INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-2: April-June -2011 IJABPT *ISSN 0976-4550*

ERYTHROCYTE ANTIOXIDANT ENZYMES IN MULTIBACILLARY LEPROSY PATIENTS

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ABSTRACT: Oxidative stress caused by reactive oxygen species incriminated to play an important role in leprosy. Antioxidants are substances capable of eliminating the injurious effect caused by reactive oxygen species. We have measured the levels of antioxidant enzymes of red blood cells like Suproxide dismutatse, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase in 25 multibacillary (highly bacilliferous) type of leprosy patients and 25 age matched healthy persons as controls. We noticed a significant ($P < 0.05$) lowering in the levels of enzymatic antioxidants in the red blood cells indicating that oxidative stress is operational and antioxidant status is depressed in the red blood cells of leprosy patients. Reduction in the efficiency of red blood cell antioxidant defense may render leprosy patients vulnerable to oxidative stress, lowering of antioxidant status, neurodegeneration, depressed immunity against the invading intracellular pathogen the *Mycobactrium leprae*. Nutritional intervention by way of exogenous supplementation of functionally efficient antioxidant could reactivate the antioxidant system and guard the red blood cells against the insult caused by reactive oxygen species.

Key words : Leprosy,erythrocyte antioxidant enzymes, Oxidative stress.

INTRODUCTION

Leprosy is a chronic granulomatous disease of mankind caused by *Mycobacaterium leprae*. The integrity of cells in affected persons is incessantly challenged by reactive oxygen species (ROS) capable of causing injury to cellular structures. ROS includes oxygen-derived free radicals like (Superoxide radical, hydroxyl radical, hydroperoxide radical and other reactive oxygen species like hydrogen peroxide). Cells do have an elaborate defense against the potentially deleterious ROS the "*Antioxidant defense system*" that comprises of enzymatic and non-enzymatic antioxidants prevent the cells from ROS mediated insult. Under normal conditions, the production of oxygen derived free radicals is compensated by the action of protective antioxidant enzymes like Superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase compartmentalized in cells and protect against the toxicity of reactive oxygen species (Yu, 1994). However, in leprosy patients this delicately maintained physiological balance is shifted in favor of ROS (Bagwat and Borade, 2002). Studies on the levels of non-enzymatic antioxidants in erythrocytes of leprosy cases reveal the low levels of red blood cell glutathione (Sheriff, 1989). Accumulating recent evidences suggest that ROS able to affect the T cell signaling pathways and also able to trigger apoptosis in leprosy patients (Cemerski et al, 2002). The reduction in red blood cell antioxidant enzymes may result in ineffective removal of ROS and one way of studying the involvement of oxidative stress driven assault is to assess the *invivo* levels of enzymatic antioxidants of the red blood cells (McRury et al, 1993). The present study is aimed to assay the levels of intra-cellular (erythrocyte) enzymatic antioxidants which reflect the *in vivo* antioxidant status during the chronic disease pathology of leprosy.

Material and methods

The studies were conducted on anti coagulated blood samples obtained by veinpuncture from leprosy in the age group of 25 to 45 years who were patients attending OPD at Central Leprosy Teaching and Research Institute, Chengalpattu, India. Informed consent of the study subjects and consent of the ethical committee was obtained from the Institute. Healthy adults (group I) comprised of 25 normal individuals were selected from staffs and students, of the institute. Person with systemic ailments, diabetes, other infectious disease taking drugs for other bacterial infections were excluded from the study. Diagnosis of leprosy was made on the clinical examination of patients and skin smear examination for the presence of the causative germ. The present study included only MB leprosy, which is highly bacilliferous (severe) form of disease.
All diagnosed MB leprosy cases were at the active stage of the disease at the time of diagnosis. Anticoagulated blood samples were collected from the study subjects was processed for the separation of haemolysate as described in the method of (Dodge et al, 1963). The enzyme Superoxide dismutate (SOD) in the haemolysate was measured by its ability to prevent oxidation of epinephrine (Misra and Fridovich, 1972). Glutathione peroxidase activity was determined as described in the method of (Rotruck et al, 1973). The activity of catalase was measured following changes in hydrogen peroxide concentration (Sinha, 1972). Activity of glutathione reductase is measured as described in the method of (Staal et al, 1969) . Enzyme Glutathione-S-transferase was measured as described in the method of (Habig et al, 1974). Haemoglobin in the whole blood was estimated by the method of (Drabkin and Austin, 1932).

