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COMPARISON OF LIPID PEROXIDATION PRODUCT AND ENZYMATIC ANTI-OXIDANTS IN NEWLY DIAGNOSED PULMONARY TUBERCULOSIS PATIENTS WITH AND WITHOUT HUMAN DEFICIENCY VIRUS INFECTION

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ABSTRACT

Tuberculosis is still a worldwide health problem and one of the leading causes of death in young adults. Acquired immunodeficiency syndrome (AIDS) is a fatal illness caused by a retrovirus known as the human immunodeficiency virus (HIV) and leads to AIDS. This study was aimed to compare oxidative stress and enzymatic anti-oxidants in patients of pulmonary tuberculosis (PTB) with and without HIV infection. The study population comprised of 50 patients, in the age group of 20 to 50 with sputum smear-positive by Ziehl-Neelsen staining for acid-fast bacilli grouped into two groups [Group 1 (PTB) and group 2 (PTB coinfected with HIV)]. Estimation of MDA, haemoglobin, enzymatic anti-oxidants superoxide dismutase (SOD) was done. There was a significant increase in oxidative stress in patients of PTB patients with HIV infection compared to seronegative PTB patients as assessed by raised MDA and significant decrease in the antioxidant enzymes like SOD, GSH Px and Catalase.

KEYWORDS : Enzymatic antioxidants; Human immunodeficiency virus; Oxidative stress; Tuberculosis;

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INTRODUCTION

Tuberculosis (TB) is a global public health problem and it is second to human immunodeficiency virus (HIV) and/or acquired immune deficiency syndrome (AIDS) as the leading infectious cause of death of adults worldwide.[1] It was declared as a global emergency in the year 1993 by the World Health Organization (WHO). There is a resurgence in the incidence of TB in developing countries, in the recent years, due to co-infection with HIV, emergence of multi-drug resistant tuberculosis, inadequate treatment, poverty, malnutrition, overcrowding, and increasing number of displaced persons

Tuberculosis is an infectious disease caused by acid fast bacillus, mycobacterium tuberculosis (MTB) in humans.[2] Tuberculosis most commonly affects the lungs, where it is called pulmonary tuberculosis (PTB). In lungs, it forms a localized infection after inhalation of bacilli. It can also affect extra pulmonary organs like lymph nodes, bones, joints, subcutaneous tissue, meninges, eyes, kidneys and gastro-intestinal tract, where it causes an insidious disease that develops without striking clinical evidence.[3]

Acquired immunodeficiency syndrome is a fatal illness caused by a retrovirus known as the HIV that breaks down the body's immune system, infects CD4+ cells initially, and progressively leads to AIDS. Among the opportunistic pathogens associated with AIDS, MTB is distinguished by its relative virulence and potential for person-to-person transmission.[4]

According to the Global Tuberculosis Control Programme 2009, there were about 1.75 million tuberculosis deaths, over 25% occurred in HIV positive persons. Tuberculosis was the most common cause of death among people living with HIV / AIDS in 2007 and also HIV positive people are about 20 to 27 times likely to develop tuberculosis.[5]

MTB is a recognized facultative intracellular bacterium that replicates and persists within macrophages.[6] MTB can induce reactive oxygen species (ROS) production by activation of macrophages,[7-9] which is an important part of host defence against mycobacterium. But generation of ROS may promote tissue injury and inflammation in affected individual. This further contributes to immunosupression,[10,11] particularly in those with impaired anti-oxidant capacity. It is widely reported that oxidative stress, an imbalance between production and elimination of chemically reactive species, such as ROS,[8] is involved in HIV infection.[9] Viral Tat protein induces an enhanced ROS production in HIV infected patients by mitochondrial generation of superoxide anion,[10] which in turn may activate nuclear factor κB (NF-κB),[9] thus increasing HIV transcription.

