Lipid peroxidation, superoxide dismutase and catalase co-relation in pulmonary and extra pulmonary tuberculosis

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ABSTRACT

Objective: Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* affecting mainly the immune system in humans. This Study determines the malondialdehyde causing oxidation stress and blood levels of superoxide dismutase, catalase act as anti-oxidant. **Materials and Methods:** Study carried out in normal control subjects (n = 100), different categories of pulmonary in newly sputum culture positive diagnosed category I (n = 100), category II (n = 100), category III (n = 100). Extra pulmonary category I (n = 35) and pulmonary category I before and after six months of directly observed treatment, short course. **Results:** Malondialdehyde levels were significantly increased in pulmonary and extra pulmonary tuberculosis patients. The activity of superoxide dismutase, and catalase were found to be significantly decreased in subjects of all categories of pulmonary and extra tuberculosis pulmonary. Negative correlation between malondialdehyde content, with superoxide dismutase, and catalase was seen in pulmonary tuberculosis, $P < 0.001$. **Conclusion:** Increased defense mechanism was due to increased oxidative stress in tuberculosis. Superoxide dismutase and catalase by scavenging of free oxygen radicals interrupt inflammatory cascades and thereby limit further disease progression. The changes were reversed after six month anti-tubercular treatment in patients with good recovery but increase oxidative stress was not completely reversed.

Keywords: Catalase, immune system, pulmonary and extra pulmonary tuberculosis, superoxide dismutase.

INTRODUCTION

The cells associated with the immune response differentiate and divide very quickly and their extreme sensitivity to oxidative damage may result in damage to cell membrane and subcellular organelles.[1,2] Peroxidative damage to the membranes manifests itself as a loss in membrane fluidity, increase fragility of the biomembranes, loss of membrane secretory functions and breakdown of tranmembrane ionic gradient. So they need higher protection from anti-oxidants.[1]

It is worth to study the total lipid peroxidation activity such as malondialdehyde, as a marker of tissue injury and

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anti-oxidant superoxide dismutase, catalase in pulmonary and extra pulmonary tuberculosis.

Human superoxide dismutases have short metabolic half-life's (<10 min) and do not penetrate into the cells.[3] Modified human superoxide dismutase, which has a longer metabolic half-life and superoxide dismutase metallic compounds or non- metallic compounds, which may have a longer metabolic half-life and penetration ability into the cells.[4] An increase in manganese superoxide dismutase expression confers protection against oxidant injury, hyperoxia and tumor necrotic factor α induced cytotoxicity. An imbalance between copper zinc superoxide and hydrogen peroxide detoxification leads to an accumulation of hydrogen peroxide and could contribute to the premature aging.[3] Superoxide dismutase is an anti-oxidative enzyme that catalyzes the dismutation of two superoxide anions to hydrogen peroxide and molecular oxygen. The toxic hydrogen peroxide is further rapidly reduced by catalase into water and molecular oxygen. Superoxide dismutase, by scavenging of free oxygen radicals, might interrupt inflammatory cascades and thereby limit further disease progression.[5] Manganese superoxide dismutase gene therapy could be applied in combination with other therapies, as chemotherapy a dual therapy achieving synergetic action for rapid breakdown of superoxide anions is needed to minimize overall tissue damage. Extracellular superoxide dismutase gene therapy, anti-inflammatory and anti-proliferative genes were found to be effective in decreasing neointimal formation.[6]

Catalase is usually located in a cellular organelle called the peroxisomes. Hydrogen peroxide is a harmful by-product of many normal metabolic processes wherein, to prevent damage, it must be quickly converted into other, less dangerous substances. Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules. Catalase oxidizes different toxins, such as formaldehyde, formic acid, phenols and alcohols. In doing so, it uses hydrogen peroxide. Hydrogen peroxide is used as a potent antimicrobial agent when cells are infected with a pathogen. Pathogens that are catalase-positive, such as *Mycobacterium tuberculosis, Legionella pneumophila* and Campylobacter jejuni, make catalase in order to deactivate the peroxide radicals, thus allowing them to survive unharmed within the host.[7] It is worth to study the total lipid peroxidation activity malondialdehyde, as a marker of tissue injury in pulmonary, extra pulmonary tuberculosis and anti-oxidant superoxide dismutase, catalase.

