Original Article

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A Study of Oxidative Stress Biomarkers and Effect of Oral Antioxidant Supplementation in Severe Acute Malnutrition

Biochemistry Section

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ABSTRACT

Background: Malnutrition represents one of the most severe health problems in India. Free radicals play an important role in immunological response, which induces the oxidative surplus in severe acute malnutrition. Severe dietary deficiency of nutrients leads to increased oxidative stress in cellular compartments.

Aim: The goal of this study was to inspect impact of oxidative stress in the form of serum malondialdehyde as product of lipid peroxidation, vitamin E, zinc and erythrocyte superoxide dismutase in patients with severe acute malnutrition.

Material and Methods: Sixty severe acute malnutrition patients were studied before and after supplementation of antioxidants for one month, and their status were compared with those of 60 age and sex matched healthy controls.

The level of serum MDA was analyzed by the Kei Satoh method, serum vitamin E concentration was measured by Baker and Frank Method, serum zinc was measured by using Atomic Absorption Spectrophotometer (AAS) and erythrocyte superoxide dismutase was measured by Kajari Das Method.

Results: Significantly increased levels of serum malondialdehyde ($p<0.001$) were found in the patients as compared to those in controls, and significant depletions were found in the levels of serum vitamin E, zinc and erythrocyte superoxide dismutase in patients with severe acute malnutrition as compared to those in controls.

After supplementation of antioxidants for one month, the levels of malondialdehyde were found to be decreased significantly ($p<0.001$) and zinc and erythrocyte superoxide dismutase capacity levels were increased significantly ($p<0.05$). Also, there was a non–significant ($p>0.05$) increase in vitamin E levels as compared to those before supplementation results.

Conclusion: Harsh deficiency of various nutrients in severe acute malnutrition leads to generation of heavy oxidative stress. These effects may be minimized with supplementation of antioxidants.

Key words: Severe acute malnutrition, Oxidative stress, Zinc

INTRODUCTION

Malnutrition is one of the most severe health problems in developing countries, including India. The World Health Organization (WHO) defines malnutrition as "the cellular imbalance between supply of nutrients and energy, along with the body's demand for them to ensure growth, maintenance, and specific functions" [1].

Malnutrition is classified into severe acute malnutrition (SAM) and Moderate Acute Malnutrition (MAM) according to WHO to the degree of wasting and the presence of oedema. The WHO and United Nations Children's Fund proposed diagnostic criteria for severe acute malnutrition in children aged 6 to 60 months [2, 3].

In the current Indian population of 1100 million, there would be about 132 million under five years of age-children (about 12% of population), of which about 8 million can be assumed to be suffering from SAM [4].

Free radical reactions have been implicated in many diseases, including atherosclerosis, cancer, diabetes mellitus, etc [5]. Earlier studies have shown that in malnutrition, there is excess production of reactive oxygen intermediates such as superoxide anion (O_2^-), hydroxyl radical ($OH^\bullet$), singlet oxygen and hydrogen peroxide (H_2O_2) within the erythrocytes. All these events lead to oxidative stress [6-8]. Malondialdehyde (MDA), a product of lipid peroxidation, is generated in excess amounts [9,10]. This oxidative stress and a possible consequential accelerated apoptosis may contribute to pathophysiology in malnutrition. Zinc deficiency caused by malnutrition is the 11th major risk factor in the global distribution of disease burden and is associated with 1.8 million deaths annually. Preliminary research correlated zinc levels with poor growth in children with malnutrition [11,12]. Superoxide dismutase (SOD) is the first line of defense against free radical damage, and is critical for maintaining optimum health and wellbeing. It scavenges free radicals and other reactive oxygen species (ROS) [1,5,13]. Along with proteins, energy, and trace elements, vitamin E deficiency is more common and it adds to mortality and morbidity of the underlying disease. The deficiency of vitamin E has been suggested to be a causative factor in the anaemia of Kwashiorkor [14,15]. It is well known fact that concentrations of plasma vitamin E of above 500 µgm/dl is indicator of adequate vitamin E nutrition and depleted levels are always associated with fragility of erythrocytes [14].

Deficiency of trace metals, selenium and zinc, especially zinc deficiency may play an important role, as it critical for the functioning of metallo-enzymes, including Zn-superoxide dismutase (SOD), which forms an integral part of the antioxidant defense system.

The prevalence of malnutrition is high in tribal and non-tribal populations in areas around Dhule, Nandurbar and Jalgaon districts in Maharashtra, India. With this background knowledge, the present study aspired to evaluate oxidative stress in malnutrition patients. We intended to investigate the status of increased oxidative stress and the zinc deficiency in relation to the antioxidant supplementation in children with severe acute malnutrition.

