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Status of Oxidants and Antioxidants in Pulmonary Tuberculosis with Varying Bacillary Load

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Abstract

When ROS production exceeds the detoxification capacity of systemic endogenous antioxidant defense, oxidative stress occurs. Severe oxidative stress has been reported in tuberculosis patients because of malnutrition and poor immunity. However, our knowledge of the antioxidant profile and its relation to lipid peroxidation in tuberculosis is very limited. We analyzed total hundred fresh untreated pulmonary tuberculosis samples with varying bacillary load and controls for oxidative stress markers viz; Malondialdehyde (MDA), Nitric oxide (NO) and Antioxidants viz; Superoxide Dismutase (SOD), Reduced Glutathione (GSH) and Vitamin C by calorimetric methods. The MDA & NO levels were high in AFB+, higher in AFB++ and with AFB+++ had the highest levels while SOD, GSH & VITC levels were low in AFB+, lower in AFB++ and AFB+++ had the lowest levels. Our findings provide the evidence of enhanced free radical mediated process corresponded with more advanced disease. It might play a role in the pathology associated with tuberculosis. Hence, it appears that suitable antioxidant supplementations are required to protect them from free radical attack.

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Key Words: Oxidative stress, Tuberculosis, Oxidants, Antioxidants

Introduction

Tuberculosis has emerged as the greatest danger to India, threatening the health of millions. More than 4,500 people die every day being unable to battle the disease. It is estimated that one third of the world population is infected with *Mycobacterium tuberculosis*.¹ Pulmonary tuberculosis is the most commonest form with onset usually insidious and illness remains unnoticed for sometime. Recently, Free radicals / Reactive Oxygen Species (ROS), the oxidants are paid particular attention as they cause lipid peroxidation; damaging the compounds of all biochemical classes; including nucleic acid, lipids, proteins, lipoproteins, carbohydrates and connective tissue micro molecules² and play an important role in the pathogenesis of tissue damage in many clinical disorders.³ These may contribute towards inflammation if not neutralized by antioxidants which are present in low concentration and significantly delays or inhibits oxidation of an oxidisable substrate. Oxidative stress results when oxidants predominate over antioxidants. Oxidative stress plays an important role in the pathogenesis of tuberculosis besides other chronic ailments; owing to the reasons: result of tissue inflammation, poor dietary intake, free radicals burst from activated macrophages, poor immunity etc.⁴⁻⁷ In TB, an increased amount of ROS and Reactive Nitrogen Intermediates (RNI) are produced by phagocytic respiratory burst.

The data on oxidants and antioxidant profile with respect to lipid peroxidation in tuberculosis patients especially in

relation to bacillary load seems to be limited. We compared the oxidants and antioxidants in fresh untreated pulmonary tuberculosis cases with varying bacillary load with healthy controls.

Material and Methods

The present study was carried out in the Department of Biochemistry at Mahatma Gandhi Institute of Medical Sciences, Sevagram. The study was designed as case control study. Total 100 Pulmonary tuberculosis cases attending District Tuberculosis Center, Wardha were included in the study. According to the AFB grading the cases were categorized into AFB+ (n=38), AFB++ (n=38) & AFB+++ (n=34). Age and sex matched healthy individuals (n=50) without past or present history of pulmonary or extra pulmonary TB or any other ailments were included as Controls.

About 5 ml venous blood was collected from each subject after obtaining informed consent procured and analyzed for malondialdehyde, nitric oxide, superoxide dismutase, reduced glutathione and Vitamin C.

Estimations

Estimation of Malondialdehyde

MDA was assessed in serum by modified TCA TBA method of Stater T. F. *et al.*⁸ 0.5 ml of serum sample of each subject was taken in test tubes and 3 ml of 10 % TCA was added, mixed well and left to stand for 10 minutes at room temperature, followed by centrifugation for 15 minutes at 5000

rpm. 2 ml of supernatant fluid from above mixture was taken to which 1.5 ml of 0.67 % TBA was added. A pale pink colour developed, where intensity was measured at 530 nm.

Estimation of Nitric Oxide

NO was estimated in plasma by Griess reagent assay of Lee D.U. et al.⁹ 50 ml of plasma sample was taken in a tube. To it 450 ml of distilled water and 500 ml of Griess reagent were added. The reading was taken at 550 nm against the blank containing 500 ml distilled water and 500 ml Griess reagent.

