOD enzyme increases. SODs and other enzymes act both as an anti oxidant and anti inflammatory in the body neutralizing the free radicals. Each type of SOD plays a different role in keeping cells healthy. CuZnSOD protects the cells cytoplasm and Mn SOD protects their mitochondria from free radical damage.

SOD helps in neutralizing free radicals along with vitamin E supplementation (Kanter 1994, Mormont et al 1997). It has been used to treat arthritis, prostrate problems, corneal ulcers, burn injuries, inflammatory diseases, inflammatory bowel diseases and long term damage from exposure to smoke and radiation and to prevent side effects of cancer drugs (Luo et al 1997). It is found in barley grass, broccoli, Brussels, sprouts, cabbage, wheatgrass and most green plants. The body needs plenty of vitamin C and copper to make this natural anti-oxidant. SOD out competes damaging reactions of superoxide thus protecting the cells from super oxide toxicity. The reaction of super oxide with non radicals is forbidden (Spin). The super oxide anion radical (O$_2^-$) spontaneously dismutates to hydrogen peroxide H$_2$O$_2$. SOD is necessary because it reacts with critical and sensitive cellular targets e.g. it reacts
with NO radical and makes toxic peroxynitrite. The dismutation rate is second order with respect to initial superoxide concentration. The reaction of super oxide with SOD is first order. The reaction rate is diffusion limited. Super oxide inactivates the citric acid cycle, enzyme aconitase can poison energy metabolism and releases potentially toxic iron which is one of the main reactive oxygen species in a cell. It was seen that in mice lacking SOD2 massive oxidative stress (Edward et al 2001) existed. Many prokaryotic SOD null mutants have been generated. Mutation in SOD 1 causes amyotrophic lateral sclerosis (ALS) a form of motor neuron disease. Most common mutation in the U.S is A4V. While the most intensely studied is G93A. The other 2 iso forms of SOD have not been linked to any human disease. In Mice inactivation of SOD2 causes potential lethality and inactivation of SOD 1 causes hepatocellular carcinoma. SOD has proved to be highly effective in the treatment of colonic inflammation in experimental colitis.

Types:

(I) Copper and Zinc Superoxide Dismutase

It is a homodimeric enzyme present in the cytoplasm, nucleus and to a lesser extent in peroxisomes (Crapo et al 1992). Copper is essential for the catalytic function of these enzymes and zinc is thought to play mainlly a structural role (Fridovich 1998, Kinnula et al 2000.) The cooper zinc SOD gene has been located on chromosome 21, mutation in this gene have been related with autosomal dominant form of familial amyotrophic lateral sclerosis (Rosen et al 1993a,b) The activity of the mutant forms of the Cu-Zn SOD enzymes in these patients have ranged forms 0.1 to 100% of normal. Down syndrome fetus have shown increased Cu-Zn SOD activity and increased lipid peroxidation in the cerebral cortex tissue and it has been suggested that an imbalance
between superoxide and hydrogen peroxide detoxification leads to an accumulation of hydrogen peroxide and could contribute to the premature aging associated with down syndrome (Haan et al 1996). The copper zinc enzyme is a homo dimmer of molecular weight 32500. These two sub units are joined primarily by hydrophobic and electrostatic interaction. The ligands of copper and zinc are histidine side chains. SOD consists of two sub units of identical molecular weight joined by a disulphide bond. There are two Cu (II) and two Zn (II) atoms per molecule (Bannister et al 1971). The amino acid sequence has a sub unit tertiary structure and zinc has a structural and stabilizing role where as Cu₂⁺ is directly involved in the catalytic activity.

