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EVALUATION OF OXIDATIVE STATUS AND ZINC LEVEL IN LEPROSY PATIENTS AFTER ZINC SUPPLEMENTATION

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ABSTRACT

Increased oxidative stress and antioxidant deficiency present in infected subjects can be related to infection progression in chronic diseases like leprosy. Zinc is an essential trace element required for human and animal nutrition and its role as an important component of body's antioxidant system has been well established. The aim of the study was to evaluate the effect of zinc supplementation on plasma malondialdehyde and serum zinc levels in leprosy patients. 50 cases, 30 belonging to Tuberculoid leprosy and 20 categorized as Lepromatous leprosy groups underwent zinc supplementation for a period of 4 months. The malondialdehyde and zinc levels were assessed at baseline, after 2 months and 4 months. Results showed that plasma malondialdehyde, the marker of oxidative stress was significantly reduced, but serum zinc levels showed a significant rise in both the groups in the post intervention period as compared to their levels before supplementation, indicating a potential beneficial role of zinc as a nutritional supplement and an anti-oxidant.

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Introduction

Leprosy is caused by invasion of *Mycobacterium leprae* bacilli occurring intracellularly. The innate methods by which the human body meets the challenge of invading live microbes is to destroy the microbes through an activation system leading to biochemical production of oxygen and other free radicals by the phagocytic cells[1]. Reactive Oxygen species (ROS) released during bacterial phagocytosis mediate peroxidation of lipids yielding lipid hydroperoxides (LOOH) that decompose with formed conjugated dienes yielding Malondialdehyde (MDA), which is one of the most widely used markers of the peroxidative process[2]. Oxidative stress ensues when the pro-oxidant antioxidant balance gets perturbed in favour of pro-oxidants.

A constellation of ROS capable of damaging cellular constituents are generated in excess during chronic, inflammatory, neurodegenerative disease process of leprosy leading to enhanced oxidative stress and lowered antioxidant status[3]. Severe oxidative stress has been reported in leprosy patients because of malnutrition and poor immunity[4]. Many factors have been attributed for the occurrence of hypozincaemia in leprosy. It is a multifactorial deficiency, in which dietary factors such as poor intake as well as high phytate intake, faulty absorption, altered metabolism and redistribution of zinc are involved[5]. Chronic zinc deprivation generally result in an increased sensitivity to the effects of oxidative stress due to inadequate activity of zinc dependent compounds and antioxidant enzymes. Studies regarding the possible role of oral zinc therapy as an anti-oxidant have been done in various diseases[6,7,8,9], while some observations revealed clinical improvement and progress in nutritional status in leprosy after zinc therapy[10,11].

Zinc is an essential trace element with a multitude of roles in human nutrition. It is one of the antioxidant minerals in animals involved as a part of the oxidant defence network that protects the cell membrane from oxidative damage[12]. It is therefore important that the status of zinc be assessed and zinc deficiency corrected by supplementation which could help in improving nutritional status and ameliorating oxidative stress and hence be an effective strategy for treatment and control of leprosy. In the present study we made an attempt to evaluate the effect of oral zinc supplementation on plasma MDA and serum zinc and tried to assess its nutritional and antioxidant property.

MATERIALS AND METHODS

Fifty untreated patients visiting the OPD, of the Leprosy Home at Naya Bazaar, Cuttack, formed the study group. All the cases were in the 20-60 years age group. After a detailed history and thorough clinical checkup all patients were subjected to slit skin smear and histopathological examination of skin lesions and were classified according to Ridley Jopling scale[13] into 30 Tuberculoid leprosy (TT) and 20 Lepromatous leprosy (LL) cases. Thirty age, sex and socio-economic matched healthy individuals constituted the controls. After obtaining informed consent, the patients received oral zinc sulphate formulation (220mg/day) for 4 months. Patients with history of Multi drug therapy (MDT), cases of chronic conditions such as leukaemia, lymphoma, hepatitis, cirrhosis and any other chronic illnesses were excluded from the study.

The present study was conducted in Hi-Tech Medical College and Hospital, Bhubaneswar. Ethical approval was given by the institutional ethical committee. Routine investigations like Hb%, total protein and lipid profile was performed in both the cases and controls. Plasma MDA was estimated as Thiobarbituric acid

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reactive substances (TBARS) by the method of Satoh[14] . The assay is based upon the reaction of TBA with MDA, which is one of the aldehyde products of lipid peroxidation. The pink colour MDA-TBA adduct so produced was measured at 532 nm and the result expressed in nmol/ml. Serum Zinc was measured by Nitro PAPS method where zinc in alkaline medium reacts with Nitro Phenol Adenosine Phospho Sulphate (PAPS) to form a purple coloured complex whose intensity is directly proportional to the amount of zinc present in the sample[15]. Estimation was done in the pre-intervention period, after 2 months and 4 months oral zinc therapy. There were 2 drop-outs from the LL cases during 2 months supplementation period, and 6 drop outs (5 TT and 1 LL) after 4 months. MDA and zinc measurement could be done in 42 cases as the subjects withdrew their consent, either because of GI side effects or due to lack of motivation.

