Tuberculosis has been an epidemic causing many deaths in the world. Of all the types of tuberculosis, pulmonary tuberculosis is most frequent and very fatal. In the modern time even when the people are made aware of the hazards and prevention of tuberculosis by the health departments, health organizations, WHO, media and education, even then it continues to be a deadly disease. With the advancements in medicinal technology it's proper diagnosis is still lacking. Researchers have proved that during the disease the status of certain proteins, vitamins, enzymes, ADA and lipids determine the gravity of disease. Therefore, more researches on tuberculosis finding out the role of such parameters as glycoproteins, antioxidants, lipid proxidation and ADA etc. are to be conducted. The present study was planned with a specific focus on studying the parameters as above, their level of variations in tubercular patients before and after the treatment for tuberculosis. Hence, the study was conducted under the following formal title:

“Studies on variation of Glyco-proteins, Vitamin E, Antioxidant Enzymes, Adenosinedeaminase and Lipid Peroxidation in Patients Suffering with Pulmonary Tuberculosis”

Objectives of the Study

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

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

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

➢ To find out the variation in levels of Lipid Peroxidation in the serum of sputum positive and sputum negative patients suffering from Pulmonary tuberculosis before and after the treatment.

**Tuberculosis**

Tuberculosis IS an air borne infectious disease and is contagious; It is a disease of respiratory system, the history of tuberculosis is as old as mankind. Robert Koch isolated the tubercle basillus in 1982 and established tuberculosis as infectious disease for which he received Noble prize. According to Med lexicon'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 where 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 many parts of the world, the disease continues to be deadly.

Tuberculosis as pulmonary tuberculosis is the most common form of tuberculosis Extra pulmonary tuberculosis is comparatively less common. Tuberculosis disease kills 2 million people each year. It can be summed up into occurring in 3 distinct phases, such as **exposure, infection and disease**.

**Types of Tuberculosis**

Tuberculosis is broadly divided into two types such as:

- Pulmonary tuberculosis: It affects the lungs
- Extra Pulmonary tuberculosis:

It's the type of tuberculosis that affects organs other than the lungs. It includes tuberculosis Meningitis, Lymph nodes tuberculosis, Abdominal tuberculosis, Laryngeal tuberculosis, Cavitary tuberculosis, Miliary tuberculosis, tuberculosis Pleurisy, and Serosal tuberculosis.

In early stages **pulmonary tuberculosis** IS silent but as it advances following symptoms get manifested (Kenneth 2010):

- Coughing outuberculosislood
- Cough producing phlegm
- Sudden weight loss
- Night sweating is a severe problem with patients of pulmonary tuberculosis and is commonly called as Hyperhidrosis.
- Wheezing
- Excess of sweating
- Chest pain
- Breathing difficulty

Pulmonary tuberculosis is marked by the formation of granuloma in infected lung tissues and by cell mediated hypersensitivity that can potentially cause inflammation and fibro-cavitary destruction in the lung, produces chronic respiratory systems and deteriorates the quality of life.

**Types of Pulmonary Tuberculosis**

Generally Primary and secondary tuberculosis are two distinct types of the disease with an entirely different natural history, onset character and localization of the lesions course and termination. Their progression and regression are governed by the law of evolution and evolution in tuberculosis.

(a) **Primary Pulmonary Tuberculosis:**

- **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.

- **Active benign primary**: This includes persons with a radiologically.

- **Quiescent benign primary**: It is manifested as a calcified nodule or round focus or no residual lesion absorption have been completed.

- **Malignant primary tuberculosis**: It is characterized by caseation in the origin lesion and its regional node as well as in the associated foci. **active malignant primary tuberculosis**: This has serious sub forms, lobar caseous pneumonia bronchopneumonia or acute miliary tuberculosis.

- **Quiescent malignant primary tuberculosis**: Characterized by calcified nodules or round foci or again no residual lesion.