MATERIALS AND METHODS

The study was undertaken in different categories of pulmonary and extra pulmonary tuberculosis of new sputum smear-positive diagnosed pulmonary category I (n = 100), extra pulmonary patients category (n = 35) before and after directly observed treatment, short course treatment of 6 months, category II (n = 100), category III (n = 100) and in normal control subjects (n = 100), were selected from Pulmonary Medicine Department, OPD and IPD of Sir J. J. Group of Hospitals, G. T. Hospital, Municipal corporation group of Tuberculosis Hospitals, Shewri Mumbai Maharashtra, India. The study was conducted with approval from the Medical Ethical committee of the institute (No-IEC/Pharm/379/07, dated 30/8/2007).

The patients with chronic diseases, hepatitis, diabetic, renal impairment, cardiovascular comorbidities, neurological psychiatric disorders, human immuno-deficiency virus (HIV) infection, various malignancies and heavy smoking, alcoholic, tobacco chewing subjects were excluded from the study. Patients with age group of 16 to 60 years were divided into groups on the basis of diagnosis. The subjects were distributed into four groups of pulmonary tuberculosis without and directly observed treatment, short course with 6 months in category I category II, and category III in each group 100 subjects were studied (50 male and 50 female), and two groups of extra pulmonary tuberculosis category I without and with 6 months directly observed treatment, short course 35 subjects (19 males and 16 females) with normal subjects of 100 (50 male and 50 female). To investigate the levels of malondialdehyde,[8] Superoxide dismutase by Randox kit, catalase[9] parameters were estimated using a UV-spectrophotometer (Jasco-V670), and fully automatic chemistry analyzer Olympus AU-400.

Blood sample collection: Venous blood samples were collected in plain and heparinized vacutainer as an anti-coagulant. Plain blood sample after 2 h of collection were centrifuged at 3000 rpm for 5 min serum was separated and collected in eppendorf sterile tubes with no sign of hemolysis used for the analysis. Statistical analysis was done using Mini tab 16 software; student t-test was applied.

OBSERVATION AND RESULTS

Table 1 Malondialdehyde superoxide dismutase and catalase in different types of tuberculosis

GROUP	MALONDIALDHYDE LEVELS n mol/ml	SUPEROXIDE DISMUTASE ACTIVITY U/gHb	CATALASE ACTIVITY U/gHb
CONTROL (n = 100)	2.46 ± 0.13	849.03 ± 120.45	12.01 ± 0.6
PULMONARY TUBERCULOSIS			
CATEGORYI UNTREATED (n = 100)	5.46 ± 0.33**	533.51 ± 41.43**	9.76 ± 0.22**
CATEGORY I AFTER 6 MONTHS TREATMENT (n = 100)	3.43 ± 0.35**	644.77 ± 52.01**	12.01 ± 0.50**
CATEGORY II UNTREATED (n = 100)	6.52 ± 0.27**	486.99 ± 31.64**	8.59 ± 1.22**
CATEGORY III UNTREATED (n = 100)	8.61 ± 0.61**	401.13 ± 42.51**	7.07 ± 0.32**
EXTRA PULMONARY TUBERCULOSIS			
CATEGORY I UNTREATED (n = 35)	5.33 ± 0.37**	575.31 ± 41.80**	9.64 ± 0.18**
CATEGORY I AFTER 6 MONTHS TREATMENT (n = 35)	3.38 ± 0.32**	689.74 ± 30.20**	10.30 ± 0.39**

***P ≤ 0.001 – Highly significant, *P ≤ 0.05 – Significant; values shown are Mean ±SD*

Table 2 Correlation between malondialdehyde and Superoxide dismutase, malondialdehyde and catalase