Results were expressed in mean ± SD. One way Analysis of Variance (ANOVA), Post Hoc Turkey test and regression analysis were used for statistical analysis by SPSS software. The significance of observed differences among the groups were evaluated with Tukey's Post hoc test, p < 0.05 was significant. Correlation study was done using Pearson correlation coefficient (r value) and p<0.01 was considered significant.

TABLE-1. CLINICAL DATA OF THE CONTROLS AND CASES

VARIABLES	CONTROL(n=30)	TT (n=30)	LL (n=20)
AGE(yrs)	33.96 ± 9.8	36.76 ± 7.28	52.8 ± 6.44 **
SEX (MALE/FEMALE) RATIO	2	4	4
WEIGHT (kg)	67.8 ± 5.21	60.6 ± 5.28 **	58.45 ± 3.95 **
BODY MASS INDEX (kg/m ²)	22.15 ± 1.29	19.75 ± 1.86 **	18.05 ± 1.60 **
Hb% (gm/dl)	16 ± 1.76	11.06 ± 2.57 **	9.05 ± 1.35 **
TOTAL SERUM PROTEIN (gm/dl)	7.77 ± 0.36	6.22 ± 0.42 **	6.01 ± 0.47 **

** (p < 0.001) - Highly Significant

The clinical data of the whole study group has been depicted in Table-1. This revealed that the mean Body weight, Body Mass Index, Hb% and serum protein levels in both the patient groups (TT and LL) was significantly decreased (p< 0.001) in comparison to controls indicating a state of undernutrition, anaemia and hypoproteinemia ,as the cases belonged to low socio-economic status suggesting the relation of inadequate nutrition in the progress of the disease process.

The lipid profile investigated in the study group. (Table-2), demonstrated that there is a significant fall in HDL cholesterol levels in TT (p < 0.05) and LL (p<0.001) cases in comparison to controls. Triacylglycerol and VLDL cholesterol (p< 0.05) as well as Total cholesterol (p< 0.001) was observed to be high in the LL group when compared with controls. Significant fall (p< 0.05) in HDL and highly significant rise (p< 0.001) in Total cholesterol observed in LL as compared to TT group revealed a different picture of dyslipidemia in 2 categories of leprosy.

TABLE-2. LIPID PROFILE OF THE STUDY GROUP

PARAMETERS	CONTROL	TT	LL
TOTAL CHOLESTEROL (mg/dl)	185.98 ± 17.98	190.96±14.98	† † 206.15 ± 19.12**
TRIACYLGLYCEROL (mg/dl)	119.89 ± 7.42	121.18 ± 7.03	124.55 ± 5.77*
HDL CHOLESTEROL (mg/dl)	41.47 ± 5.11	36.98 ± 2.19*	† 32.65 ± 5.59**
LDL CHOLESTEROL (mg/dl)	150.93 ± 21.24	151.56 ± 18.89	147.90 ± 6.83
VLDL CHOLESTEROL (mg/dl)	23.98 ± 1.49	24.23 ± 1.41	24.91 ± 1.15 *

** p< 0.001 - Highly significant as compared to control

* p< 0.05 - Significant as compared to control

† † p<0.001 - Highly significant as compared to TT

† p<0.05 - Significant as compared to TT

The pre-supplementation plasma MDA in both the patient groups was observed to be high (p<0.001), whereas the serum zinc was low(p<0.001) in LL cases which was found to be statistically significant. Only a moderate decrease in serum zinc was revealed in the TT group (p < 0.05).This indicated a state of oxidative stress and anti-oxidant deficiency in leprosy cases (Table-3).

TABLE-3. BASELINE PARAMETERS OF THE STUDY GROUP

PARAMETERS	CONTROL	TT	LL
MDA (nmol/ml)	1.12 ± 0.07	3.25 ± 0.39**	4.10 ± 1.21**
Zn (µg/dl)	107.86 ± 11.11	100.13 ± 7.65*	66.19 ± 9.26**

** p<0.001 -Highly Significant

* p< 0.05 - Significant

Significant ($p < 0.05$) decrease in plasma MDA and increase in serum zinc was seen in post supplementation period in both the diseased groups (Table-4) indicating the beneficial effect of oral zinc treatment. +