In normal conditions, during cellular metabolism, due to production of ROS like superoxide anion and hydrogen peroxide, the lungs are exposed to a basal oxidative stress.[6] In PTB oxidative stress results when reactive oxygen species are not adequately removed and can lead to peroxidation of membrane lipids, depletion of nicotinamide nucleotides, rise in intracellular calcium ions (Ca2+), cytoskeleton disruption and deoxyribo nucleic acid (DNA) damage.[12] These ROS induce lipid peroxidation (LP), a chain process which affects unsaturated fatty acids mainly localized in cell membranes leading to generation of Malondialdehyde (MDA).[13] Lipid peroxidation products (LPPs) diffuse from the site of inflammation enters in circulation and can be measured in the blood If the antioxidant system is not adequate and the free radical level is greater than the antioxidant capacity of the organism, free radical reactions become very toxic and may harm lungs. This will lead to acute and/or chronic pulmonary damage.[14]

This study hypothesized that tissue inflammation, oxidative stress and continuous production of free radicals in pulmonary tuberculosis patients may cause lower levels of enzymatic antioxidants and also compared the oxidative stress and enzymatic anti-oxidants patients of pulmonary tuberculosis without and with HIV infection.

MATERIALS AND METHODS

This one year cross sectional study was conducted in the Department of Biochemistry, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2009 to December 2009. Prior to the commencement of study, ethical clearance was obtained Ethical Clearance Committee, Jawaharlal Nehru Medical College, Belgaum.

The study population comprised of 50 patients of pulmonary tuberculosis, in the age group of 20 to 50 with sputum smear-positive by Ziehl-Neelsen (ZN) staining for acid-fast bacilli newly diagnosed pulmonary tuberculosis patients attending the respiratory medicine out-patient department of KLES Dr. Prabhakar Kore Hospital and Medical Research centre, Belgaum, Karnataka.

They were further grouped into two groups. Group 1 (n=25) patients of pulmonary tuberculosis only), group 2 (n=25) patients with pulmonary TB co-infected with HIV. Fifty age and sex matched healthy controls were included in the study. Patients with history of smoking, chronic alcoholism, diabetes mellitus, rheumatoid arthritis, hepatic, renal, cardiovascular, neurological, gastrointestinal disorders, neoplasia and extra pulmonary tuberculosis were excluded from the study. Written informed consent was obtained from the participants of this study.

Under aseptic precautions, fasting blood samples (2 ml) were collected from antecubital venipuncture, kept in glass tube with lithium heparin anticoagulant. One ml of whole blood was used for the estimation of MDA as a marker of oxidative stress and one ml for preparation of hemolysate. Enzymatic anti oxidants Superoxide dismutase, Glutathione peroxidase, Catalase were analysed within one hour from the hemolysate.

Estimation of MDA in the serum was done by the method of Yagi (1987). The color produced by the reaction of thiobarbituric acid with MDA was measured spectrophotometrically (Shimadzu UV-1201 Spectrophotometer) at 533 nm. The results were expressed as nmoles/ml.[15]

Hemoglobin was estimated by the method of Drabkin and Austin (1932). The hemoglobin was oxidized to methemoglobin with alkaline cyanide reagent to produce a brown colored compound.[16]

Enzymatic anti-oxidants were measured from hemolysate. First red blood cells (RBCs) were isolated using cellulose and microcrystalline cellulose. Hemolysate was prepared from one volume of RBC and nine volume of stabilising reagent containing ethylene diamine tetra acetic acid (EDTA) and β mercaptoethanol.[17,18]

Superoxide dismutase (SOD) was estimated by method of Mishra and Fridorich. Epinephrine was auto oxidised to adrenochrome by superoxide radical at pH maximum of 10.2. Superoxide dismutase prevents this auto oxidation, was the basis for assay measured at 480 nm. Results were expressed in IU/gHb.[19]

Glutathione peroxidise (GSH Px) was estimated by method of Beutler. Reduced glutathione was oxidised in presence of t-butylhydroperoxide catalysed by GSH Px. Oxidised glutathione was again reduced in presence of NADPH which gets oxidised, was catalysed by glutathione reductase. The oxidation of NADPH was measured at 340nm and results were expressed in IU/gHb.[20]

Catalase was estimated by method of Beutler. The rate of decomposition of hydrogen peroxide catalysed by Catalase was measured spectrophotometrically at 230nm and the results were expressed in IU/gHb.[20]

Statistical analysis

The data obtained was tabulated and statistical analysis was done using student unpaired‘t’ test and comparison between the groups was done by analysis of variance.