GROUP	r-values MALONDIALDEHYDE/ SUPEROXIDE DISMUTASE	r-values MALONDIALDEHYDE/ CATALASE
PULMONARY TUBERCULOSIS		
CATEGORY I UNTREATED (n = 100)	–0.930**	–0.956**
CATEGORY I AFTER 6 MONTHS TREATMENT (n = 100)	–0.944**	–0.929**
CATEGORY II UNTREATED (n = 100)	–0.957**	–0.897**
CATEGORY III UNTREATED (n = 100)	–0.955**	–0.976**
EXTRA PULMONARY TUBERCULOSIS		
CATEGORY I UNTREATED (n = 35)	–0.946**	–0.857**
CATEGORY I AFTER 6 MONTHS TREATMENT (n = 35)	–0.960**	–0.932**

** P ≤ 0.05 – Significant ** P ≤ 0.01 – Highly significant*

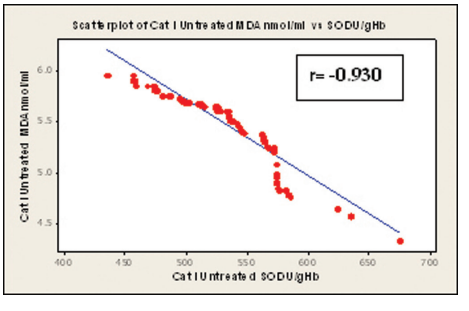

Figure 1. Scatter diagram of serum malondialdehyde and in superoxide dismutase category I pulmonary tuberculosis untreated.

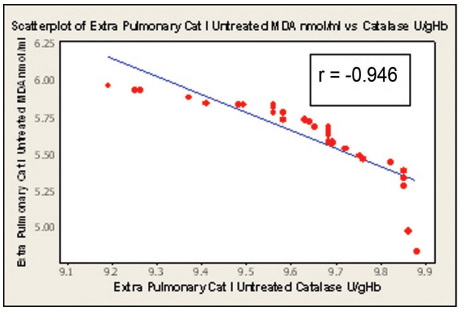

Figure 3. Scatter diagram of serum malondialdehyde and in catalase category I extra pulmonary tuberculosis untreated.

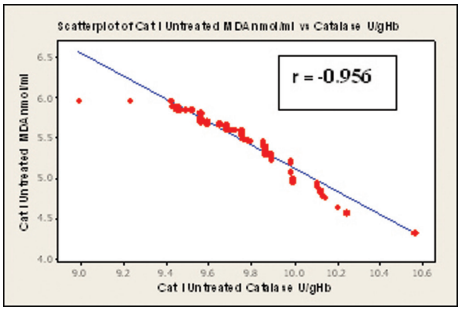

Figure 2. Scatter diagram of serum malondialdehyde and in catalase category I pulmonary tuberculosis untreated.

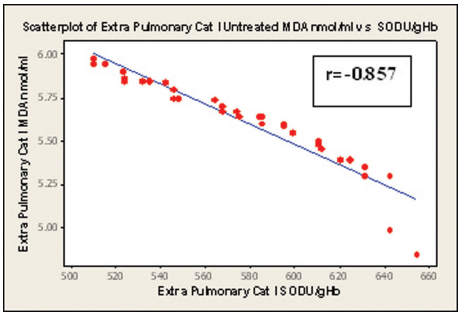

Figure 4. Scatter diagram of serum malondialdehyde and in superoxide dismutase category I extra pulmonary tuberculosis untreated.

DISCUSSION

Tuberculosis remains one of the top killers among infectious diseases. It is the most feared diseases in the world and spread from person to person via aerosols.[10] It is a chronic granulomatous infectious disease caused by *Mycobacterium tuberculosis*; the disease affects almost all the organs, lungs being primary.[11,12]

The pathophysiology of the disease is very well understood. In modern medicine, more and more emphasis are being laid on biochemical changes such as hyperoxidant stress leading to increased concentration of lipid peroxidation products anti-oxidant superoxide dismutase, catalase that act to inhibit and neutralize "free radicals."[13]

The present study is a comprehensive evaluation of concentrations of circulating anti-oxidants and markers of oxidative stress in pulmonary tuberculosis and extra pulmonary tuberculosis patients in different categories with and without anti-tuberculosis therapy compared with healthy human volunteers. According to our study, there is significant increase in malondialdehyde, significant reduction in superoxide dismutase, catalase showing increasingly protective effect.[14–18] Several factors such as low food intake (proteins), nutrient malabsorption and inadequate nutrient release from the liver, acute infections and an inadequate availability of carrier molecules may influence circulating anti-oxidant concentrations.

