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Research Article

Assessment of oxidative stress in serum of pulmonary tuberculosis patients

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ABSTRACT

Background: Tuberculosis (TB) remains a human health issue and often deadly infectious disease in low-middle income nations. In TB, oxidative stress is a result of tissue inflammation, poor dietary intake of micronutrients due to illness, free radical burst from activated macrophages. This study was conducted prospectively to evaluate the oxidative stress in TB.
Methods: The study included 30 newly diagnosed TB positive patients and 30 healthy individuals. Pro-oxidant markers like the thiobarbituric acid reactive species (TBARS) and nitric oxide were studied from serum. Antioxidant parameter like serum total-SH was also assessed.
Results: Levels of pro-oxidants were significantly increased whereas antioxidant defense markers were significantly impaired in the TB group. Nitric oxide and TBARS were increased ($p<0.0001$) where glutathione was decreased ($p<0.0001$) in TB population compared to healthy controls.
Conclusions: Marked oxidative stress were seen in the TB population as compared to the healthy cohort. The role of antioxidant therapy may therefore be evaluated in the management of TB.

Keywords: Tuberculosis, Oxidative stress, TBARS, Nitric oxide, GSH

INTRODUCTION

Mycobacterium tuberculosis (MTB), the responsible agent of tuberculosis (TB), remains a issue of high morbidity and mortality worldwide. TB causes 1.9 million deaths annually among a pool of infected individuals close to 2 billion people.[1] TB occurs because of dysregulation of the immune system and/or poor immune response against the infection. Innate immune response critically acts against MTB infection. MTB is recognized intracellular bacteria that replicates and grow within macrophages. MTB can stimulate activated macrophages to produce reactive oxygen species (ROS), which is an important part of host defense against mycobacterium.[2] Oxidative stress has been implicated as a significant contributor to the development and prognosis of TB.[3,4] Oxidative stress parameters are intricately involved in the homeostasis of the immune system. Imbalance in oxidative levels results in cellular damage due to the oxidation of amino acid residues on protein.[5]

Critical immunological functions like inflammation are regulated by reduced glutathione (GSH).[6] Decreased in glutathione levels indicates the potential of oxidative damage to erythrocyte and erythrocyte membrane of pulmonary TB patients.[7] In HIV infected individuals, the risk of developing TB is high due to decreased level of

GSH which affects the capacity of monocyte to kill MTB.[8] Biomarkers of lipid oxidation such as thiobarbituric reactive substances (TBARs), NO among others have been studied as indicators of oxidative stress. Oxidative stress also has been shown to be associated with TB infection through activation of phagocytes by mycobacteria which may further contribute to immunosuppression.[9] Both nitrogen intermediates and oxygen radicals may also play an important role in the suppression of the infection through mycobacterial killing. Nitric oxide is also an important mediator of immune homeostasis.[10] Oxidative stress increases susceptibility of MTB to isoniazid, suggesting importance of oxidative stress in physiology.[11]

The present study was conducted to assess the levels of these oxidative parameters can be useful in predicting and diagnosis markers need to be evaluated. The levels of three important markers of oxidative stress in patients suffering from TB were estimated.

METHODS

The study was approved by the Research and Review Committee (RRC) and Institutional Ethical Committee (IEC) of the MGM Institute of Health Science, Kamothe, Navi Mumbai vide Certificate No. MGM/HIS/RS/2014/112 dated: 11.08.2014 Written informed consent was obtained from each participants prior to sample collection.

Study groups

The study population consists of 30 newly diagnosed TB positive patients and 30 Healthy individuals were included in the study. Patients belonging to both sex i.e. male and female of age of 16 years and above included. AFB staining was used for the diagnosis and confirmation of TB infection. Informed consent of the patients was taken before participation in the study.

Blood collection

5 milliliters of whole blood from TB positive patients and healthy volunteers were collected in plain vacuum tubes and was used immediately for the determination. The serum was separated from plain vacuum tube, aliquoted and stored at -20°C and used for the following assays.

