Indian J Lepr 2013, 85 : 5-9

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**Original Article**

# Status of Free Radicals and Antioxidants in Leprosy Patients

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Received : 24.05.2012 Revised : 24.11.2012 Accepted : 25.11.2012

Oxidative stress is a condition associated with an increased rate of cellular damage induced by the oxygen derived oxidants commonly known as reactive oxygen species (ROS). ROS are capable of damaging cellular constituents generated in excess during the chronic, inflammatory, neurodegenerative disease process of leprosy. Severe oxidative stress has been reported in leprosy patients because of malnutrition and poor immunity. The decreased levels of SOD, glutathione and total antioxidant status in leprosy patients may indicate a degradation of these antioxidant enzymes by free radicals during detoxification processes.

The subjects for this study comprises of Normal human volunteers (NHV, n= 20) and treated MB patients (MB, n=20). The levels of lipid peroxidation products are increased in MB Patients (*P<0.001). SOD (**P<0.0001) and glutathione levels (***P<0.0001) decreased in MB Patients in comparision with normal human volunteers. The present study of estimation of antioxidants conclude that the free radical activity was increased and the total antioxidant status was decreased in all MB patients, indicating that there was an oxidative stress even after the treatment with MDT. The decreased levels of SOD, glutathione indicate a link between oxidative stress and leprosy. Since the MB patients are unable to produce sufficient amount of antioxidant to cope up with the increased oxidative stress in them. Providing nutritional supplementation may present a novel approach for fast recovery. Administration of exogenous antioxidants like vitamin C, tocopherols would prevent tissue damage and make the patient therapeutically benefited.

**Keywords**: Free radicals, Oxidative stress, Leprosy, Lipid perroxidation

## Introduction

Oxidative stress is a condition associated with an increased rate of cellular damage induced by the oxygen derived oxidants commonly known as reactive oxygen species (ROS) (Reddy and Bansal 1987; Reddy et al 2003). ROS are highly reactive oxidizing agents belonging to the class of free radicals (Prasad 2003; Reddy et al 2003; Sahu et al 1991). The possible reason for the decreased antioxidant status in leprosy cases may be increased production of ROS (Dharmendra and Mukherjee 1952), deranged liver function, and the free radical producing ability of drugs used in MDT of leprosy (Ellman 1959; Siez and Stahl 1995).

The most common ROS that have potential implication include super oxide anion, hydrogen peroxyl radicals and the very reactive hydroxyl

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radicals (Marklund and Marklund 1974). The objectives of the present study is to investigate the levels of lipid peroxidation products (MDH), superoxide dismutase (SOD) and Glutathione levels in normal human volunteers and treated MB patients (Vijayaraghavan et al 2009). Nutritional rehabilitation by way of exogenous supplementation of functionally efficient anti-oxidants like vitamin E (Di Massio et al 1991; Goulart and Goulart 2009, Lockwood and Suneetha 2005, Prasad 2003) reactivates the enzymatic anti-oxidant system and guards against the insult caused by ROS during the pathogenesis of the disease and antileprosy chemotherapy (Dharmendra and Mukherjee 1952, Reddy and Bansal 1987, Siez and Stahl 1995).

## Materials and Methods

The subjects for this study comprised of Normal human volunteers (NHV, n=20) and treated MB patients (MB, n=20). Patients included in this study are from the Leprosy colony, Bhimavaram. The blood samples were collected from all the subjects using double syringe method (Rao et al 1982, Okhawa et al 1979, Prabhakar 1989) and the following parameters are estimated (Reddy and Bansal 1987).

### Estimation of Lipid Peroxidation Products (Malanodialdehyde)

Acetic acid detaches the lipid and protein of the tissue. The protein in the reaction mixture is dissolved by the addition of lauryl sulphate. Thiobarbituric acid (TBA) reacts with lipid peroxides, hydroperoxide and oxygen double bond to form the colour adducts with absorption maxima at 532 nm using Ohkawa et al 1979.

To 0.2 ml of plasma sample, 0.2 ml of 8.1% sodium lauryl sulphate, 1.5 ml of 0.8% thiobarbituric acid and 1.5 ml of 20% acetic acid (pH 3.5) were added. Volume was made up to 4 ml with double distilled water and heated at 95$^0$C for 60 min. After cooling, 1 ml of double distilled water and 5 ml of butanol: pyridine mixture was added. The solution was shaken vigorously in a vortex and centrifuged at 1000 rpm at room temperature for 10 minutes. Organic layer was separated and absorbance was read at 532 nm in the spectro-photometer (UV-VIS Spectrophotometer, SL 150, Elico, Hyderabad). Tetramethoxy propane 1 to 10 nM is used as external standard.

### Glutathione level in blood

Glutathione can be determined by several colorimetric, chromatographic and enzymatic procedures. Direct determination of sulphydryls in protein-free tissue extracts using Elman's reagent (DTNB.5, 5′ dithiobis (2-nitro benzoic acid)) gives a rapid approximation of GSH content. Bis (p-nitrophenyl) disulphide reacts with aliphatic thiol compounds at pH 8.0 to produce one mole of p-nitrothiophenol anion per mole thiol. Since this anion is highly colored (em 13, 6000 at 412 nm), it can be used to measure the thiol concentration.

Based on Ellman's method (Ellman 1959), to 2ml of buffer was added 100µl of sample followed by the addition of 500µl DTNB and volume is made up to 3ml with distilled water. Mixed well, kept for incubation for 10 minutes and absorbance was read at 412nm against the reagent blank.