(II) Manganese Super Oxide Dismutase (MnSOD)

Is also called as SOD2 and is also a homo tetrameric enzyme located in the mitochondrial matrix near the electron transport chain. (Fujimura et al 1999). It is encoded by a nuclear gene in the long arm of chromosome 6 and translated extra mitochondrially as a precursor containing a mitochondrial targeting sequence to enable mitochondrial separation. MnSOD's biosynthesis is complicated by the existence of two distinct MRNAs of 4 and 1 kb of which 4 kb species is produced and eliminated faster than 1 kb. MnSOD production and activity is induced by a wide variety of factors such as hyperoxia, irradiation, tumor necrosis factor α, interleukin-1α, lipopolysaccharide, interferon γ oxidized LDL and the cellular redox state. Many of the factors including MnSOD can also activate NF-KB and a close association has been suggested between the activation of NF-KB and elevation of MnSOD gene expression (Das et al 1995). In most of the cells an increase in MnSOD (Marklund 1992) expression confers protection against oxidant injury as hyperoxia and TNF induced cytotoxicity. It is most commonly used by eukaryotes,
the cytosoles of virtually all eukaryotic cells contains an SOD enzyme with copper and zinc for e.g. Copper, Zinc SOD available commercially is normally purified from bovine erythrocytes. Both copper and zinc have been removed from Cu-ZnSOD to yield the apoenzyme. SOD is unusually stable enzyme and it's apoenzymes are very unstable (Forman and Fridovich1973). Worthington SOD retains its-activity for upto a year at 5°C.

\[2O^{2-} + 2H^+ \rightarrow O_2 + H_2O_2\]

(III) Extra Cellular superoxide dismutase:

Extra Cellular superoxide Dismutase (Marklund 1992): It is an extracellular form of SOD, (EC-SOD) and is a secretory homo tetrameric glycoprotein with the high affinity for heparin sulphate. The gene for human EC-SOD has been located to chromosome 4. EC-SOD is found in extra cellular fluids and in extra cellular matrix of all human tissues especially the heart, placenta, pancreas and lung. It functions to scavenge super oxide released from the cell surfaces, and helps in the regulation of nitric oxide bio availability. It's expression may be induced by interferon α and reduced by TNF α as well as tumor growth factor β but the regulation of EC-SOD is inadequately understood. EC-SOD is controlled concurrently with inducible nitric oxide synthase by cytokines exposure offering a protective mechanism during inflammation (Brady et al 1997).

1.6.3 Glutathione Peroxidase

Glutathione peroxidase is a selenium dependent enzyme (Tamer) and it decomposes \(H_2O_2\) and various hydro and lipid peroxides (Kinnula et al 1995). The classical form of GPx is cellular and dispersed throughout the cytoplasm but GPx activity is also found in mitochondria.
Peroxidase requires a separate acceptor. Extra cellular form of GPx is genetically distinct from intracellular GPx (Yoshimura et al 1994) and has been detected in several tissues including lung (Chu et al 1992). The Km value for GPx is lower than that for catalase and is considered more important in physiological conditions. It is essential for protein synthesis. Several selenium deficiencies can cause degenerative heart disease and necrosis. GPx requires reduced glutathione as a cosubstrate and the role of glutathione reductase is to derive glutathione from an oxidized form to reduced form (Beutler et al 1963).

**Glutathione Peroxidase**

Glutathione is abundant in almost all cells and is an important substrate for glutathione peroxidase and glutathione transferases, it acts by destroying the free radicals (Rotruck et al 1973). The main function of Glutathione peroxidase is to eliminate various hydroperoxides but not hydrogen peroxide. It is also known as gamma glutamylcysteinylglycine or GSH (Valencia et al 02001), it is body's primary anti oxidant which is found in almost every cell. It is made up of amino acids and gamma glutamic acid, cysteine and glycine, it is also considered as a tripeptide and is found in large concentrations in liver (Amstad et al ). It helps to
maintain the red blood cells (Gerald et al 1963)\textsuperscript{167}, white blood cells and helps in carbohydrate metabolism and break down of oxidized (Suter et al 1974)\textsuperscript{168} fat. It's a general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage (Lepore et al 2004)\textsuperscript{169}. GPx reduces lipid hydroperoxides to their corresponding alcohols and reduces free hydrogen peroxide to water (Braven et al 1989)\textsuperscript{170}

