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Research Article

Oxidative stress and antioxidant vitamins in leprosy

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ABSTRACT

Background: Leprosy is a disease of great antiquity and it still continues to be a significant public health problem in few countries including India .Of the various mechanisms that influence the pathogenesis of leprosy, oxidative stress is important which occurs due to derangement in the balance between ROS and natural antioxidants. Hence **t**his study attempted to assess the oxidative stress and antioxidant status in terms of MDA and vitamin E, vitamin C respectively in leprosy.
Methods: Hundred untreated leprosy patients (50 PB and 50 MB) were studied and compared with 50 healthy controls. Serum Malondialdehyde (MDA) and vitamin E, vitamin C was measured by spectrophotometric method. Serum malondialdehyde (MDA) was measured as an indicator of lipid peroxidation and antioxidant status was assessed by estimating serum vitamin E and vitamin C levels**.**
Results: Significant rise in serum MDA (P <0.001) in both PB and MB leprosy was seen when compared with controls. The vitamin E level was significantly decreased in both PB and MB leprosy patients as compared to controls. The vitamin C level was significantly decrease (P<0.001) in MB leprosy patients as compared to controls.
Conclusions: Elevated MDA levels indicate oxidative stress in leprosy patients, denoting its crucial involvement in the pathogenesis and tissue damage in leprosy. Hence MDA levels can be used to monitor prognosis, treatment and control of leprosy. Decreased vitamin E, C levels in leprosy can be improved by oral vitamin E, C supplementation.

Keywords: Leprosy, Oxidative Stress, MDA, Vitamin E, Vitamin C

INTRODUCTION

Leprosy is a disease of great antiquity and it still continues to be a significant public health problem in few countries including India. Recently, the number of cases has decreased considerably as a consequence of opportune detection and effective multidrug treatments .However, the incidence rate (new cases / year) has been stubbornly consistent. It remains a public health problem in Asia, Brazil and tropical Africa (WHO 2010).[1]

Leprosy is a chronic granulomatous infection caused by Mycobacterium leprae, an obligate intracellular bacillus that attacks cutaneous tissues and peripheral nerves producing skin lesions, nerve degeneration, anesthesia, infection and deformities.

The major defense against the microbial infection in leprosy is macrophage system. The infected foamy macrophages in skin and nerve show increased phagocytosis, enzyme activity and oxygen consumption

known as respiratory burst.[2] Burst of respiratory activity leads to production of a variety of molecules and free radicals called reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide, hydroxyl radicals etc.[3] These ROS can damage lipids, proteins and nucleic acids. Extensive damage can lead to death of the cell.

Humans are well endowed with antioxidant defense mechanism against reactive oxygen species; among the antioxidants are vitamins A, C and E enzymes such as superoxide dismutase and catalase. One of the roles of Vitamins C,A and E is that of scavenging free radicals in the aqueous and lipid phase of cells and the circulatory system to mop up generated reactive oxygen species ROS.[4,5]

Oxidative stress (OS) is an expression used to describe various deleterious processes resulting from an imbalance between free radical generating and scavenging systems. OS leads to metabolic impairment and cell death. It occurs if ROS are not adequately scavenged by antioxidants.[6]

The objective of present study was to assess the oxidative stress in leprosy patients by estimating levels of MDA as lipid peroxidation products and vitamin E, vitamin C for antioxidant status.

METHODS

Selection of cases

The study was designed as a case control study. The present study comprised of 100 newly diagnosed cases of various types of leprosy. The patients were selected from Skin – V.D. Department, OPD and IPD of MIMER Medical College and BSTRH Hospital. The diagnosis was done on clinical grounds and bacterial examination by skin clip method. Patients were classified into two groups Paucibaillary (PB) and Multibacillary (MB) based on the WHO guidelines.[7] Paucibaillary (PB) group includes tuberculoid (TT) and borderline tuberculoid (BT) leprosy patients while Multibacillary (MB) group includes borderline (BB), borderline lepromatous (BL) and lepromatous (LL). The patients were not taking any vitamins like tocopherol, ascorbic acid etc. Written Informed consent was taken from each patient and control before procedure. The control group consisted of age and sex matched 50 healthy individuals.