**(b) Secondary Pulmonary Tuberculosis**

Secondary Pulmonary T.B is the advanced stage of disease and is characterized bi-lateral fibrocaseous disease followed by the presence of an associated foci in the opposite lung to that of initial lesion

**The another set of types of pulmonary tuberculosis is as follows:**
➢ **Primary tuberculosis pneumonia:**—affects people with low immunity, children and elderly etc, gets manifested as pneumonia and is contagious.

➢ **Laryngeal tuberculosis:**—Affects throat, vocal chord area, and is contagious.

➢ **Cavitary tuberculosis:**—Forms cavities in the lungs, it's highly contagious.

➢ **Miliary tuberculosis:**—Affects the young or anyone with weak immune system leads to a dangerous fever, small granules are formed in lungs seen through chest X-ray.

➢ **Tuberculosis pleurisy:**—It develops shortly after catching the infection, characterized by shortness of breath, chest pain and fluid in the lungs.

**Based on the Pathogenetic classification pulmonary tuberculosis is classified into two forms or types as given by (Milosh Sekulich)**

**Fibrous Forms**

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

**Active forms**

- Benign primary
- Malignant primary
- Minimal secondary
- Moderately advanced secondary
- Advanced secondary
- Fibrous secondary
- Fibrocavitaria secondary
- Non pulmonary

**Quiescent forms:** residual lesions of any of the above.

**Classification of Pulmonary Tuberculosis**

It is very difficult to sort out the forms of pulmonary tuberculosis from that of general tuberculosis as both have the same chief manifestations.
Albrecht 1907, gave the first pathological classification based on description of pathological lesions. The lesions were divided into three groups:

- Indurating, Cirrhotic, heading
- Nodular (Productive)
- Caseous pneumonic (Exudative)

First clinico pathological classification was given by Bard 1828.

He classified pulmonary tuberculosis into 4 forms:

- Parenchymatus
- Interstitial
- Bronchial and
- Post pleuritic

Classification of pulmonary tuberculosis has been given in five digit code. (B. Papanicolou 1951)²²

- Digit No.1 - concerns tubercule bacilli.
- Digit No.2 - Type and forms of the disease.
- Digit No.3 - Localization of Lesions.
- Digit No.4 - Cavities and Effusion in Pleura.
- Digit No.5 - Clinical status with Symptomatology.
Pulmonary Tuberculosis is also further divided or classified into two categories on the basis of development of infection:

<table>
<thead>
<tr>
<th>Primary Tuberculosis</th>
<th>Reinflection or Exogenous or Endogenous Tuberculosis</th>
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Transmission of Pulmonary Tuberculosis

The infectious agent of tuberculosis mycobacterium tuberculosis is carried on airborne droplet nuclei. It is transmitted through:

(A) Transmission of Infection from Person to Person

(B) Transmission with in the Body

Pathogenesis

The step by step development of a disease is called as Pathogenesis and takes place via known and unknown pool of infection

- **Known pool of infection:** It has diagnosed patients with a +ve sputum and it consist of potential infectors.

- **Unknown pool of infection:** It has diagnosed patients with a +ve sputum.

Discripation of Parameters

Glyco Proteins

These are complexes in which carbohydrates are attached covalently to asparagines (N-Glycans) or serine/ threonine (o-
glycans) residues of peptides (Lennarz et al. 1980). Most proteins synthesized in mammalian cells are glycosylated. The N and O-glycans contain different core structures but biologically active Oligo Saccharides are often found on outer chains attached to these cores.

**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 anti oxidants properties. In particular vitamin is associated with α-tocopherol (also written as alpha-tocopherol). Generally eight basic forms of vitamin E are recognized, four tocopherol and four tocotrienols (Harrara and Barbas 2001; and Packer et al. 2001). Like wise there are four isomers of tocotrienols, α-Alpha, β-Beta, γ-Gama and δ delta tocopherols, α-tocopherol is the form most commonly associated with vitamin E and used in vitamin E supplements. It has been claimed that α-tocopherol is the most important lipid soluble antioxidant and that it protects cell membranes oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reactions.