**Iso Enzymes of glutathioneperoxidase**

There are several Iso enzymes encoded by several genes which vary in cellular location and substrate specificity, the GPx1 (Gouaze et al)\textsuperscript{171} is the most abundant form and is found in cytoplasm of all mammalian tissues whose preferred substrate is hydrogen peroxide (Muller et al 2007)\textsuperscript{172}.Glutathione peroxidase 4 (GPx4) has a higher preference for lipid hydroperoxide. It is expressed in every mammalian cell though at much lower levels. GPx2 is an intestinal extra cellular enzyme, GPx3 is an extra cellular enzyme especially abundant in plasma, SO far 8 different iso forms of glutathione peroxidase such as GPx 1 to 8 have been identified in humans. GPx 1 being an enzyme found in cytoplasm, GPx2 as gastrointestinal enzyme, GPx3 in plasma and GPx 4 as phospholipid hydroperoxidase have been shown to be containing selenium where GPx6 is a selenoprotein in humans with cysteine containing homologues. GPx5 exists as epididymal androgen related protein, GPx in olfactory, GPx 7 and 8 in putative membranes (Ran et al 2007)\textsuperscript{173}.Gpx 1, GPx2, GPx3 are homo tetrameric proteins, where as GPx 4 has a monomeric structure. The integrality of cellular and sub cellular membranes depends on glutathione peroxidase. It's an anti oxidative protective system depending on the presence of selenium (Sies et al)\textsuperscript{174}
Glutathione peroxidase reacts at the selenocysteine site where the resting state is SE(-) state (Epp et al 1983)\(^{175}\) this is oxidized by peroxide to SE-OH which is trapped by a GSH molecule to yield SE - SG and by one more GSH molecule to give SE(-) again there by releasing a GS- SG by product.

\[ 2 \text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GS} - \text{SG} + 2\text{H}_2 \]

Where GSH represents reduced monomeric glutathione, GS-SG represents glutathione disulfide.

It was discovered by Gordon C Mills in 1957\(^{176}\)

\[ \text{GS-SG} + \text{NADPH} + \text{H}^+ \rightarrow 2 \text{GSH} + \text{NADP}^+ \]

### 1.7 Adenosine Deaminase

ADA is an enzyme (EC 3.5.44) involved in purine metabolism and is needed for breakdown of adenosine from food and for the turn over of nucleic acids in tissues (Cunha et al, 1992)\(^{177}\) It in-vitro catalyzes the deamination of both adenosine to 2'deoxyadenosine and inosine to 2' deoxyinosine respectively. It initiates T-cell activation and is associated with the production of reactive oxygen species by neutrophils with the production of NO\(^\circ\) O\(^2\circ\) H\(_2\)O\(_2\) and OH\(^\circ\). The enzyme is produced in all cells but highest level is found in immune system cells called lymphocytes which develop in lymphoid tissues (Franco et al 1997)\(^{178}\), lymphoid tissues include thymus, lymphnodes and ADA's primary function is to eliminate a molecule called deoxyadenosine which is generated when DNA is broken down (ADA converts deoxyadenosine to deoxyinosine which is not harmful). ADA can localize to the cell surface through it's interaction with CD-26 using CD-26 transfected cells, it could be demonstrated as cell surface ADA. EctoADA can regulate
adenosine receptor engagement by degrading extra cellular adenosine to inosine Ecto ADA and has the potential to regulate adenosine receptor – mediated cAMP responses in vivo in tissues with CD26+ cells and sufficient cell death caused by apoptosis or inflammation to provide a source of ADA to bind to CD26 (Aran et al 1991)\(^{179}\)

**Adenosine Deaminase**

Levels of adenosine deaminase found in most cells are increased during TB (Chottiner EO et al 1987)\(^{180}\) and TB pleurisy, it's estimation in body fluids has attained popularity due to it's non invasive and non expansive nature. ADA is involved in the proliferation and differentiation of lymphocytes especially T-lymphocytes (Murray JL 1986)\(^{181}\). T-lymphocytes release ADA when stimulated by intracellular microorganisms. ADA is therefore looked on as a marker of cell mediated immunity. Several isoforms of ADA are there and prominent ones are ADA1 and ADA2. ADAI is present in all cells where as ADA2 is found only in monocytes. High levels of ADA have been reported in various diseases in which cell immunization is stimulated e.g., infectious condition being Pulmonary tuberculosis, non infectious conditions
associated with pleural fluid lymphocytosis including malignant condition and collagen vascular diseases.

