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RESEARCH ARTICLE

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STUDY OF PROTEIN OXIDATION AND ANTIOXIDANTS STATUS IN PULMONARY TUBERCULOSIS PATIENTS.

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ABSTRACT

Imbalance in the oxidants and antioxidant status indicates on going oxidative stress is considered as one of the etiological factor for development of lung function abnormalities. However no study has been performed to assess the oxidative stress in pulmonary tuberculosis. That's why this study was over taken. Protein Carbonyl (PC) and advanced oxidation protein product (AOPP) as protein oxidation products (PPP) and antioxidants such as reduced glutathione (GSH),total thiol (TT) levels were analyzed in 25 patients with diagnosed pulmonary tuberculosis (TB) and 25 normal healthy age and sex matched controls. Statistically significant increased (p-0.001) levels of PC and AOPP were noted; GSH (p-0.013) and TT (p-0.001) levels were decreased .Hence increased oxidative stress signified by increased protein oxidation product and altered antioxidant levels may have a role in etiopathogenesis of pulmonary tuberculosis.

KEYWORDS

Pulmonary tuberculosis, Protein oxidation products ,Antioxidants.

INTRODUCTION

Tuberculosis is a leading health problem worldwide and remain one of the leading cause of death from infectious disease (1). It is highly prevalent in India in terms of morbidity and mortality. It is estimated that India accounts for 1/5 th of worlds new TB cases (2). High levels of free radicals are produced in the lungs by activated inflammatory cells, i.e. neutrophils , eosinophils and alveolar macrophages. If not detoxified, they may cause cellular damage, an important part of which is the oxidation of amino-acid residues on proteins, forming protein Carbonyls. Protein carbonyl content is a widely used marker of oxidative modification of proteins; in inflammatory disease like TB (3).

The inner surface of the lung is covered by a thin film of fluid that contains a broad array of proteins, which clearly contribute to antioxidant capacity (4). In healthy individual, a delicate balance exists between free radicals and antioxidants. In some pathological conditions such as diabetes and in critically ill patients, oxidative stress causes the level of antioxidants to fall below normal. Antioxidant supplements for such conditions are beneficial (5). Recent research suggest that in pulmonary Tuberculosis there is increase in several circulating markers of free radical activity, indicating ongoing oxidative stress and decrease in the antioxidant activity which may contribute to development of lung function abnormalities (6).

The aim of the present study was to determine the oxidants and anti-oxidant status in pulmonary TB patients and values were compared with normal healthy controls.

MATERIALS AND METHODS

The present study was conducted in the department of biochemistry KMC, Mangalore. 25 pulmonary TB patients and 25 age and sex matched healthy controls were selected. Patients were from Govt Wenlock Hospital Managalore. Sputum positive pulmonary TB patients were included in study, i.e. at least two sputum smear positive for Acid Fast Bacilli (AFB) or at least one positive smear for AFB along with X-ray abnormalities or at least one positive smear for AFB and one Sputum culture positive for AFB.

The TB patients who were on antitubercular treatment (ATT), HIV infected TB patients, liver and renal diseases, extra pulmonary TB patients and patients on immunosuppressive drugs and those who were suffering from malignancy and other diseases which are effecting the oxidative stress were excluded from the study. The ethical committee of Kasturba Medical college Mangalore approved the study.

Under all aseptic precautions 5 ml of venous blood was collected in the heparinised vaccutainers after taking inform consent. Plasma was separated after centrifugation at 3000 rpm for 5 min into separate sterile vials.

The following parameters were estimated in our study;

Protein carbonyl and advanced oxidation protein product (AOPP) are protein oxidation products; reduced glutathione (GSH), total thiol are antioxidants.

Protein carbonyl content was determined by method of Levin et al (7).Introduction of carbonyl group into amino acid residues of proteins is the hallmark for oxidative modification that is mediated by free

radicals.Reaction of the carbonyl group with the 2,4-dinitrophenyl hydrazine(DNPH) forms a yellow colored 2,4-dinitrophenyl hydrazone which is measured spectrophotometrically.

AOPP Was Estimated By Modified Witko's Method (8**).** Concentrations of AOPP were expressed as mmols/L by measuring absorbance in acidic conditions at 340nm in the presence of potassium iodide (KI).

Plasma glutathione was measured by method of Ernest Beutler(9).Virtually all the non-protein sulphydryl group of erythrocytes is in the form of reduced glutathione. DTNB is a disulfide readily reduced by sulphydryl compounds to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412nm and is directly proportional to the glutathione concentration.

Total thiols measured in the plasma by G.L.Ellmans' procedure(10).The sulfhydryl groups in the plasma react with 10mM 5-5 ′ dithiobis 2-nitrobenzoic acid (DTNB) in abosolute methanol (Ellman's reagent) to produce a yellow colored compound whose absorbance is read at 412nm.

Statistical analysis of the data was done using Mann-Whitney U test.