RESULTS

Values are expressed in mean ± Standard Deviation. Student "t" test was used to evaluate the difference between the groups. The criterion for significance was $P<0.05$. Activities of red cell antioxidant enzymes in the haemolysate preparations of control and experimental subjects were presented in table 1.

Table 1. Activities of enzymatic antioxidants in control and multibacillary leprosy patients

Antioxidant enzyme	Control	MB leprosy cases
Superoxide dismutase *(U/g Hb)*	1865 ± 503	1119 ± 108
Catalase *(μ moles of H_2O_2 consumed / g Hb/ min)*	1032 ± 156	646 ± 79
Glutathione Peroxidase *(U/g of GSH utilized /g Hb/ min)*	5645 ± 650	3496 ± 455
Glutathione reductase *(U/ g Hb)*	6.8 ± 0.6	4.2 ± 0.3
Glutathione-S-transferase *(U/ g Hb)*	2. 60 ± 0.32	1.23 ± 0.2

Multibacillary leprosy patients (group II) had significantly decreased activities of antioxidant enzymes when compared to controls (compare group I vs group II). The activities of enzymatic antioxidants found decreased with the severity of the disease. This may be largely due to the excess generation of ROS during the chronic inflammatory and infectious disease process of leprosy. The main observation of the present study was decline in the enzymatic antioxidant status signaling that oxidative stress-related mechanism is operational in leprosy patients.

DISCUSSION

Erythrocytes are susceptible for oxidative stress because of the high oxygen content in them. ROS affect two main component of the red cells they are: 1. The red cell membrane and 2.The haemoglobin molecule. The studies were conducted on the red cell haemolysate preparation which is reasonable in terms of oxidative stress modification of the red cell in response to reactive oxygen species (ROS) in leprosy patients. Enzyme superoxide dismutase is a primary antioxidant enzyme which specifically scavenge ROS like superoxide radicals (Halliwell and Gutteridge, 1985). The low levels of superoxide dismutase as seen in leprosy patients could enhance the production of superoxide radicals in multibacillary leprosy patients. Catalase is primarily a major antioxidant enzyme that primarily work to catalase the decomposition of hydrogen peroxide to water formed during the free radical chain reactions. Decrease in the activities of catalase together with glutathione peroxidase could favor the accumulation of highly deleterious hydrogen peroxide. Glutathione peroxidase serves as a major protective enzyme against the accumulating organic peroxides (ROOH) which are potential radical forming species within the cell (Cheng et al, 1981). Glutathione reductase catalyses the converstion oxidized glutathione to reduced glutathione. It is possible to slow down the ROS mediated damage and progression of chronic diseases through diet rich in green leafy vegetables, fruits, nuts and grains which seems to be protective due to the presence of antioxidants. However, leprosy affected persons do not always get antioxidant rich diet regularly. Hence exogenous supplementation of antioxidants will be an added advantage for leprosy patients.

The low levels of *invivo* free radical scavengers (enzymatic 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. . The concept that oxidative stress occurs in leprosy may be due to the infection with *Mycobactrium leprae,* the inflammatory response of the patients to infectious agent and defective cell mediated immunity to infection of *M.leprae*. Apart from the severity of the disease on the one hand, malnutrition and deranged liver function frequently seen in leprosy cases could affect the homeostasis of antioxidants (Foster,1988). It is now increasingly evident that antioxidants are involved in many body process and they operate as rejuvenators. Measurement of antioxidant provides evidence of susceptibility to free radical mediated insult during the disease process of leprosy. The assessment of free radical mediated toxicity in leprosy patients will provide a strong experimental basis for the development of new diagnostic, therapeutic and prevention strategies. One of the approaches to the disease prevention is to use specific nutrients that protect tissues against the toxic injury caused by ROS. Since cell mediated immuno deficiency appears to be involved in determining the disease type in leprosy, mere killing of the bacteria using anti leprosy chemotherapy (MDT) without correcting the antioxidant status could aggravate inflammatory process in affected individuals. Nutritional intervention with functionally efficient, immuno enhancing essential micro-nutrients like alpha lipoic acid, vitamin C, vitamin E, vitamin A is an attractive approach in combating oxidative stress mediated insult during the chronic course of the disease process of leprosy.

Acknowledgements
We acknowledge the Director, Central Leprosy Teaching and Research Institute for providing support for this study. The study subjects receive our thanks for their kind co-operations.

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