RESULTS

Table 1
Correlation of the anthropometric measurements and Haemoglobin levels

Parameters	Controls (n=50)	Pulmonary tuberculosis without HIV (n=25)	Pulmonary tuberculosis with HIV(n=25)
Age	33.68 ± 7.30	33.92 ± 9.80	34.28 ± 8.96
Males	25 (50%)	15 (60%)	18 (72%)
Females	25 (50%)	10 (40%)	7 (28%)
BMI(kg/m2)	23.83 ± 4.318	21.83 ± 1.82	20.37 ± 2.90
Hb (g/dl)	12.69 ± 1.16	10.06 ± 2.45	9.76 ± 1.16

There was no significant difference in the age groups of the patients and cases. But the BMI was significantly low in PTB patients as compared to controls. There was further significant lower BMI in HIV infected TB patients than only TB patients. The haemoglobin levels were lower in HIV infected PTB patients compared to PTB.

Table 2
Comparison of oxidative stress and antioxidant enzymes

parameters	Controls (n=50)	Pulmonary tuberculosis without HIV (n=25)	Pulmonary tuberculosis with HIV(n=25)
Malondialehyde (nmol/L)	5.20 ± 1.42	13.20 ± 2.23	15.10 ± 1.75
SOD (IU/L)	882.10 ± 116.81	578.90 ± 110.63	429.10 ± 60.41
GSH Px (IU/L)	19.20 ± 1.67	10.20 ± 1.68	7.40 ± 1.74
Catalase (IU/L)	5.90 ± 1.40	3.70 ± 0.83	3.70 ± 1.21

There was significant increase in the MDA levels in patients with tuberculosis with and without HIV infection compared to controls ($p<0.001$). Further, significant increase in MDA levels was seen in patients with both TB and HIV infection compared to those with only TB infection ($p<0.001$).

There was significant decrease in the SOD, GSH Px, catalase in patients with tuberculosis with and without HIV infection compared to controls ($p<0.001$). Further, significant decrease in SOD and GSH Px levels was seen in patients with both TB and HIV infection compared to those with only TB infection ($p<0.001$). But no significant difference was seen in catalase levels in patients of PTB with HIV and without HIV.

DISCUSSIONS

Tuberculosis was announced by WHO as a global emergency in the year 1993. It is still a worldwide health problem and one of the leading causes of death in young adults. Mycobacteria are intra cellular pathogens and replicate in the host macrophages. In an attempt to kill mycobacteria, host cells generate huge amounts of reactive oxygen species which also contribute to inflammatory injury to host cells. These ROS cause

membrane lipid peroxidation leading to oxidative stress which can be assessed by measuring malondialdehyde levels.[6]

In the present study the BMI was significantly lower in HIV infected tuberculosis patient and tuberclosis patients without HIV, compared to healthy individuals, suggesting they were malnourised to some extent. Also the haemoglobin levels were lower in tuberculosis patients. Malnourishment is one of the factor known to enhance the severity of anemia and infection. Due to poor intake of micronutrient, malabsorption and poor immunity free radical generation is observed in patients with tuberculosis.[1]