Inclusion criteria

Cases: Newly diagnosed leprosy patients in the age group 21-60 years.

Control: Healthy controls in the age group 21-60 years.

Exclusion criteria

The patients with history of smoking, diabetes mellitus, rheumatoid arthritis and other concomitant bacterial infections and major illness were excluded.

Sample collection

About 5 ml of fasting venous blood was collected with all aseptic precautions in plain bulb. Separated serum was used for measurement of MDA (an Index of lipid peroxidation) and vitamin E, vitamin C (antioxidant vitamins). Serum MDA levels were assessed by the method of Buege et al using thiobarbituric acid reaction.[8] Vitamin E (Tocopherol) was estimated by the method of: Baker and Frank.[9] Serum Vitamin C levels were assessed by the method of DNPH method.[10]

The biochemical data was expressed as mean ± standard deviation. Significance was analyzed using student paired 't' test. Correlations between MDA and vitamin E, C were also calculated by Pearson Correlation test.

RESULTS

Table 1 shows the rise in MDA levels was found to be highly significant in both groups of leprosy patients than in control individuals ($p<0.001$). The rise was also significant in MB leprosy patients (5.90±0.76 nmol/ml) than PB patients (4.11±0.43 nmol/ml).

Table 1: MDA levels in control and different types of leprosy.

Group	Sample Size(n)	Malondialdehyde level (n mole/ml)
Control	50	2.81±0.25
PB	50	4.11 ± 0.43*****
MB	50	5.90 ±0.76*****

***** $p<0.001$-significant

Table 2 shows significant decrease in vitamin E levels in both groups of leprosy patients than control (0.93±0.15 mg/dl). The decrease was more in MB leprosy patients (0.50±0.06 mg/dl) than PB leprosy patients (0.64±0.04 mg/dl). The decrease in vitamin C levels were not statistically significant in PB leprosy groups compared to controls. It also shows that vitamin C levels were significantly decreased in MB groups of leprosy patients than in control subjects (0.77±0.07 mg/dl).

Table 2*:* Serum vitamin E and vitamin C levels in controls and PB, MB leprosy patients.

Group	Sample Size(n)	Vit E mg/dl	Vit C mg/dl
Control	50	0.93±0.15	0.77±0.07
PB	50	0.64±0.04*****	0.70±0.07
MB	50	0.50±0.06*****	0.58±0.06*****

***** $p<0.001$- significant

Table 3 shows significant negative correlation between MDA and vitamin E in PB leprosy patients while it is not statistically significant between MDA and vitamin C levels. In MB leprosy patients there is significant negative correlation between MDA and vitamin E, also between MDA and vitamin C levels.

Table 3: Statistical correlation between serum MDA, serum vitamin E and vitamin C levels in patients group. (r values).

Correlation variables	PB	MB
MDA with Vitamin E	-0.5 *	-0.7 *
MDA with Vitamin C	-0.1	-0.8 *

***** p<0.001-significant

DISCUSSION

In recent years the phenomenon of lipid peroxidation has attracted considerable attention in several pathologic conditions. The major defense against the microbial infection in leprosy is macrophage system. Microbial killing by macrophages is associated with a burst of respiratory activity that leads to production of free radicals called reactive oxygen species (ROS). Prime targets of peroxidation by ROS are the polyunsaturated fatty acids (PUFA) in membrane lipids .PUFA is degraded by free radicals to form malondialdehyde (MDA).The level of MDA in serum serve as a marker of cellular damage due to free radicals.[11] Since MDA serves as an index of lipid peroxidation, it was estimated in leprosy patients to estimate the extent of lipid peroxidation. MDA levels in both PB and MB leprosy patients were significantly elevated compared to those in normal controls. This indicates that increased lipid peroxidation due to 'free radical'-mediated injury occurs in leprosy patients. Increased lipid peroxidation can occur if the rate of production of reactive oxygen species is higher or the antioxidant level is low.[12]

The present study shows significantly higher MDA levels from TT to LL thus the maximum increase in MDA level was in LL. In lepromatous leprosy there is defective monocyte –macrophage function .The macrophage in LL shows normal Phagocytosis, but they are unable to kill the M. leprae due to inadequate superoxide production.[13] In LL the source of ROS could be some other subpopulation of phagocytes in which normal respiratory burst occurs like immunologically activated macrophages, neutrophils and some other sources.