### Superoxide dismutase activity in erythrocytes

Pyrogallol (1,2,3-benzenetriol)autoxidizes rapidly in alkaline medium to a yellow-brown colored compound. The absorbance increases linearly with time at 420nm. Superoxide anion radical ($O_2$) is involved in this autoxidation process estimated using Marklund and Marklund method[9]. SOD by dismuting the $O_2$ slows down the autoxidation of pyrogallol (Rock et al 1996, Vijayaraghavan et al 2005). The rate of increase in absorbance is inversely proportional to the amount of SOD in the system.

To 0.9 ml buffer was added 0.05 ml of 4 mM

pyrogallol followed by addition of 0.05 ml of sample. Zero reading was taken by adding 0.05 ml of 4 mM pyrogallol to 0.95 ml buffer. Absorbance was read at 420nm for 3min at 30 sec intervals. Standard SOD was used as external standard.

## Results

### Lipid Peroxidation Products (MDA)

The serum levels of MDA are expressed as nmol/ml in fig. 1A. The (Mean ± S.D) values were found to be NHV 5.810±0.513, MB patients 8.655±0.726 (*P<0.001). The MDA values were increased in MB patients with clinical improvement after treatment with antileprotic treatment.

### Superoxide Dismutase (SOD) levels

The levels of SOD expressed as IU/ml in fig. 1B. The (Mean ± S.D) values were NHV 73.50 ± 7.1, MB patients 57.80± 8.1 (**P<0.0001). The SOD values are lower in MB patients in comparision to NHV. These increased gradually with clinical improvement after treatment with antileprotic treatment.

### Glutathione levels (GSH)

The levels of Glutathione are expressed as IU/ml in fig. 1C. The (Mean ± S.D) values were NHV 0.782 ± 0.29, MB patients 0.443 ± 0.298 (***P<0.0001). The glutathione levels are significantly lower in MB patients in comparision to NHV. These increased gradually with clinical improvement after treatment with antileprotic treatment.

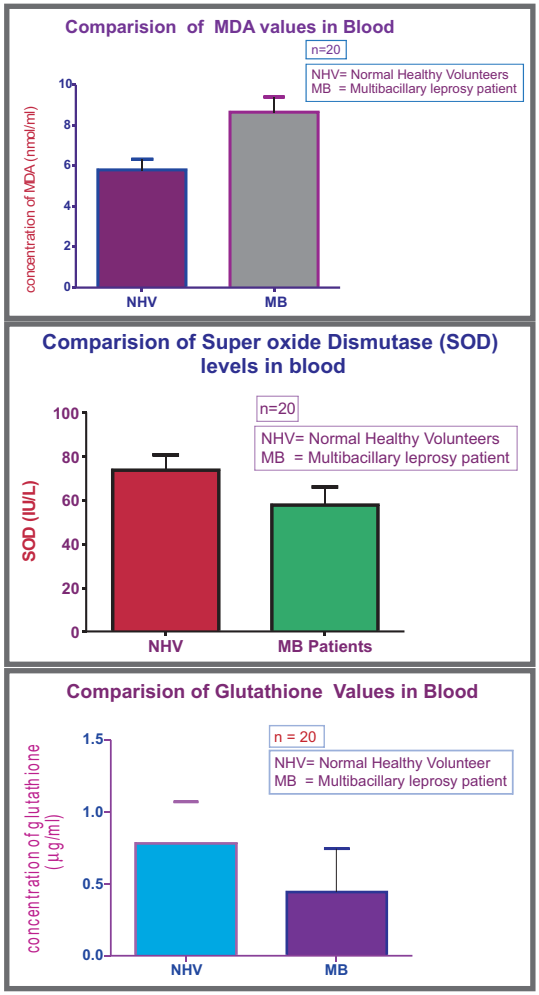


**Fig 1 : Levels of (A) Lipid peroxides, (B) Superoxide dismutase, (C) Glutathione status of treated MB patients in comparision with normal human volunteers (Mean ± S.D).**

## Discussion

In our present study in Bhimavaram we had investigated the serum lipid peroxidation products (MDA) and important free radical scavenging enzyme superoxide dismutase (SOD) and antioxidant glutathione levels in Normal Healthy volunteers (NHV) and MB patients. In our study of comparison of oxidative stress in Normal Healthy volunteers (n=20) and MB patients (n=20) results showed decrease in blood SOD levels between NHV and MB patients.

Blood levels of Glutathione were decreased in MB cases, in comparison with NHV even after treatment with MDT. When total lipid peroxidation products were estimated the results showed increased levels of MDA in MB patients in comparison with NHV.

Severe oxidative stress has been reported in leprosy patients because of malnutrition and poor immunity (Reddy and Bansal 1987). The decreased levels of SOD, glutathione and total antioxidant status in leprosy patients may indicate a degradation of these antioxidant enzymes by free radicals during detoxification processes (Vijayaraghavan et al 2005).

In the present study of estimation of antioxidants conclude that the free radical activity was increased and the total antioxidant status was decreased in all MB patients, indicating that there was an oxidative stress even after the treatment with MDT. The decreased levels of SOD, glutathione indicate the degradation of these antioxidant enzymes by free radicals during detoxification process (Marklund and Marklund 1974; Reddy and Bansal 1987). These findings further support a link between oxidative stress, and leprosy. The MB patients are unable to produce sufficient amount of antioxidants to cope up with the increased oxidative stress in them (Reddy and Bansal 1987, Siez and Stahl 1995). Hence nutritional supplementation may present a novel approach for fast recovery (Sahu and Das 1994, Sethi et al 1996, Siez and Stahl 1995). Administration of exogenous antioxidants like vitamin C, tocopherols would prevent tissue damage and make the patient therapeutically benefit.

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**How to cite this article :** Prabhakar MC, Santhikrupa D, Manasa N and Rao OU (2013). Status of Free Radicals and Antioxidants in Leprosy Patients. *Indian J Lepr.* **85** : 5-9.