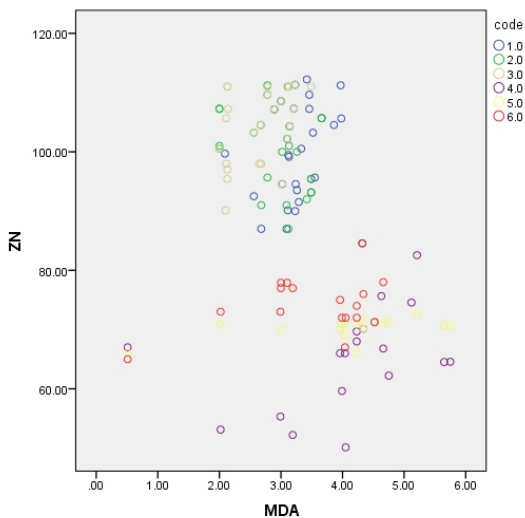
TABLE-4. COMPARISON OF PLASMA MDA AND ZINC LEVELS AFTER ORAL ZINC SUPPLEMENTATION

STUDY GROUP	MDA	MDA	MDA	ZN	ZN	ZN
	Baseline	2month	4month	Baseline	2month	4month
TT	3.25±0.39	2.89±0.48	2.15±0.46*	100.13±7.65	101.64±7.07	109.35±6.32*
LL	4.10±1.21	4.02±1.25	3.13±1.06*	66.19±9.26	70.63±2.35	74.27±4.55*

* $p < 0.05$ - Significant as compared to Baseline value

Graph-1 shows a negative correlation ($r = -0.39$) between the two parameters MDA and Zinc.

GRAPH-1 CORRELATION BETWEEN MDA AND ZINC



Correlation Coefficient $r = -0.39$

- Code-1 Baseline TT
- Code-2 2 month TT
- Code-3 4 month TT
- Code-4 Baseline LL
- Code-5 2 month LL
- Code-6 4 month LL

DISCUSSION

Oxidative stress is an expression used to describe various deleterious processes resulting from an imbalance between free radical generating and scavenging system. Induction of macrophages in response to bacterial infection is associated with a burst of respiratory activity which contributes to an increase in MDA levels since phagocytosis is a potential mechanism in ROS production[16]. The ROS can diffuse from the site of generation and damage the structural and functional integrity of cells causing tissue damage[17]. Bhadwat and Borade reported a progressive

increase in mean serum MDA levels in lepromatous leprosy with minimum increase at the tuberculoid pole[18]. Some authors noted a significant increase in serum MDA levels in multibacillary (MB) with significant decrease in anti-oxidant status, but changes were non-significant in paucibacillary (PB) group[19], while others revealed a significant increase in oxidative stress index (MDA/SOD ratio)[20,21]. Our study corroborated with the above findings indicating a steady increase in MDA along the bacterial spectrum from TT to LL. The bactericidal activity of macrophages in TT are activated but an overwhelming bacterial load in LL, stimulates phagocytic activity in the macrophages which are unable to kill the bacilli. It is likely that the source ROI in such case could be some other subpopulation of phagocytes like neutrophils.

Nutritional zinc deficiency is seen secondary to inadequate dietary intake and malnutrition[22]. The complex lepromatous processes occurring inside various organs may effect the serum trace elements, which play a vital role in metabolism and also act as catalysis in biochemical reactions of the body leading to deterioration of trace element supplies[23]. It has been suggested that the increasing bacterial load in macrophages through the leprosy spectrum could produce hypozincaemia by increasing the liberation of leukocyte mediator (LEM) like substance which stimulates zinc distribution with hepatic uptake and decreased serum levels[24]. Dysproteinemia of varying degrees is a feature of all subtypes of leprosy, therefore decreased carrier protein in plasma along with dietary factors could be among the contributory factors. A decrease in serum zinc levels was revealed in the present study, in comparison to healthy controls, with maximum decrease in LL cases suggesting that there is a correlation between serum zinc and severity of the disease. This is in general confirmation with observations of various studies[25,26,27].

The role of zinc in modulating oxidative stress has recently been recognized. It is involved in several components of the oxidant defence system. It has been shown to stabilize membrane structures, it is required for the adequate formation and function of the antioxidant enzyme Cu-Zn SOD, through its association with metallothionein (MT), which is rich in thiolate groups and an excellent scavenger of hydroxyl radicals and also by its competition with Cu and Fe for membrane binding sites, thus reducing the potential for hydroxyl radical formation via redox cycling [12]. It protects vitamin E stores [28] and prevents LDL and VLDL oxidation [29]. Despite having such important functions, the body does not store zinc and requires a constant dietary intake. In concordance with the observations made by various authors[6,7,10,11] we observed that following oral zinc therapy in leprosy, the serum zinc levels increased, oxidative stress marker decreased therefore retarding the oxidative process and improving nutritional status in comparison to their pre-supplementation values.

This study was done to draw attention to the possible importance of zinc nutrition in leprosy. The unique properties of zinc may have significant therapeutic benefits. In leprosy, zinc deficiency increases oxidative stress, which may complicate and adversely affect the disease process. It is therefore important that the status of zinc be assessed and its deficiency corrected by developing a supplementation protocol in conjunction with routine modalities of treatment in order to maximize the health benefits of the supplement and hence present a novel approach for faster recovery and better prognosis.

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