Estimation of Superoxide dismutase

SOD was assayed in serum utilizes the principle of inhibition of auto-oxidation of pyrogallol by SOD enzyme using the method of Marklund S, Marklund G et al.¹⁰ The assay mixture in a 3 ml volume consisted of 300 ml of Pyrogallol (0.2 mM), 300 ml of EDTA (1 mM), and varying concentration of standard SOD enzyme or 100 ml of serum in air equilibrated Tris HCl buffer (50 mM, pH - 8.2). Pyrogallol was added after the addition of all other reagents to start reaction. Initial 60 sec period was considered as induction period of enzyme. So after 60 sec change in absorbance at 420 nm at 10 sec interval was recorded to a period of 4 mins. Change in absorbance per mint was calculated.

Estimation of Reduced Glutathione

GSH was assayed in serum by the method of Beutler et al.¹¹ Two test tube marked test and blank were taken. In test marked test tube, 1.5 ml plasma followed by addition of 2 ml distilled water, 8 ml of Di-sodium phosphate buffer, then 1ml of DTNB reagent added to it, and mixed well. The colour developed rapidly, stable for 10 min. A reagent blank was made using 2 ml of distilled water, 8 ml of phosphate buffer and 1 ml of DTNB. Reading was taken at 412 nm in the spectrometer.

Estimation of plasma ascorbic acid

Vitamin C was assayed by colorimetric method as described by Aye Kyaw.¹² Take 1 ml of fresh plasma in a centrifuge tube, 1 ml of colour reagent was added and mixed thoroughly with a glass rod and allowed to stand for 30 min. at room temperature . (The reaction is complete within 30 min and colour is stable). The mixture was then centrifuged at

3000 rpm for 15 min. The blue colored supernatant was transferred to another test tube carefully with the help of pipette without disturbing the precipitate. Absorbance at 700 nm was read against a blank, constituted with distilled water, which is subjected to all treatment simultaneously as test sample. For every set a standard and a blank were run through the procedure.

Statistical analysis

For comparison analysis of data between cases and control group was carried out using student 't' test and the relationship between two quantitatively measured parameters was observed by establishing correlation. All statistical analysis has been carried out using statistical software SPSS 10-0 version.

Result

Mean plasma levels of MDA were high in all the three stages of fresh untreated PTB(TB1) cases. The values were high in AFB+ (7.58±0.95); those were higher in AFB++ (8.08±1.01) and highest in AFB+++ (9.21±1.36), all the values were statistically significant (P<0.01) as compared to Controls (4.50±.043). Mean plasma levels of nitric oxide (NO) were high in all the three stages of TB1 cases. The values were high in AFB+ (0.46±0.09); those were higher in AFB ++ (0.58±0.10) and highest in AFB +++ (0.60±0.10) all the values were statistically significant (P<0.01) when compared to Controls (0.19±0.05) shown in Table

I. Mean serum levels of SOD were low in all the stages of TB1. The values were low in AFB+ (2.29±0.63); those were lower in AFB++ (2.07±0.57) and lowest in AFB+++ (1.94±0.54), all the values were statistically significant (P<0.01) when compared to Controls (3.37±0.52). Mean serum levels of GSH were low in all the three stages of TB1. The values were low in AFB+ (0.12±0.02); those were lower in AFB++ (0.11±0.01) and lowest in AFB+++ (0.09±0.01), all the values were statistically significant (P<0.01) as compared to controls (0.31±0.07). Mean plasma levels of Vit C were low in all the three stages of TB1. The values were low in AFB+ (0.33±0.06); those were lower in AFB++ (0.29±0.030) and lowest in AFB+++ (0.23±0.02), all the values were statistically significant when compared to controls (0.61±0.1) shown in Table II.