RESULTS

1. The levels of PROTEIN CARBONYL were very high significant increased in pulmonary TB patients when compared to controls with the 'p'value.0.001.
2. The levels of AOPP were Very high significantly increased in pulmonary TB patients when compared to controls with the 'p' value 0.001.
3. The levels of GLUTATHIONE were significantly decreased in pulmonary TB patients when compared to controls with the 'p' value 0.013.
4. The levels of TOTAL THIOLS were decreased very high significantly in pulmonary TB patients when compared to controls with the 'p' value 0.001.

Table 1:

Comparison of protein oxidation products and antioxidant levels in pulmonary TB patients and normal controls.

PARAMETERS	MEAN±SD Controls n=25.	MEAN±SD TEST n=25.
AOPP (mmols/L)	0.067±.0039 (0.03 -0.19)	0.282±.0160*** (0.14-.043)
Protein Carbonyl (μmol/ml)	2.80± 0.632 (1.7-3.8)	10.08±2.58*** (4.8 -13.2)
Glutathione (mg/dl)	10.87± 4.13 (6 -20)	8.00± 4.49* (2.08- 20)
Total thiols (mmols/L)	0 .547± 0.172 (0.26-0.85)	0.226±0.117*** (0.05 -0.46)

n= Number of samples , SD=standard Deviation , P=Test of Significance ,P =0.362 non significant, * P =0. 013 significant , *** P =0.001 Very highly significant, Value in parenthesis indicates normal range.

DISCUSSIONS

Oxidant–antioxidant balance is essential for the normal lung function. Both, an increased oxidants and / or decreased antioxidant may reverse the physiologic oxidants–antioxidant balance in favor of oxidants, leading to lung injury (11).

Recent research suggests that oxygen and its relative species (oxidants) may contribute to the pathogenesis of a number of important lung diseases (6).

The lung exists in a high- oxygen environment and together with its large surface area and blood supply, is susceptible to injury mediated by these oxidants (6).

Increased production of reactive oxygen species / reactive nitrogen intermediates(RNI) secondary to phagocyte respiratory burst occur in pulmonary TB. Evidence suggest that increased circulating levels of free radical activity are found in pathogenesis of active pulmonary TB and hence play a role in resultant fibrosis (12).

In the present study protein oxidation products, AOPP and protein carbonyls in TB patients were significantly increased as compared to controls. Result obtained in present study are in agreement with earlier studies on TB.

Serum protein carbonyl level was significantly increased in pulmonary and extra pulmonary TB patients, as compared to normal (3).

RNI has damaging effects on proteins converting them in to protein carbonyls. Peroxynitrite has cytotoxic and genotoxic effects. Introduction of carbonyl group in the proteins make them susceptible to degradation, by proteolytic enzymes leading to deficiency of proteins. Oxidatively modified proteins are not repaired and must be removed by proteolytic degradation and a decrease in the efficiency of proteolysis will cause an increase in the cellular content of oxidatively modified proteins, hence increase in the disease process (3).

One of the advantages of protein carbonyls as a marker of oxidative stress is that it is widely used, and can thus be compared between laboratories. Although not absolutely specific, protein carbonyls represents a gold standard in the assessment of protein oxidation(4). Protein carbonyl level was also elevated in urogenital TB patients, as compared to normal (13).

AOPP as terminal products of protein exposure to free radicals without oxidants properties are reliable markers of the degree of oxidative stress(14). Increase levels of AOPP was also found in urogenital TB patients (13).

In the present study plasma GSH and total thiols were also very significantly decreased in pulmonary TB patients as compared to normals. The result obtained in the present study are in agreement with earlier studies on pulmonary TB, by Y.N Reddy et al (15)

Several factors such as low food intake, nutrient malabsorption and inadequate nutrient release from the liver, acute infection and inadequate availability of carrier molecules may influence circulating antioxidant concentrations. Lower concentration of antioxidants suggest increased utilization by reactive oxygen species (ROS)as an important contributing factor to the lower concentration of anti-oxidants in TB(16).

Reduced glutathione levels indicates the potential of oxidative damage to erythrocyte and erythrocyte membrane of pulmonary TB patients(17).

Erythrocyte glutathione level was also decreased in Tubercular meningitis(TBM) patients as compared to normals (18). GP /GSH/GR system is more important in catabolising H_2O_2 in RBC and leukocytes. This system is very important in the protection of phagocytic leukocytes against their own products. Erythrocyte GR activity is low in TBM patients. This decrease GR activity may be a predominant cause for GSH depletion within RBC leads to haemolysis (18).

GSH levels were decreased in patients with HIV–1 infection, and this decreased GSH causes increased risk of developing tuberculosis in HIV infected patients. This decrease would be associated with reduced capacity of monocytes to kill intracellular M.TB (19). Pulmonary TB patients also had significant lower blood total thiols in this study. These thiols represent the major intracellular redox buffering compounds (20).

CONCLUSION

In conclusion there is a significant increase in protein oxidation, decrease antioxidants in pulmonary TB patients. These findings establish a link between oxidative stress and tuberculosis infection. Alarming signals of TB infection warrant global attention for more extensive research need rapid outcomes to control the massive rate of infection in the present situation and also nutritional supplementation may represent a novel approach for recovery. Hence the therapeutic benefit of exogenously administered antioxidants like copper, vitamin -C, vitamin–E etc need to be assessed under carefully controlled clinical setup.

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