It acts in the maturation of monocytes and transforming them to macrophages. It is a significant indicator of active cellular immunity. It is a cytosolic enzyme and has been the object of considerable interest mainly because in humans a congenital defect in the enzyme causes SCID (Severe combined Immune Deficiency Disease).

Further metabolisation of these deaminated nucleosides leads to hypoxanthine which can be either transformed into uric acid by xanthine oxidase or salvaged into mono nucleotides by the action of hypoxanthine – guanine phosphoribosyl – transferase.

ADA occurs as a soluble monomer in all human cells, it also exits as Ecto-ADA bound to the membrane glycoprotein CD26/dipeptidyl peptidase IV, this type of ADA regulates extra cellular adenosine levels. Adenosine is not just a metabolite it is also a signaling molecule that regulates numerous cellular functions by binding to G-protein coupled protein, adenosine receptors (A1, A2A, A2B, A3 in mammals) that can regulate intra cellular cyclic adenosine monophosphate. Deoxyadenosine is a cytotoxic metabolite released by various cell population that undergo programmed cell death. It can kill cells via some mechanism that includes obstruction in deoxynucleotide metabolism.

ADA may appear also on cell surface called as Ecto-Ada No differences are seen in catalytic activity between cytosolic ADA and Ecto-ADA. Ecto ADA could bind directly to atleast 3 different cell surface molecules.

ADA is a purine nucleoside adenosine, a plural functional mediator and modulator of myriad physiological process that serves as a substrate
for ATP and is found to be elevated in injured and inflamed sites. This molecules and its receptors elevate levels of C-amp playing a crucial role in regulation of inflammatory responses and in limiting inflammatory tissue destruction on signaling via it’s specific Gs protein coupled A2A adenosine receptor, it suppresses the immune system by inhibiting lymphoid or myeloid cells including neutrophills, macrophages, lymphocytes and platelets.

It is an endogenous inhibitor of neutrophil induced endothelial cell injury and β2 integrin expression on polymorphonuclear leukocytes, that mediate adhesion to the N-lymphocytes and is important for proper functioning of immune system without ADA the toxin derived from the metabolic by-products kill the T-cells shortly after they are produced in bone marrow. T-cells of individuals with ADA deficiency live only a few days and gradually their numbers are reduced and body’s entire immune system is weakened.

The ADA exists in two Iso forms:-

Iso enzyme 1 (Andreasyan et al)–ADA deaminates mainly two nucleosides, Adenosine and 2’ deoxy adenosine with many effects in human cells. Iso enzyme ADA1 down regulates substrates adenosine and 2’ deoxyadenoisne. It is present in red cells that capture and internalize 2’ deoxydenoisne. The importance of ADA 1 in cells is revealed by the dysfunction of immune response in subjects congenitally lacking ADA 1. ADA 1 is highly efficient in deaminating the substrates (Adenosine and 2’ deoxyadenosine) in biological sites where the PH is optimal for this isoenzyme even though the concentration is low.

Iso enzyme 2 (Keagen et al 2004)182 – It is non ubiquitous but co-exists with ADA 1 only in monocytes–macrophages. ADA 2 and ADA
1 are coded by different gene loci. It is necessary to consider ADA 1 and ADA 2 system required for homeostasis of ADA and 2' deoxyadenosine in monocytes-macrophages. This homeostasis mechanism involves two substrates and two isoenzymes. Both iso enzymes have same affinity for substrate adenonsine while ADA 2 has a different affinity rather very weak for the substrate 2' deoxyadenosine. It has been demonstrated that in macrophages–monocytes the ADA level is low where as in some conditions 2' deoxyadenosine level rises dramatically due to increase of ADA 2 this causes homeostatic mechanism to up regulate 2' deoxyadenosine inside monocytes– macrophages. Increase of ADA2 in monocytes macrophages occurs when these cells are infected by intracellular micro organisms and the parasite is still alive. Monocytes and macrophages especially in activated states tolerate high levels of 2' deoxyadenosine. Monocytes-Macrophages are crucial cells in immune defense. 2’deoxyadenosine is deleterious for nucleic acid and makes us conclude that ADA1– ADA2 system may by a tool in the production of a weapon of monocytes– macrophages against offending parasites. CD-26 is a lymphocyte marker that can anchor ADA on the T-cells surface. We found that ADA is regulated by cytokines on the cell surface during T-cell activation. By means of flow cytometry, immunefluorescence and immunoblotting techniques, we found that interleukin IL-2 and IL-12 up regulate Ecto-ADA and CD-26 expression. ADA catalyzes the irreversible hydrolytic deamination of adenosine and 2'deoxyadenosine as under-