Vitamin E or Alpha tocopherol is a fat-soluble antioxidant. It protects lipid peroxidation efficiently through its chain breaking antioxidant action. Apart from the antioxidant action vitamin E also has membrane stabilizing effects.

The present study has demonstrated statistically significant decrease (p<0.001) in the levels of vitamin E (0.64±0.04 in PB and 0.50±0.06 in MB). Osadolor HB et al[14] found that there were significant decreases in the plasma vitamin E (P<0.05) relative to controls in patients with leprosy. R Vijayaraghavan et al[15] studied protective role of vitamin E in leprosy. They found that exogenous supplementation of vitamin E guards against the insult caused by ROS during the pathogenesis of the disease and antileprosy chemotherapy.

Vitamin-C or Ascorbic Acid is a water-soluble non-enzymatic antioxidant. Its role as an antioxidant is indicated by its known free radical scavenging action. As a reducing and antioxidant agent, it directly reacts with superoxide and hydroxyl radical and various lipid hydroperoxides. In addition it can restore the antioxidant properties of oxidized vitamin-E, suggesting that a major function of vitamin-C is to recycle the vitamin-E radicals.

The levels of vitamin C in leprosy patients were significantly low when compared with controls. Sinha SN.et al[16] observed low levels of vitamin C in all types of leprosy. We found lower values of vitamin-C in MB leprosy patients only. Osadolor HB. et al[14] found that there were significant decreases in the plasma vitamin E (P<0.05) relative to controls in patients with leprosy.

Plasma levels of antioxidant vitamin α-tocopherol are significantly reduced in PB and MB leprosy and ascorbic acid levels are significantly reduced in MB patients when compared to controls. The level of α-tocopherol is significantly low in MB leprosy as compared to PB leprosy. Even though there is a fall in PB leprosy as compared to control group, the level was found within normal range for majority of the patients in this group. On the contrary the reduction was found clinically significant in majority of MB leprosy patients.

The decreased levels of serum vitamin E, vitamin C may be due to their increased utilization in scavenging lipid peroxides. Low antioxidant vitamins may support the involvement of oxidative stress in leprosy.

As the pathological spectrum of leprosy shifted from TT to LL end there was a progressive fall in serum vitamin E and vitamin C level. As these vitamins are known for their immune system stimulant action and cell mediated immunity is severely impaired in LL leprosy group, the severe reduction in the concentration vitamins E, C in plasma may be associated with immune compromised state found in this group of leprosy.

The low levels of antioxidants may expose the tissues to oxidative stress mediated modifications of cells and biomolecules, and could mediate inflammatory episodes, organ damage, depressed cell mediated immune response and degeneration of nerves in leprosy patients.[17]

The *'antioxidant hypothesis'* proposes that antioxidants like vitamin C, vitamin E, carotenoids present in fruits and fresh leafy vegetables afford protection against the oxidative damage to cells and bio-molecules.[18] As leprosy patients are mostly from low socioeconomic status, they do not get antioxidant rich diet like fruits and fresh leafy vegetables every day. Thus the enhanced lipid peroxidation observed in leprosy patients can also be attributed to a large extent to the paucity of vitamins in the diet.

From above discussion, it becomes clear that, increased state of lipid peroxidation seen in leprosy patients is most likely to be due to inadequate scavenging of ROS due to decreased level of antioxidant defense such as vitamin E and vitamin C leading to increased oxidative stress. Of the various mechanisms that influence the pathogenesis of leprosy, oxidative stress is important which occurs due to derangement in the balance between ROS and natural antioxidants. Oxidative stress may have an upper hand which may be responsible for the clinical manifestations seen in patients of leprosy.

This study confirms the presence of oxidative stress in leprosy which is maximum in LL. Decreased vitamin E, C levels in leprosy patients may be improved by vitamin E and vitamin C supplementation. This can be proving to be a cost effective measure to minimize tissue damage. Improvement in the general conditions may help in boosting of the mental status of patients.

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