Table I: Status of Oxidants in fresh untreated PTB cases with varying bacillary load as compared to controls

Parameters	AFB+ (n=38)	AFB++ (n=38)	AFB+++ (n=34)	Control (n=50)
MDA (nmol/ml)	7.58 ± 0.95*	8.08 ± 1.01*	9.21 ± 1.36**	4.50 ± 0.43
NO (M)	0.46 ± 0.09*	0.58 ± 0.10*	0.60 ± 0.10**	0.19 ± 0.05

* Significant (P value <0.05) as compared to controls

** Significant (P value <0.01) as compared to controls

Table II: Status of Antioxidants in fresh untreated PTB cases with varying bacillary load as compared to controls

Parameters	AFB+ (n=38)	AFB++ (n=38)	AFB+++ (n=34)	Control (n=50)
SOD (unit/ml)	2.29 ± 0.63*	2.07 ± 0.57*	1.94 ± 0.54**	3.37 ± 0.52
GSH (mg/dl)	0.12 ± 0.02*	0.11 ± 0.01*	0.09 ± 0.01**	0.31 ± 0.07
Vit- C (mg/dl)	0.33 ± 0.06*	0.29 ± 0.03*	0.23 ± 0.02**	0.61 ± 0.1

* Significant (P value <0.05) as compared to controls

** Significant (P value <0.01) as compared to controls

Discussion

Oxygen the very essence of life, an aerobic life cannot sustain without oxygen, but even too much of the best is bad. Oxygen indispensable of maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as ROS (reactive oxygen species). Antioxidants defense system acts as defender against the ROS for the inactivation and removal.

Hence, in the present study we estimated the concentration of these oxidants and antioxidants in fresh untreated pulmonary tuberculosis cases with varying bacillary load as compared to controls to rule out the oxidative stress and if so, its relation with severity of disease. In the present study, Malondialdehyde levels an indicator of lipid peroxidation and Nitric oxide being an important reactive nitrogen species reported to have strong implication in tuberculosis, were significantly elevated in all PTB cases with varying bacillary load compared to controls ($p < 0.01$). This suggests the association of oxidative stress with tuberculosis and its possible involvement in the pathogenesis of the disease process. Reddy et al¹³ reported, high free radical activity in patients of tuberculosis. Beside that we have demonstrated the imbalance between oxidants and antioxidants is more pronounced in patient with AFB +++ bacillary load followed by AFB ++ & AFB +, which provides the evidence of enhanced free radical mediated process corresponded with more advanced disease. Deveci F & Lihan N¹⁴ observed patients with active pulmonary TB showed increased circulating levels of MDA and decreased trace metal (zinc & albumin) as compared to inactive PTB patients and controls. There are a considerable number of reports suggesting stepwise increase in oxidative stress with clinical severity or inflammation. S. Kwiatkowska et al,¹⁵ reported significant increase in LPO, in the form of MDA and CA (conjugated dienes) with marked clinical manifestation (sputum positive & advanced x ray finding) than in patients with small changes of x-ray and negative sputum smear. In tuberculosis, the increase in reactive oxygen species may lead to the consumption and reduction of antioxidants, thus causing oxidative damage. Plasma Vitamin C is the only endogenous antioxidant that can completely protect the lipids from detectable peroxidative damage induced by aqueous peroxy radicals. These significantly reduced antioxidants: Glutathione, vit C, a tocopherol in tuberculosis patients are integral components of a regenerating redox cycle have also reported by different workers.¹⁶⁻¹⁸ In another study Kaur et al,¹⁹ evaluate the concentration of MDA, Vit-C&E according to radiological extents, sputum grading and cavity status in PTB cases which were associated with level of clinical severity and oxidative stress was more pronounced in advanced disease. Our observations are also in concordance with others as well. In tuberculosis several factors like low food intake, nutrients malabsorption and inadequate nutrient released from the liver, acute infection and an inadequate availability of carrier molecules may also influence circulating antioxidant concentrations.^{20, 21} Infact, the combination of malnutrition leading to decreased supplementation of antioxidants and enhanced ROS generation leading to increased utilization of these compounds may represent a pathogenic loop that results in markedly enhanced oxidative stress during tuberculosis infection and it gradually increased with severity of disease.⁵

Our findings of a significant correlation between high oxidants concentration and low concentration of antioxidants with varying bacillary load, suggest increased utilization by ROS as an important contributing factor to the lower concentration of antioxidants in TB patients also it interfere with severity of disease. The TB patients are unable to produce sufficient amount of Antioxidants to cope up with the increased oxidative stress in them. Anti-oxidants supplementation may prove beneficial and may help in fast recovery.

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