MATERIAL AND METHODS

This research was conducted in the Department of Biochemistry, ACPM Medical College Dhule, Maharashtra and Department of Biochemistry PDVVPF's Medical College, Ahmednagar, Maharashtra. Prior to study, institutional ethical committee clearance was

obtained and utmost care was taken during experimental procedure according to the Declaration of Helsinki 1975.

This study was performed on total 120 subjects who included 60 age and sex matched (27 males and 33 females) healthy controls and 60 (24 males and 36 females) severe acute malnurioushed children. All patients were under the strict supervision of medical professionals during the study period. The patients were aged between 0-5 years. All the patients having history of cardiovascular diseases, hypertension, thyroid dysfunction and diabetes mellitus, which induced oxidative stress were excluded from the study.

After obtaining written consents from all the participants who were included in the study, total 5ml blood was withdrawn aseptically from the antecubital vein from each subject. Out of this, approximately 2 ml blood was collected in EDTA (0.47mol/l K3-EDTA) container and 3 ml blood was collected in plain blub. The samples were centrifuged at 3000 rpm for 10 min to separate serum and RBCs respectively. The separated sera were collected in polythene tubes with corks and stored at –20^{0}C. The sera with no signs of haemolysis were used for analysis of all the parameters.

Serum malondialdehyde, a product of lipid peroxidation, was measured by a thiobarbituric reaction described by Kei Sathoh [16]. Serum zinc estimation is based on flame emission photometry by absorption of atomic absorption spectrophotometry (AAS) method [17]. Serum Vitamin-E was determined by Baker and Frank Method, based on reduction of ferric to ferrous ions which form a red coloured complex with α- α'-bipyridyl [18]. The erythrocyte SOD activity was measured by Kajari Das' method, based on the superoxide radicals generated by the photoreduction of riboflavin, which subsequently react with napthylethylenediamine, and form red azo-compound [19].

The analysis of all parameters was done manually using the chemicals of Qualigens Fine Chemicals Co., Mumbai, India. The parameters were run on UV visible Spectrophotometer (Systronics).

The assessment of the above parameters in baseline except controls was conducted before and on 30th day of the antioxidant supplementation in the form of an antioxidant syrup, A-Z 5ml b.i.d., which was predominantly composed of antioxidants, vitamins and trace elements.

The statistical analysis was carried out by using the SYSTAT software, version 12.0 for Windows. The Student's 't' test was applied for the statistical analysis and the results were expressed in mean ± SD. p <0.001 was considered as highly significant.

RESULTS

[Table/Fig-1] shows significantly elevated (p<0.001) baseline characteristics of serum MDA and that the mean value of serum vitamin E (p<0.05) decreased significantly while serum zinc and erythrocyte SOD were lowered (p<0.001) in SAM patients than in healthy controls. After one month of antioxidant supplementation in the SAM patients, it was observed that levels of MDA decreased significantly (p<0.001) as compared to baseline values. Similarly, serum zinc and erythrocyte SOD levels were significantly higher (p<0.05). Also, serum vitamin E (p>0.05) non significantly increased on the 30th day of the antioxidant supplementation as compared to its values before supplementation .

Parameters	Controls (n=60)	SAM patients (n=60) Before supplementation of antioxidants. (Group I)		SAM patients After 30th day Supplementation of antioxidants. (Group II)	
	(Mean± SD)	(Mean ± SD)	p–value	(Mean ± SD)	p–value
Serum MDA (nmols/ml)	1.2 ± 0.23	2.9 ±0.35	p<0.001	1.81±0.32	p<0. 001
Serum Zinc (ug/dl)	106.1 ± 16.6	55±9.6	p<0.001	85.7±11.3	p<0.05
ESOD (unit/gm Hb)	1978±212	1464±142	p<0.001	1782±169	p<0.05
Serum vit. E (mg/dl)	1.38±0.37	0.72±0.22	p<0.05	0.97±0.28	p>0.05

[Table/Fig-1]: Indices of oxidative stress and antioxidant status in blood samples of control and Severe Acute Malnourished children before and after treatment

Statistical comparison was done between controls, Group I and II
Values were expressed in mean with Standard Deviation (mean ± SD)
p<0.001- Highly significant.
p<0.05- Significant
p>0.05- Non significant
n= number of subjects

DISCUSSION

The scientists, Golden and Ramdath postulated that there was involvement of oxidative stress more than 10 years ago in the severe oedematous malnutrition syndrome [20]. Free radicals are unstable and very reactive. Due to lack of any "Gold Standard Assay" for calculating the activity of reactive oxygen species, three main approaches have been used: 1. Determination of various endogenous antioxidant levels, 2. Measurements of oxidation by products, 3. Direct detection of free radicals [13].