Determination of NO (Nitric oxide)

1% Sulfanilamide Solution in 5% o-phosphoric acid and 0.1 % N-(1-Naphthyl) ethylene diamine dihydrochloride solution was allowed to equilibrate to room temperature. 50µl of standard and 50µl of serum was added. To this then, 50µl of the 1% sulfanilamide solution was added. 50µl of 0.1% NED Solution was added. A purple/magenta color of Azo-compound will begin to form immediately. The absorbance was taken at 520 nm.[12]

Estimation of TBARS

Lipid peroxides were estimated by measurement of thio barbituric acid reactive substances (TBARS) by the method of Brown and Kelly.[13] The pink chromogen produced by the reaction of Thiobarbituric acid with TBARS, a secondary product of lipid peroxidation was measured at 532 nm. Results was estimated as nmole/mL.

Estimation of reduced glutathione

Serum Total-SH was determined with slight modification by method described by Ellman and Sedlak and Lindsay.[14,15] Concentration of SH groups was measured colorimetrically with modified Ellman method in blood serum. To 445µl of PBS buffer (pH) 7.4, 25 µl of 2 mM of dithionitrobenzoic acid (DTNB) and 50 µl of standard or sample were added. Tubes were centrifuged at 15,000 rpm for 10 minutes and absorbance was measured at 412 nm against blank with DTNB.

Statistical analysis

Each result was expressed as Mean±SEM. The statistical significance of the data was determined by t-test. Statistical analysis was done using Graph Pad Prism 7 Software.

RESULTS

Demographic characteristics of the TB Patient

The demographic characteristics of the participants are showed in Table 1. A total of 60 participants were included in this study. These include newly diagnosed TB infected subjects and healthy volunteers. Average age from the TB group and healthy control was 32.46 and 28.71 years.

Table 1: Demographic characteristic of TB patient and healthy control.

Characteristic	Group	
	Control	TB (Naïve)
Number of participants	30	30
Age (Year)	28.71±6.34	32.46±12.03
Height (ft)	5.4±0.38	5.3±0.37
Weight (Kg)	56.85±11.82	49.52±8.52
Male:Female (%)	43:57	85:15

Levels of pro-oxidants in TB group

The levels of TBARS and NO were elevated in the TB as compared to healthy population (Figure 1). The mean serum TBARS levels were found to be 8.32 nmole/mL in TB population whereas 1.17 nmole/mL in healthy population. This difference was statistically significant. The mean serum NO levels were found to be 35.96 uM in TB population whereas 14.77 uM in healthy controls. The

levels of NO showed significantly increase while compared to healthy controls (Table 2).

Levels of antioxidants in TB group

Levels of GSH in TB infected population were estimated. The levels of GSH were decreased in the TB as compared to healthy population (Figure 2). The mean serum GSH levels were found to be 258.8 uM in TB population whereas 556.5 uM in healthy controls. The levels showed significant increase while compared to healthy population (Table 2).

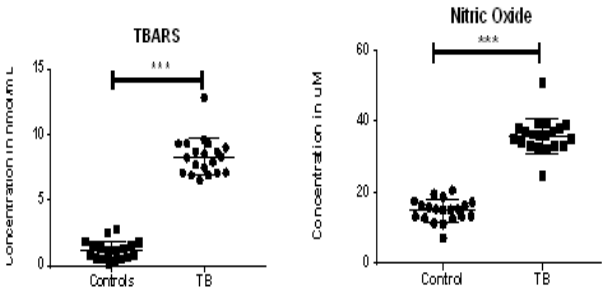

Figure 1: Levels of TBARS and nitric oxide in TB group and healthy control; (*P<0.0001).**

Table 2: Biochemical parameters for pro-oxidants and antioxidants (*p<0.0001).**

Parameters			Control	TB
Pro-oxidants	**TBARS** (nmole/mL)	Mean±SEM	1.175±0.1573	8.323±0.3178
		P value	***	
	NO (uM)	Mean±SEM	14.77±0.7106	35.96±1.09
		P value	***	
Antioxidants	**GSH** (uM)	Mean±SEM	556.5±26.2	258.8±8.795
		P value	***	