**Anti-Oxidant Enzymes**

Antioxidant enzymes are compounds that may reduce the energy of free radical from forming in the first place, or interrupt an oxidizing chain reaction to minimize the damage caused by free radicals (Reddy et al. 2004). Free radicals are believed to play a major role in more than 60 health condition including the ageing process, cancer, atheroscleroris 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.

**Catalase**

Catalase is an antioxidant enzyme which is like SOD and GPX. Catalase is one of those antioxidant enzyme found in nearly all living organisms that are exposed to oxygen where it catalyzes the decomposition of hydrogen peroxide to water and oxygen. 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.

**Superoxide Dismutase**

Superoxide dismutase is an enzyme that catalyzes the dismutation of super oxide anion into hydrogen peroxide. The most popular of the enzyme forms are copper, zinc, super oxide dismutase Superoxide dismutase: SOD EC1.15.1.1 is important antioxidant defense in nearly all cells exposed to oxygen. 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.

There are two types of SOD.

- **Copper and Zinc Superoxide Dismutase:** It is a homodimeric enzymes present in the cytoplasm, nucleus and to a lesser extent peroxisomes (Crapo et al 2003). Copper is essential for the catalytic function of the enzymes and zinc is thought to play
mainly a structural role. 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.

- **Manganese Super Oxide Dismutase (MnSODY):** Is also called as SOD2 and is a homo tetra meric enzyme located in the mitochondrial matrix near the electron transport chain. (Fridovich 1998). 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 sepration.

**Glutathione Peroxidase**

Glutathione peroxidase is selenium dependent enzyme and it decomposes H$_2$O$_2$ and various hydro and lipid peroxides( Kinnula et al 1995)$^{91}$ the classical form of GPx is cellular and dispersed throughout the cytoplasm but GPx activity is also found in mitochondria (Buettner 1998)$^{92}$. Extra cellular form of GPx is genetically distinct from cellular GPx (Takahashi et al 1987$^{93}$, Yoshimura et al 199494) and has been detected in several tissues including lung. It is also known as gamma glutamylcysteinylglycine or GSH, it is body's primary anti oxidant which is found in almost every cell.
ADA (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 turnover of nucleic acids in tissues. In vitro catalyzes the deamination of both adenosine-2’ deoxyadenosine to inosine and 2’ deoxyinosine respectively. It indicates T-cell activation and is associated with the production of reactive oxygen species by neutrophils with the production of NO* O (2)* H (2)O(2) and OH*. The enzyme is produced in all cells but highest level is found in immune system cells called lymphocytes which develop in lymphoid tissues.

- It acts in the maturation of monocytes and transforming them to macrophages. It is a significant indicator of active cellular immunity, its level in serum increases in various diseases in which cell immunization is stimulated for e.g. pulmonary tuberculosis (Dunlap NE, Briles DE 1993)\textsuperscript{59}.

- ADA occurs as a soluble monomer in all human cells also exits as ectoADA 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 with 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 populations that undergo programmed cell death. It can kill
cells via some mechanism that includes obstruction in deoxynucleotide metabolism (Ungere JP et al 1994).61

The ADA exists in two Iso forms:

ADA iso enzyme 1 — The importance of ADA 1 and ADA 2 of the enzyme, ADA deaminate mainly two nucleosides, Adenosine and 2’ deoxyinosine adenosine and 2’ deoxy adenosine are molecules 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’ deoxyadenoisne. The importance of ADA 1 in cells is revealed by the dysfunction of immune response in subjects lacking 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 ADA 2 (Ungere JP et al 1994, Gaga M et al 2005, D Mishra et al 2000) - It is a 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.

Lipid-peroxidation

It is a process by which free radicals steal away electrons from lipids present in cell membranes causing cell damage and increase in
production of free radicals. Lipid peroxidation leads to oxidative degradation of lipids (Gutteridge JMC 199598 Bandyopadhyay et al 1999). The process proceeds by a free radical mechanism which is a chain reaction. It basically affects poly unsaturated fatty acid 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
2. Propagation
3. Termination

MATERIALS AND METHODS

Pre-test post-test Design with Control Group method of research was used to study the variation of parameters viz. Glycoproteins, Vitamin E, Anti-oxidant enzymes, Adenosine deaminase and lipid peroxidation in sputum positive and sputum negative pulmonary tubercular patients versus normals.