\[
\text{Adenosine} + H_2O \leftrightarrow \text{Inosine} + \text{NH}_3
\]

Ecto ADA protects activated lymphocytes from the toxic effects of extra cellular adenosine. It is widely distributed in human tissues, it's specific role is in maturation of immunological system. Accumulation of
ADA and 2′ deoxyadenosine is widespread in many tissues. Human ADA exists in at least 3 isoforms ADA 1 ADA 2, ADA1 + ADA-complexing protein (ADAcp). ADA1 is a monomer of 41KD with gene assignment on chromosome 20. Although location of ADA 1 is mainly cytosolic, the enzyme has been found on the surface of a high percentage of B lymphocytes and macrophages in some T-lymphocytes from peripheral blood. ADA2 is coded by a different gene locus of unknown chromosomal position, it can only be detected in monocytes and it is the pre-dominant isoenzyme in the sera of normal individuals.

In some lymphocytes and in the peripheral blood two ADA1 molecules renamed Ecto-ADA are connected via a dimmer of ADAcp. The importance of ADA1 and ADA2 of the enzyme is that ADA deaminates mainly 2 nucleosides viz., adenosine and 2′deoxyadenosine, producing inosine and 2′deoxyinosine. Adenosine and 2′deoxyadenosine are molecules with many effects on human cells. ADA downregulates substrates adenosine and 2′deoxyadenosine. It is present in red blood cells that capture and internalize 2′ deoxyadenosine. The importance of ADA 1 in cells is revealed by the dysfunction of immune response in subjects congenitally lacking ADA1. ADA1 is highly efficient in deaminating the substrates (adenosine and 2′ deoxyadenosine) in biological sites where the PH is optimal for this isoenzyme.

Functional importance of CD-26ag in the T-cell activation cascade together with essential role of ADA in development of normal immunological responses, suggests a direct involvement of Ecto-ADA in T-cells activation. Ecto-ADA was regulated by exogenous cytokines through T-cells activation, positively by IL-2 and IL-12 and negatively by IL-4 and this regulation involves a translocation based mechanism. ADA's activity increases during antigenic and mitogenic responses of lymphocytes. Therefore, it is considered as an important immunoenzyme
marker for assessing cell mediated immunity in diseases characterized by T-lymphocytes proliferation and maturation (KOSE at al 2001.) The ADA genecodes for the enzyme adenosine deaminase which is required for proper functioning of immune system. It’s enzyme activity is inversely proportional to the degree of cell differentiation. In diagnosis of tuberculosis microbiologic, genetic immunologic and biochemical method are used.

ADA helps in maturation of monocytes and transforms them to macrophages. It is a significant indicator of active cellular immunity. ADA enhances T-cell response elicited by dendritic cells loaded within activated HIV adenosine deaminase enhances Anti-HIV responses. In the absence of ADA, T cell - lymphocytes and white blood cells are unable to remove toxins derived from metabolic by-products kill t cells after they are produced in bone marrow. ADA regulates the lymphocyte metabolism and important for lymphocytic differentiation and growth. It is present in lymphocytes in high concentration, it's activity seems to be necessary for an effective immune response as shown by many studies like in combined immunodeficiency disease. Increased activity of serum ADA level has been demonstrated in TB pleural effusion, peritoneal TB, AIDS and Cancer patients.