Malondialdehyde is a three carbon aldehyde released due to free radical attack on the poly-unsaturated fatty acids present in the biological membranes.[21] In the present study the mean MDA levels were significantly increased in patients HIV positive patients compared to the seronegative patients of pulmonary tuberculosis. There are a considerable number of reports suggesting stepwise increase in oxidative stress with clinical severity or inflammation. S. Kwiatkowska et al,[22] reported significant increase in lipid peroxidation in the form of MDA and CA (conjugated dines) with marked clinical manifestation (sputum positive and advanced x-ray finding) than in patients with small changes of x-ray and negative sputum smear. Tuberculosis has been reported to enhance HIV replication and the progression to AIDS in co-infected patients possibly involving enhancement of inflammatory cytokines such as tumour necrosis factor α.[23] Oxidative stress has shown to enhance HIV replication and known to induce the production of several inflammatory cytokines, and to promote lymphocyte apoptosis and T cell dysfunction which further enhance the immunodeficiency in HIV positive tuberculosis patients. Friis-Moller et al[24] have shown that HIV-infected patients are in oxidative imbalance early in the disease; serum and tissue anti-oxidants levels are low and peroxidation products elevated. High plasma levels of MDA, reduced plasma glutathione (GSH), and decreased SOD activities are normally found. They also showed that, HIV and TB infection also result in considerably reduced vitamin C concentrations. Formation of free radicals from mitochondrial leakage due to increased oxygen consumption, inflammatory and reperfusion processes, and leukocyte activation elevate oxidative stress in erythrocytes. The pathogenesis of immunodeficiency during chronic HIV infection includes apoptosis of CD4 T cells that is influenced by ROS production.[4]

To defend the oxidative stress cells have enzymatic anti oxidants like SOD, GSH Px and catalase. Superoxide dismutase, a zinc and magnesium containing enzyme, is one of the antioxidant enzymes which protects the lungs against oxidants by converting superoxide radical to hydrogen peroxide.[25] Glutathione peroxidase essentially utilises glutathione as a reductant in disposing the free radicals. It is a metallozyme associated with selenium as a metal prosthetic group. Glutathione peroxidase activity spares the requirement for vitamin E in the cytosol.[26,27] In the present study, it was observed that free radical activity increased leading to oxidative stress. Superoxide dismutase and glutathione peroxidase levels though were lower in pulmonary tuberculosis patients compared to healthy controls but compared to HIV positive TB patients their levels were high in HIV negative TB patients, as SOD and gluthathione peroxidase were near completely utilised to scavenge the excess free radicals in sero-positive patients. Our study is in accordance to various studies reported in literature.[25,26,27] Our results were contradictory to a study done by Safarian et al[28] who showed compensatory increase in SOD and GSH Px levels. Accordingly, the combined deficiency of these antioxidants may markedly increase oxidative stress, possibly adversely affecting the immune response and predisposing to drug toxicity.

Catalase is an enzyme present in animal tissue, characterised by its power to decompose hydrogen peroxide into water and oxygen. Catalase is constitutively expressed especially in type II pneumocytes along with SOD in the alveolar regions suggesting their role protective role against damage caused by free radicals.[29,30] In the present study there

was a decrease in catalase levels in all pulmonary tuberculosis patients, but there was no significant difference in HIV positive and HIV negative TB patients suggesting that mycobacteria are resistant to hydrogen peroxide and organic peroxide because mycobacteria also release catalase which neutralises the hydrogen peroxide and hence increase the infectivity of mycobacteria especially in immunocompromised patients.

CONCLUSIONS

Overall findings of the study suggest that there was significant increase in oxidative stress in patients of pulmonary tuberculosis patients with HIV infection compared to sero-negative pulmonary tuberculosis patients as assessed by raised MDA and significant decrease in the antioxidant enzymes like SOD GSH Px and Catalase. Low levels of these enzymes may be due to heavy load of free radicals which damage the membranes leading to oxidative stress. Also malnutrition due to lower BMI may enhance the oxidative stress. Nutritional supplementation may be a novel approach for faster recovery and also reducing free radical load in these patients along with drug therapy. Supplementation of micronutrients like selenium, zinc and magnesium along with α tocopherols which enhance the anti oxidant levels further need to be assessed under carefully controlled clinical setup on a larger sample.

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