Sample

Sample consisted of 150 subjects I.e. 50 normals volunteers (control group) and 50 sputum positive and 50 sputum negative pulmonary tuberculosis patients were selected randomly from a pool of pulmonary tuberculosis patients in L.L.R. Medical College, Meerut and District Hospital, Meerut. The study was carried out on three groups:
(a) Normal Healthy Persons (control group).

(b) Sputum positive and sputum negative pulmonary tuberculosis patients.

(c) The same group of patients (as in category b) after the treatment with anti-tuberculosis therapy.

The parameters whose variation has been studied:

- Glycoproteins: Protein bound Hexose, Protein bound Hexosamine and Protein bound Sialic Acid.
- Vitamin E.
- Anti-oxidant enzymes: SOD, Catalase and GPX
- Adenosine Deaminase.
- Lipid Peroxidation.

The study was carried out in the Deptt. of tuberculosis, LLR Medical College, Meerut and OPD of tuberculosis, P.L. Sharma Govt. District Hospital, Meerut. A pool of 100 patients registered with Medical College and 100 patients registered with District Hospital was drawn by using simple random technique respectively. From that pool of patients, 25 sputum positive pulmonary tuberculosis patients and 25 sputum negative pulmonary tuberculosis patients were identified by applying tuberculin skin test/ Montoux, obtaining sputum smear, X-ray reports and bronchoscopy. 50 normal volunteers were taken as the control group. Hence, the variation among the above parameters in the serum of patients suffering from pulmonary tuberculosis was studied against normals.
**Glycoprotein:**

Several theories have been proposed to account for the rise in serum protein bound, glycoprotein fractions occurring in infection, cancer and non specific stress (Catchpole et al). All the serum samples were stored at –70 degree centigrade until analysis. Biochemical analysis was performed on serum samples for estimation of protein bound hexose, hexosamine, and sialic acid. All the reagents were of analytical reagent grade.

- Protein-bound Hexose
- Protein-bound Hexosamine
- Protein-bound Sialic Acid

**Protein-bound Hexose:** Protein bound hexose was estimated by the method given by Niebes used by various researchers.

**Protein bound Hexosamine:** Protein bound hexosamine estimated by the method of Elson and Morgan (1933) modified by Niebs (1972).

**Protein-bound Sialic Acid:** Protein bound sialic acid was estimated by Thiobarbturic method given by Warren.

**Vitamin E**

Vitamin E was estimated by the method given by Desai (1984).

**Antioxidant Enzymes**

**Super Oxide Dismutase (SOD)** was estimated by the method given by Kakkar.
Catalase

Catalase was estimated by method given by Sinha et al (1972)

Glutathione Peroxidase

Glutathione Peroxidase was measured by Paglia & Valentine (1967)

Adenosine Deaminase

ADA was estimated by method of Martinek (1963) based on Berthelot reaction.

Lipid Per oxidation

Lipid peroxidation was observed by the formation of tuberculosis ARS by the method of Niehaus and Samuelsson (1992).

Statistical Methods Used

The choice of appropriate statistical method is governed by the design of the study, the type of data collected and the type of relationship being evaluated. Based on these facts the statistical calculations for the present study involved the following:-

- Mean
- S.D
- Percentage
- t-test

CONCLUSIONS

Level of Variation of Glycoproteins: PBHX (protein bound hexose), PBHXA (protein bound hexosamine), SIAL (Sialic Acid), in
the serum of sputum +ve and sputum -ve pulmonary tuberculosis patients versus normals before and after the treatment.

**Protein Bound Hexose**

A significant increase in mean levels of serum PBHX was observed in sputum +ve and sputum -ve pulmonary tuberculosis patients against normals. With the treatment for tuberculosis the level of serum PBHX decreased but still remained higher than that of normals.