ADA binds to cell surface by means of either CD26, A adenosine receptors or A2B adenosine receptors. For many years it was considered to be cytosolic, but it has been found on the cell surface of many cell types. It is considered as Ecto enzyme and has been proposed to be having a catalytic independent function as a co-stimulatory molecule and lymphocytes. So two types of cell surface anchoring proteins for Ecto–ADA have been described.
– The first type is with only one member CD-26, a multifunctional protein of 110 is strongly expressed on epithelial cells, fibroblasts and on leukocytes subsets.

–The second type being those that cover the adenosine receptors (AR) A1, (A1 R) and A2B (A2BR) These two types of Ecto–anchoring proteins have been identified in DC. Adenosine receptor are expressed in myeloid DC as such as in plasmacytoid DC.

1.8 Lipid-peroxidation:

It is a process by which the free radicals steal away electrons from lipids present in cell membranes causing cell damage and increase in the production of free radicals (Timothy et al 1991).³⁸⁴

Mechanism of Lipid-peroxidation:
Lipid peroxidation leads to oxidative degradation of lipids. The process proceeds by a free radical mechanism which is a chain reaction (Bandyopadhyay et al. 1999). When a radical reacts with a non-radical it produces another free radical i.e. why it is called as a chain reaction mechanism. It basically affects poly unsaturated fatty acids because they contain multiple double bonds in between which there are methylene groups that possess reactive hydrogens. The entire mechanism follows three major steps:

1. Initiation-It starts when a fatty acid radical is produced (Cross et al. 1987). The initiators are reactive oxygen species (ROS) (Parke 1996) such as OH⁻ that combine with a hydrogen atom to make water and fatty acid radical.

2. Propagation–The fatty acid radical is not very stable therefore, it very readily reacts with molecular oxygen creating a peroxyl fatty acid radical which too is an unstable species and it reacts with another free fatty acid radical producing a different free fatty acid radical and lipid peroxide (Angel 2010) or a cyclic peroxide. The cycle continues as new fatty acid radical too react in the same way.

3. Termination-The radical reaction stops when two radicals react and produce a non-radical species. When there is a high concentration of free radicals there is a high probability of two radicals to actually collide. There are certain vitamins that act as free radical scavengers one such non-enzymatic anti-oxidant is vitamin E (Vijayamalini et al. 2004) other anti-oxidants may be Superoxide dismutase, Catalase and Glutathione peroxidase.

If the cycle is not terminated fast there can be a severe damage to cell membrane. The end products of lipid peroxidation may be mutagenic and carcinogenic, it’s end products preferably are malondialdehyde and
thiobabituric acid. Thiobarbituric acid reacts with malondialdehyde to give a fluorescent product which is called the common **TBARS Assay**

Lipid peroxidation is nothing but a cell injury in both plants and animals and is indicative of oxidative stress in cells and tissues (Kaneda\textsuperscript{190} 1982 and Wagner\textsuperscript{191} et al 1998). The lipid peroxides are unstable and decompose to form complex series of compounds such as carbonyl compounds e.g. Polyunsaturated fatty acid peroxides which upon decomposition yield malondialdehyde and 4-hydroxyalkenals (HAE).

The oxygen radical produced by lipid peroxidation react with polyunsaturated fatty acid residues resulting in so many products, many of them being reactive towards protein and DNA. Site specific and random mutagenesis experiment indicate that MDA-DNA adducts are mutagenic in bacteria and in mammalian cells. Hence, Lipid peroxidation appears to be a major source of endogenous DNA damage in humans that can lead to cancer and other dangerous diseases.

Free radicals are basically atoms, molecules, or ions with unpaired electrons on an open shell configuration (Hua et al 2001)\textsuperscript{192}. They may have positive, negative and zero charge they are highly reactive species. A necessary part of life and body has a number of mechanisms to minimize free radical damage and repair the free radical damage. They also help in intracellular killing of bacteria by phagocytic cells they have also been helpful in cell signalling processes this is called as redox signalling. Although the free radicals are short lived due to their reactivity, long lived radicals (Rahman2002)\textsuperscript{193} also exist and may be categorized as:
Stable Radical—stable radical is a molecular dioxygen and it shows remarkable thermodynamic stability e.g. thiazyl radical and organic radical having conjugated \( \pi \) system such as that produced by vitamin E (tocopherol).