MDA is the oxidized byproduct often used as a reliable marker of lipid peroxidation in malnutrition. The serum MDA level in severely acute malnutrition was extremely higher (p<0.001) as compared to that in control subjects. Mehmet Bosnak et al., [13] explained several mechanisms which could contribute to enhanced oxidative stress in SAM. The most important one was the subnormal intake of nutrients such as carbohydrates, proteins, vitamins, which could lead to accumulation of ROS. Depleted concentrations of enzymic and non enzymic antioxidants, along with trace elements, have been reported in malnutrition. The second mechanism for increased oxidative stress in malnutrition may be a non specific chronic activation of the immune system due to chronic inflammation. The elevated activity of MDA in malnourished children may be due to depletion in overall antioxidant enzymes as a compensatory mechanism for protection of cell membrane from hazardous effects of free radicals [1,10,13]. Our finding implies that in malnourished cells, the lipids and proteins are more susceptible to auto-oxidation.

Serum zinc was found to be significantly lowered (p<0.001) in SAM patients as compared to that in healthy controls. The findings of this study were similar to those of previous studies done by other countries, which stated that zinc deficiency was greater in malnourished patients. They further explained that hypoalbuminaemia contributed to low zinc levels, which supported the fact that there was poor food intake and over utilization of zinc during infections. Mushi S et al., and Gerardo Weisstaub et al., described that, the zinc deficiency in patients with diarrhoea was caused by zinc loss, pneumonia and impairment of intestinal absorption which was caused by mucosal damage and prolonged reduction in dietary intake due to anorexia [21,22]. Savitri Thakur reported that zinc scarcity was mainly due to common factors like less food intake, lack of breast feeding and formation of measles [23]. It is a well known fact that, copper and zinc are required to maintain proper immune response. Our findings are in agreement with those of other researches, that supplementation of antioxidants including zinc restricts diarrhoeal episodes and other infections. Also, it leads to accelerated regeneration of intestinal mucosa, enhanced levels of intestinal brush border enzymes, which results in improved intestinal permeability, and cellular immunity in SAM patients. After supplementation, we observed that zinc level was increased significantly, as zinc plays a crucial role in development and expression of T and B cell functions. It acts as a cofactor for various metalloenzymes, which plays an important role in antioxidant protection [23,24].

Vitamin E, particularly α-tocopherol, functions in vivo as a lipid soluble, chain breaking antioxidant and is potent peroxyl radical scavenger. The serum vitamin E status was found to be extremely lower in patients. The reduced concentration of vitamin E may be

due to enhanced generation of free radicals, that causes inflammation, leads to vascular leakage, which thereby results in oedema in Kwashiorkor [10,14,15].

Erythrocytes are protected from oxidative stress by intracellular preventive antioxidant enzyme, ESOD and are the 1st line of defense against oxidative stress. Earlier studies and our findings suggest that decreased ESOD activity was found in the patients with SAM. Elevated reactive oxygen species lead to disintegration of polyunsaturated fatty acids on the cell membrane, which causes lipid peroxidation [10]. The depleted levels of ESOD suggest that energy deficient state may result in enhanced lipid peroxidation. This alteration could be due to insufficient intake of micronutrients such as zinc, copper selenium etc, antioxidants like vitamin E, C, A, etc. The normal activity of erythrocyte dismutase is essential for the dismutation of superoxide radical to H_2O_2, which is subsequently detoxified by glutathione peroxidase [10,13].

CONCLUSION

The essence of the current study lies in the fact that there was enhanced oxidative stress in the form of serum MDA and depleted activities of serum zinc, vitamin E, ESOD in patients before treatment. The low activity of ESOD and vitamin E may affect bacterial killing capacity, which leads to inadequate handling of free radicals reactive oxygen metabolites. The deficiency of trace elements hosts the susceptibility to various infections. One way to increase the survival and rapid growth rates of such patients is inhibiting the effect of oxidative stress and improvement in the nutritional status. Regular antioxidant supplementation to SAM patients improves the nutritional as well as antioxidant status by neutralizing the free radicals formation, followed by protection of RBCs from anaemia and boosting of the immune system.

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