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A STUDY OF ASCORBIC ACID AND ALPHA TOCOFEROL IN DIFFERENT FORMS OF LEPROSY

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ABSTRACT
Leprosy, an infectious disease caused by *Mycobacterium leprae*, is still a public health problem in India. It causes oxidative stress, and antioxidant, like ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E) may play a significant role in its progression and cure. 116 newly diagnosed cases of Lepromatous leprosy (LL) and 79 cases of Tuberculoid leprosy (TL) were selected as subject, and 35 random age and sex matched healthy individuals as controls. Antioxidant status was assessed by estimating ascorbic acid and α-tocopherol levels, along with enzymes malondialdehyde (MDA), superoxide dismutase (SOD) and ratio of MDA/SOD as the indicator of oxidative stress. Both the ascorbic acid and α-tocopherol was significantly decreased in both LL and TL leprosy as seen when compared with healthy controls ($p \leq 0.001$). But there was a statistically significant increase in the value of MDA level and ratio of MDA/SOD ($p \leq 0.001$). and a sharp decrease in SOD activity in leprosy patients compared to healthy controls ($p \leq 0.001$). Decreased level of ascorbic acid and α-tocopherol in both the group of leprosy patients may be improved by supplementation of ascorbic acid and α-tocopherol and prevent the tissue injury.

KEYWORDS: Leprosy, Oxidative Stress, Ascorbic acid, α-tocopherol, Malondialdehyde, Superoxide dismutase.

INTRODUCTION
Leprosy is a chronic granulomatous disease caused by the intracellular acid resistant bacillus *Mycobacterium leprae* (*M. leprae*). The *M. leprae* attacks mainly skin and peripheral nerves, the mucosa of the upper respiratory tracts and the eyes of the infected persons, provoking severe deformities and leading to many cases of mutilation and social stigma.[1] The prevalence of the disease is still high in India, Brazil and tropical Africa.[2]

In leprosy, six spectrums of clinical phenotype have been classified by Ridley-Jopling; but commonest in India are Lepromatous leprosy (LL) and Tuberculoid leprosy (TL).[3]

Nutritional deficiencies are common in countries in which leprosy is predominant. Poverty, poor education, dietary inadequacy, reduced intake of nutritional vegetables, fruits, fish, etc. are the significant risk factors of leprosy.[4] It is probable that deficiency of trace elements and vitamins affect the adaptive immune response, causing in imbalance of the host response to pathogens.[5]

In *M. leprae* infection macrophage activation is important for the control of this microorganism in which the main mechanism of destruction is mediated by reactive oxygen species (ROS). These ROS can damage lipids, proteins and nucleic acid; also, extensive damage can lead to death of the cell. One of the roles of ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E) are that of scavenging free radicals in the aqueous and lipid phase of cells and the circulatory system to combat generated ROS.[6] The level of malondialdehyde (MDA) in plasma serves as a marker of cellular damage due to free radicals.[7] An enzymatic antioxidant superoxide dismutase (SOD) is catalyses the dismutation of superoxide ion into oxygen and hydrogen peroxide. The prognosis and treatment of the leprosy can be benefited by the evaluation of oxidative stress.

The present study was aimed to evaluating oxidative stress in leprosy patients by estimating levels of MDA as

lipid peroxidation products and ascorbic acid, α-tocopherol for antioxidant status.

MATERIALS AND METHODS

This study was designed as a case-control study with leprosy patients as cases and healthy volunteers as controls. Newly diagnosed leprosy patients before the start any treatment, attending the outpatient department of North Bengal Medical College and Hospital, Hathighisa Leprosy Clinic and Jeshu Ashram, a Leprosy Clinic run by the Missionaries; all under the jurisdiction of Darjeeling District, West Bengal, India. The diagnosis was done on clinical grounds and bacterial identification, and these diagnosed patients were classified into two groups – LL and TL based on the WHO guidelines.[8]

This study was approved by the ethical committee. After prior written informed consent from the patients, we included 116 cases of LL and 79 cases TL in this study. These patients did not have history of any antioxidant vitamins medications, like, ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E), etc. Also, 35 healthy age and sex matched individuals, without any past history of leprosy disease were included as controls.

People with history of smoking, alcoholism, any skin disease, rheumatoid arthritis and systemic diseases like diabetic mellitus or hypertension or any major illness were excluded from this study.

After overnight fasting, 5 ml of venous blood samples were collected from all the subjects in vacutainer gel vials. The samples were analysed for ascorbic acid (Vitamin C) levels by Dinitrophenyl-hydrazine (DNPH) method[9] and α-tocopherol (Vitamin E) was estimated by Baker and Frank method.[10] Serum MDA levels werequantitative assessed by using thiobarbituric acid reactive substances (TBARS) reaction.[11] and SOD levels on serum using a kit supplied by Randox LaboratoriesLtd., U.K., along with appropriate control. Also, total protein was estimated by biuret method.[12]

This data was analysed by using SAS Software (6.12 Version).[13] The results were expressed as Mean ± Standard Deviation (SD).

RESULTS

The baseline characteristics of control and leprosy patients are summarised in Table 1.