Persistent Radical—These are those compounds whose longevity makes it difficult to react with other molecule e.g. nitroxides, triphenylmethyl radical, tempo. The longest lived free radical is melanin. They are generated during combustion and are responsible for oxidative stress causing cardiopulmonary disease.

Diradicals—These are molecules having two radical centers having high reactivity. Radicals are stabilized by complexes as carbocations, the more substituted the radical center the more stabilized. The radicals attack double bond resulting in a radical which is relatively stable and can couple with other molecule or be oxidized. The two most important oxygen centered free radicals are super oxide and hydroxyl radical, because of their reactivity they can participate in many side reaction causing cell damage. The cells cycle gets altered to produce malignancy. Therefore, enzymes such as SOD, CAT, GPX and glutathione reductase are the major anti-oxidants that stabilize the free radicals. Bilirubin and uric acid are good anti-oxidants. ROS and RNS are a family of antimicrobial molecules, ROS are like molecules, ions radicals and anions (both ion and radical ) hydrogen peroxide \( \text{H}_2\text{O}_2 \), hypochlorite ion \( \text{OCl}^- \), hydroxyl radical \( \cdot \text{OH} \), \( \cdot \text{OH} \) and superoxide anion \( \text{O}_2\cdot^- \) species react with \( \text{O}_2 \) to give peroxyl radical, RNS are derived from free radicals such as nitric oxide \( \text{NO}^- \) and superoxide species \( \text{O}_2\cdot^- \) with the formation of peroxynitrite \( \text{ONOO}^- \), both RNS and ROS are associated with cell damage and are a natural by product of normal metabolism of oxygen. They do have an important role in cell signalling. The free
radical markers are the NMR spectroscopy, chemical labeling, lipid peroxidation products, amino acid oxidation products, peptide oxidation products, measurement of the decrease in the amount of antioxidants, use of chemical species that reacts with free radicals to give a stable adduct.

1.9 Objectives of the Study:

1. To study the variation in levels of glycoproteins in the serum of sputum positive and sputum negative patients suffering with Pulmonary tuberculosis before and after the treatment.

2. To study the status of variation in levels of vitamin E in the serum of sputum positive and sputum negative patients suffering with Pulmonary tuberculosis before and after the treatment.

3. To find out the variation in levels of anti-oxidant enzymes in the serum of sputum positive and sputum negative patients suffering with Pulmonary tuberculosis before and after the treatment.

4. To find out the variation in levels of Adenosine Deaminase in the serum of sputum positive and sputum negative patients suffering with Pulmonary tuberculosis before and after the treatment.

5. To find out the variation in levels of Lipid Peroxidation in the serum of sputum positive and sputum negative patients suffering with Pulmonary tuberculosis before and after the treatment.
1.10. **Rationale:**

Tuberculosis has been an epidemic causing many deaths in the world. Of all the types of tuberculosis, Pulmonary Tuberculosis is most frequently occurring and vary fatal. In the modern time where even if the people are made aware of the hazards and prevention of tuberculosis by the health departments, health organizations such as WHO etc, media and education, T.B continues to be at it's rampant. With the advancements in medicinal technology a proper diagnosis still takes a backseat. Researches have proved that during the disease the status of certain Proteins, Vitamins, Enzymes, ADA and Lipids determine the gravity of disease. Therefore, it becomes imperative to carry out a study that will help in identifying the status of the disease through estimation of various biochemical parameters, so that the treatment may be effectively provided to the patients suffering with pulmonary tuberculosis at the right time as soon as possible and the symptoms can be subsided to a certain extent.

An alternative form of treatment may also be discerned over besides the routine treatment to make the prognosis rewarding. This study may be an incipient study for studies on many other diseases, hence, being highly instrumental in not only curing this deadly disease, but also other such diseases of such nature.
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