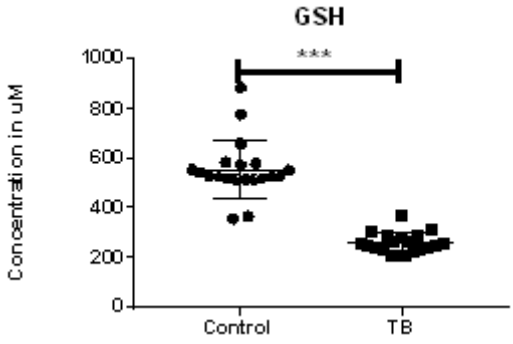

Figure 2: Levels of GSH in the study population; (*p<0.0001).**

DISCUSSION

Oxidative stress results from an imbalance between the generation of reactive oxygen and protective mechanisms. Free radicals, the main causes of oxidative stress, may react with variety of biomolecules including lipids, carbohydrates, proteins, nucleic acids and macromolecules of connective tissue. The oxidative stress is known to be a component of molecular and cellular tissue damage mechanisms in a wide spectrum of human diseases. Reduced glutathione (GSH) is the most prevalent non-protein thiol in animal cells. GSH levels have an impact on many immune functions, including activation of lymphocytes. Consequently, it was postulated that GSH deficiency could lead to the progression of immune dysfunction, a hallmark of TB.[16,17]

In the present study levels of GSH were found to be significant decreased in serum of TB infected individuals compared to healthy controls. Various studies showed that GSH play important role in TB.[8,18,19] Venketaraman V et al showed that H37Rv grown in vitro is sensitive to glutathione (GSH) at physiological concentrations. Data showed that GSH at a 5 mM concentration is bacteriostatic to TB strain H37Rv, suggesting the possibility that the presence of a high concentration of GSH may result in an imbalance in a bacterium containing an alternative thiol for regulating reduction or oxidation activity.[18] In another study, blood cultures from human immunodeficiency virus infected subjects treated with N-acetyl cysteine, which is a precursor of glutathione, caused improved control of intracellular MTB infection.[8] Examination of the effects of GSH in improving the ability of neutrophils to control intracellular MTB infection was evaluated. Findings indicated that increasing intracellular levels of GSH with a liposomal formulation of GSH (L-GSH) resulted in reduction in the levels of free radicals and increased acidification of MTB containing phagosomes leading to the inhibition in the growth of MTB.[19] Decreased GSH levels have been shown to activate NFκB, leading to a series of downstream signal transduction events that facilitate TB survival and growth. The low GSH levels in serum of TB infected patients may be a survival mechanism that pathogen employs.

TBARS are a product of lipid peroxidation and an important marker of oxidative stress. In this study, levels of TBARS exhibited a significant increased in TB population in comparison with healthy controls. Rashmi Kulkarni R et al conducted a study of serum malondialdehyde (MDA) and TNF-α in TB patients. TNF α and MDA levels in serum were significantly increased in pulmonary TB patients as compared to those of controls.[20] Madebo T et al also studied the lipid peroxidation products in untreated TB patients in Ethiopia. Data showed that serum MDA concentrations, were significantly higher in patients with TB than in healthy Ethiopian control subjects.[21] Kandukuri RE et al suggested that MDA level are increased indicating progression of TB. They also concluded that in addition to serum adenosine deaminase (ADA) levels, estimation of MDA are useful biochemical parameters to assess whether the TB in progression.[22]

Nitric oxide is an important molecule to study the oxidative stress markers in the bacterial infections. The nitric oxide serves as a pro-oxidant molecule. In the present study, NO levels were significantly raised in TB population as compared to healthy control ($p<0.0001$). Pearl JE et al demonstrated that the level of nitric oxide within the lesion site can dramatically impact the local protective and immune-pathological response by reducing accumulation of specific subsets of activated effector cells and by altering the potency of the lymphocytes with regard to accumulation within the lesion and cytokine production.[23] Jonna Idh et al showed correlation between resistance to first-line anti-TB drugs and reduced NO susceptibility in clinical strains of MTB.[24]

In summary, the results of the present study demonstrated that TB (naïve) infected patient, there is increase oxidative stress with concomitant reduction in antioxidative machinery. Since oxidative environment is crucial for survival of the MTB, it appears that MTB altered the host physiology biasing towards pro-oxidative environment. Such an adaptation of the host by the bacteria would be beneficial for the survival and proliferation of the pathogen. Since high oxidative stress is also favorable for the other pathogen like HIV, it is possible that modulation of the host oxidative stress machinery might further aid in developing co-infections. It will be of interest to determine the levels of oxidative stress molecules in patients with and without co-infection to understand the role of oxidative stress in TB pathophysiology.

CONCLUSION

Although preliminary, present data strongly suggest occurrence of oxidative stress upon TB infection. Wherever the levels of these oxidative parameters can be useful in predicting and diagnosis markers need to be evaluated. Our data also suggest the possible use of antioxidant therapy for treatment of TB.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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