Parameter	Unit	Control (n = 35)	Lepromatous leprosy (LL) (n = 116)	Tuberculoid leprosy (TL) (n = 79)
Ascorbic acid (Vitamin C)	mg/dl	1.08 ± 0.22	0.62 ± 0.18	0.72 ± 0.19
α-tocopherol (Vitamin E)	mg/dl	0.98 ± 0.15	0.65 ± 0.09	0.88 ± 0.11
Malondialdehyde (MDA)	nmol/dl	198.28 ± 3.12	596.07 ± 29.12	638.64 ± 21.61
Superoxide dismutase (SOD)	Unit/gm Protein	99.12 ± 2.61	40.21 ± 0.82	49.47 ± 0.61
MDA/ SOD		2.09 ± 0.91	15.01 ± 0.99	13.37 ± 1/04

Values as Mean± Standard Deviation (SD)

59.5% patients had LL, whereas 40.5% patients had a TL disease. There was no difference in age distribution of leprosy cases and healthy control groups. Similarly, there was no difference in sex distribution of cases and controls.

Comparison of ascorbic acid and α-tocopherol levels in both the cases of leprosy and controls is seen in table 1. The levels of ascorbic acid and α-tocopherol were significantly lower in both the cases of leprosy as compared to healthy controls. The decrease was more in LL patients (0.62 ± 0.18 mg/dl) and (0.65 ± 0.09 mg/dl) than TL patients (0.72 ± 0.19 mg/dl) and (0.88 ± 0.11 mg/dl) respectively. But, the decrease of both ascorbic acid and α-tocopherol levels were rather of similar levels in LL patients when compared with TL patients.

The mean value of serum MDA and MDA/ SOD ratio were significantly increased and SOD was significantly decreased in leprosy patients when compared to healthy control groups. The mean values of serum MDA and MDA/ SOD ratio were non-significant when compared to the two groups of leprosy patients. Same features for SOD also, when compared in LL patients in comparison with TL patients.

DISCUSSION

In this case-control study, a significant difference in ascorbic acid and α-tocopherol levels that were measured. All levels were low in both the groups of leprosy patients; also, LL patients had lower levels of these two vitamins level. But no significant difference was found between LL and TL forms of leprosy. Our findings are consistent with finding of previous studies demonstrating an expected result as all vitamins level were lower in leprosy patients.[14] These lower levels of vitamins were attributed to poor nutrition.[15] Leprosy is commonly known as a disease of poverty. India is one of the countries where good number of new cases of leprosy were still identified. A perfect concentration of micronutrients like ascorbic acid, α-tocopherol, etc. are important to enhance the immune response against leprosy.[16] Ascorbic acid plays crucial role in healing of trophic ulcers, which are quite common in leprosy.[14]

This study has demonstrated statistically significant decrease in the levels of ascorbic acid (0.62 ± 0.18 mg/dl in LL and 0.72 ± 0.19 mg/dl in TL).

In recent years, some studies suggested that oxidative stress was to play a key role in the pathogenesis of leprosy and phenomenon of lipid peroxidation in several pathological conditions.[17] The microbial infection in leprosy is macrophage system.Microbial killing by macrophages is associated in burst of respiratory activity that trends to production of free radical, called ROS. The excess production of ROS as seen in leprosy. The possible reason for the decreased antioxidant status in leprosy patients may be enhanced production of ROS, abnormal liver function and the free radical producing ability of drugs. Severe oxidative stress has been repeated in leprosy patient become of malnutrition and poor immunity. The α-tocopherol has ability to prevent oxidative stress mediated nerve damage.[18]

The level of MDA in plasma serve as a as a marker of cellular damage due to free radical generation.[19] MDA levels in both the group of leprosy patients were significantly elevated compared to those in healthy normal controls. This indicate the increased level of lipid peroxidation due to free radical mediated injury occurs in leprosy patients.

The α-tocopherol is a fat-soluble antioxidant, it protects lipid peroxidation efficiently and also has membrane stabilising effects. This study has demonstrated statistically significant decrease in the levels of α-tocopherol (0.65 ± 0.09 mg/dl in LL and 0.88 ± 0.11 mg/dl in TL).

The decreased value of both ascorbic acid and α-tocopherol levels may be due to increased utilization in scavenging lipid peroxidase. Also, low antioxidant vitamins support the involvement of oxidative stress in leprosy. Some components of leprosy might be down regulating the SOD gene in the host macrophages and other tissues. In this study supports the existence of oxidative stress in leprosy from increased MDA and decreased SOD activity in leprosy patients, like other studies.[20]

In conclusion,it becomes clear that increased state of MDA seen in leprosy patients is most likely due to inadequate scavenging of ROS due to decreased level of antioxidant defense e.g., ascorbic acid and α-tocopherol leading to increased oxidative stress.

Thus, administration of nutritional antioxidants such as, ascorbic acid and α-tocopherol may benefit in the treatment and a cost-effective means to minimise tissue damage, which may establish by follow up studies.

Ethical Approval
Ethical Committee clearance was obtained from Institutional Ethical Reviewed Board of Hospital before commencement of study. Informed consent was obtained from all individual patients included in this study.

Informed Consent
Informed consent was obtained from all the participants individually who were included in the study.

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Nil

Competing Interests
The authors declare that they have no competing interests.

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