Tuberculosis, lipid peroxidation, tissue injury, DNA damage and anti-oxidant status

The malondialdehyde content of plasma in control subjects and experimental groups of different categories of pulmonary and extra pulmonary tuberculosis demonstrate that there is a significant elevation in contents of malondialdehyde in various categories of tuberculosis as compared to control.[19–22]

Natural anti-oxidants strengthen the endogenous anti-oxidant defense from ROS and restore the optical balance by neutralizing the reactive species.[23] They are gaining immense importance by virtue of their critical role in disease prevention. As the generation of free radicals is a normal physiological process, the cell has evolved a number of counteracting anti-oxidant defenses. These anti-oxidant defense mechanisms can be categorized under the heads of free radical scavenging and chain-breaking anti-oxidants.[24]

Hyperoxidant stress evident in various categories of pulmonary tuberculosis and extra pulmonary tuberculosis is associated with a consequent depletion of anti-oxidant enzymes mitigating oxidative stress.[14] The erythrocytic activity of superoxide dismutase and catalase activity were found to be significantly decreased in subjects of all categories of pulmonary tuberculosis and extra pulmonary tuberculosis as compared with controls. The level was lowest in subjects of category III tuberculosis.[14,25–28]

The toxic radicals produced by activated phagocytes during reaction cause maximal damage to membrane because they are active in a lipid phase. Lipid peroxide is obviously more polar than anything that should be present in the hydrophobic interior of biological membrane. The microbicidal ability of phagocytes through reactive oxygen intermediates (ROI) such as H_2O_2, O^-_2 and OH^- is a basic defense mechanism of the human host against microbial infection. Some mycobacteria are also identified as susceptible to H_2O_2. Reactive oxygen intermediates such as H_2O_2, O^-_2 and OH^- radicals are important microbicidal components and they could play a role in an infection. The ability of the patient's phagocytes to respond to infection produces reactive oxygen intermediates such as superoxide to kill the bacteria, which lead to increased number of neutrophils as a result of infection. The increase in lipid peroxide level could be due to an increase in the formation of superoxide radical with in cells.[14]

Superoxide dismutase, and catalase constitute a mutually supportive team of defense against ROS.[23–29] Superoxide dismutase is a metalloprotein and is the first enzyme involved in the anti-oxidant defense by lowering the steady-state level of O_2^-. The insertion of superoxide dismutase genes into an individual's cells and tissues to treat disease.[30–35]

Catalase is a hemoprotein, localized in the peroxisomes.[36] Catalase becomes more important when the concentration of hydrogen peroxide far exceeds the physiological level. Thus, the precipitous fall in catalase activity in pulmonary and extra pulmonary patients indicates that there may be marked decrease in the ability of these patients tissue to detoxify hydrogen peroxide and protect the cell from oxidative damage by hydrogen peroxide and hydroxy radical a similar pattern of results was observed in different disease.[13,37–39]

CONCLUSION

Lipid peroxidation and production of free radical plays a major role in the pathogenesis of tuberculosis. Oxygen and its derivatives, hydrogen peroxide, singlet oxygen,

hydroxyl radical could be the primary sources of oxidative damage to number of tissues and organs in tuberculosis. Peroxidation products could be correlated to tissue damage. The toxic effect of the reactive species of oxygen is neutralized by anti-oxidant defenses including superoxide dismutase, Catalase.

Equilibrium between the free radical generating and the free radical quenching mechanisms is the characteristic of a healthy individual if this equilibrium is disturbed free radical increases and after six months of directly observed treatment, short course treatment in tuberculosis patients when this equilibrium is not maintained leads to shift of disease to category II and so on therefore along with treatment of directly observed treatment, short course check point of anti-oxidant superoxide, catalase and malondialdehyde levels assessed will give guideline to increase treatment for few more months so as not to land up in chronic (MDR, XDR) state of diseases.[40]

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