**Protein Bound Hexosamine**

Mean level of serum PBHXA increased significantly in sputum +ve and sputum -ve pulmonary tuberculosis patients from the normals. After the treatment for tuberculosis a significant decrease in serum PBHXA levels was observed in sputum +ve and sputum –ve tuberculosis patients as being even lower than that of serum mean level of PBHXA in normals.

**Sialic Acid**

The mean values of serum Sialic Acid showed a significant increase in sputum +ve and sputum –ve pulmonary tuberculosis patients from that of normals, which decreased significantly after the treatment but still the serum mean values in sputum +ve and sputum –ve tuberculosis patients were higher than the normals.

Thus, the mean level of serum glycoprotein: PBHX, PBHXA and Sialic Acid increased significantly in sputum +ve and sputum –ve tuberculosis patients from normals. After the treatment for
tuberculosis the serum mean level for SOD and Sialic acid decreased yet remained higher than the serum mean values in normals but the level of sialic acid decreased to the level below of normals.

**Level of variation of vitamin E** in serum of sputum +ve and sputum –ve pulmonary tubercular patients before and after the treatment from Normals

**Vitamin E**

The serum mean levels of Vitamin E in sputum +ve and sputum –ve pulmonary tuberculosis patients decreased significantly as compared to serum mean value of Vitamin E in normals. But the decrease was more in sputum +ve tubercular patients. With treatment significant increase was found in the serum mean level of Vitamin E in sputum +ve and sputum –ve tuberculosis patients yet still were less as compared to the corresponding serum mean value of Vitamin E in normals.

**Level of variation of antioxidant enzymes:** SOD, CATALASE, and GPX in the serum of Normals and sputum + ve and sputum –ve pulmonary tuberculosis patients before and after the treatment:

**Antioxidant enzymes:**

The mean level of serum SOD decreased significantly in sputum +ve and sputum –ve pulmonary tuberculosis patients but increased with the treatment yet still showed persistent decrease in serum SOD level from that of normals.
**CATLASE**

A significant decrease in serum mean level of CATLASE in sputum +ve and sputum –ve pulmonary tuberculosis patients from normals was reported but with treatment its level increased yet did not approach the closer proximity with the serum mean value in normals.

**GPX**

The mean level of serum GPX in sputum +ve and sputum –ve pulmonary tuberculosis patients decreased significantly from normals that increased with the treatment but still was less than the serum mean value of GPX for normals.

Thus, the levels of anti oxidant enzymes viz SOD, CATALAS and GPX decreased in sputum +ve and sputum –ve tuberculosis patients, with the treatment, though their level increased but could not come closer to the serum mean level of anti-oxidant enzymes in normals.

**Level of Variation of Serum ADA in Normals and sputum +ve and sputum –ve pulmonary tubercular patients before and after the treatment**

**ADA**

Serum ADA level in sputum +ve and sputum –ve pulmonary tuberculosis patients increased significantly as compared to the serum mean value of ADA in normals. With the treatment for tuberculosis serum ADA level decreased but was still higher than that of normals.
Level of variation of lipid peroxidation level in sputum +ve and sputum –ve pulmonary tubercular patients before and after the treatment of tuberculosis.

Lipid Peroxidation

The level of lipid peroxidation increased significantly in sputum positive and sputum negative pulmonary tuberculosis patients as compared to that of normals. After the treatment for tuberculosis a significant decrease in the level of lipid peroxidation in both types of pulmonary tuberculosis patients was observed.

Suggestions of Further Research

Following suggestions were made for further research ill the area of tuberculosis:

1. The present investigation has been conducted by taking samples from tuberculosis patient form LLRM Medical College and PL Sharma Govt. Hospital. The further research may be conducted by taking samples from other Hospital of tuberculosis.

2. A study of parameter such as taken in the study may be further study in relation to demographic factors.

3. The present study may be extended to find out the extent of variation of parameters in male and female suffering with tuberculosis.

4. A Study to find out the role of the parameters taken in the study with regard to various age groups.
5. Health awareness contributes towards the prevention of infectious disease therefore; a research study may be design to make a comparative analysis of parameters in tuberculosis patent having and not having awareness about the diseases.