Evaluation of Oxidative Stress in Malnourished Children at Nutritional Rehabilitation Centre of Bhopal

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ABSTRACT
The aim of this study was to evaluate the effect of oxidative stress in severe acute malnourished children. Serum Malondialdehyde (MDA), vitamin C and serum zinc were determined in 60 severe acute malnourished children (age 6 months - 5 years) and sixty age and gender matched healthy controls. Mean serum MDA was found to be significantly raised and serum vitamin C and zinc were significantly decreased in malnourished children as compared to healthy controls. A significant positive correlation was found between vitamin C and zinc and negative correlation between vitamin C and MDA. From the present observations, it is evident that stress is created as a result of deficiency of nutrients in severe acute malnourished children. This stress leads to production of excess reactive oxygen species (ROS). These ROS lead to lipid peroxidation and consequent formation of MDA.

KEY WORDS: malnutrition, MDA, oxidative stress, vitamin C

INTRODUCTION:
In a developing country like India, Protein energy malnutrition constitutes one of the major nutritional and health problems in children under five years of age. It has a significant contribution to the mortality and morbidity in this age group of children. World Health Organization (WHO) defines malnutrition as 'the cellular imbalance between supply of nutrients and energy and the body's demand for them to ensure growth, maintenance, and specific functions'.

Malnutrition is one of the largest factors suppressing India's spectacular growth. With vast forests and several of India's famous game parks, Madhya Pradesh is geographically the second largest state in India and has a population of about 72 million (provisional figure, Census 2011). There are a large number of tribal communities here and almost 40 per cent of the state's inhabitants live below the poverty line, many in rural areas where they subsist on tiny farm plots. While high rates of malnutrition, child and maternal mortality have challenged this state, UNICEF and the State Government are making a positive impact with a range of programs. These include training thousands of village health workers to recognize and treat sick babies, and encouraging women to rest and eat well-balanced meals during pregnancy. New hospital units for sick newborns are also saving lives. Hence, there is need to understand the nature of antioxidants and their resultant benefits in the larger interest of the deprived population of developing countries. It's worth noting that the free radicals are very short lived and unstable, so they are difficult to measure. However, their detrimental effects can be measured by estimating their by-products. Marker of oxidative stress is MDA, a by-product of lipid peroxidation. This oxidative stress has to be counteracted by antioxidants. Capacity of body to defend itself from free radicals can be measured by assessing the blood levels of antioxidant micronutrients zinc and ascorbic acid and also endogenous antioxidants such as albumin and bilirubin. Zinc plays a critical role in the functioning of metalloenzymes including Zn-superoxide dismutase, which plays an important role in antioxidant defence mechanism. Lipid peroxide radicals formed by peroxidation of polyunsaturated fatty acids react with vitamin E to form tocopheroxo...
radical which is reduced back to tocopherol by reaction with vitamin C from plasma. Thus vitamin C forms an important antioxidant of aqueous phase.

MATERIALS AND METHODS:

The study was conducted after the approval of the ethical committee, in the Department of Biochemistry and Paediatrics at People’s College of medical Sciences & Research Center, Bhopal. The research protocol was in agreement with the Helsinki declaration. The present study included 120 children between the age of 6 months to 5 years with the help of Paediatrician. Out of 120, sixty children were diagnosed and suffering from severe acute malnutrition according to the diagnostic criteria proposed by WHO and admitted to the nutritional rehabilitation centre, PCMS & RC. Sixty samples of healthy children were taken as controls. The controls were age and gender matched to cases, with ratio of case to control was 1:1.

Inclusion and exclusion criteria: Severe acute malnourished children having no clinical evidence of any infectious disease at the time of blood collection were taken as subjects. Children taking antioxidant supplements were excluded from the study.

Informed consent was taken from parents of study participants. Random blood samples. Five ml of venous blood was withdrawn from each subject and collected in plain bulb and allowed for spontaneous blood clotting for 20-30 minutes. Then the samples were centrifuged at 3000 rpm for 10 minutes at room temperature to separate serum from blood cells. The separated sera was stored at - 20°C in eppendorf tube vials until assay. The analysis of all parameters was done using chemicals and reagents of analytical grade. Spectrophotometer was used for the measurement of the parameters like malondialdehyde, vitamin C and zinc. Malondialdehyde was measured by thiobarbituric acid reaction serum ascorbic acid by Ayekyaw method and serum zinc by colorimetric method.

RESULTS:

Statistical analysis was carried out using SPSS software, version 20. The results were expressed as mean±SD. Student ‘t’ test was applied for statistical analysis. p value (p<0.001) were considered as highly significant. The observations and inference obtained from this study were summarized in the following tables.

Table no.1 shows significantly elevated (p<0.001) levels of antioxidants Vitamin C and zinc in healthy controls as compared to SAM patients. While a significantly increased levels of MDA is noticed in SAM patients p<0.001 as compared to healthy controls. Table 2 shows that vitamin C and zinc are positively highly correlated while vitamin C AND MDA are negatively correlated r=-0.503 in cases and r=-0.849 in controls.

DISCUSSION:

In the present study malnourished children were found to have less antioxidant levels and raised levels of products of oxidative damage.

Table 1: Indices of oxidative stress and antioxidant among Controls and Patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls (Mean±SD)</th>
<th>Severe acute malnourished patients (Mean±SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 60</td>
<td>n = 60</td>
<td></td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>1.59±0.24</td>
<td>2.98±0.60</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Vitamin C (mg/dl)</td>
<td>1.62±0.32</td>
<td>0.46±0.04</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Zinc (µg/dl)</td>
<td>105±11.56</td>
<td>53.10±4.33</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

(* Highly significant, p<0.001)

Table 2: Correlation analysis: (a) Vitamin C and MDA, (b) Vitamin C and Zinc level.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MDA</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=60)</td>
<td>-0.849</td>
<td>0.909</td>
</tr>
<tr>
<td>Cases (n=60)</td>
<td>-0.503</td>
<td>0.969</td>
</tr>
</tbody>
</table>

(Correlation is significant at the 0.001 level)
Malondialdehyde (MDA), a product of lipid peroxidation is generated in excess amounts. This oxidative stress and a possible consequential accelerated apoptosis may contribute to pathophysiology of malnutrition. A significant increase in the level of MDA in malnourished as compared to controls indicates the occurrence of lipid peroxidation.

Graph 1: Mean MDA levels in Cases as Compared to controls.

Lipid peroxidation leads to loss of membrane fluidity and integrity. Loss of membrane integrity, in case of mitochondria, undermine the efficiency with which the electron transport chain converts reducing equivalents to ATP, thus further aggravating the adverse effect of already reduced energy intake in malnourished children. The high levels of MDA are in agreement with those of other studies carried out by Khare Perampalli, Bosnak, and Ghone et al.

Ferhat Catal et al (2007) & Jain Anuradha et al (2008) reported that significantly increased level of serum MDA in patients with malnutrition as compared to controls. In such condition, depletion of endogenous antioxidants may be expected. A peroxidative damage of lipids is indicated by the increase in serum MDA levels. Among the many peroxidative effects of Nitric Oxide (NO), protein modification by nitrosylation or oxidation of -SH groups has been reported. NO plays an important role in regulation of vascular tone and endothelial function, with respect to pathophysiology of malnutrition.

The body has developed several endogenous antioxidant defense systems which includes enzymes, minerals and vitamins. Particularly, vitamins C and E are non enzymatic endogenous antioxidant also exist within normal cells and react with free radicals to form radicals themselves which are less reactive than the radicals. They break radical chain reactions by trapping peroxyl and other reactive radicals.

In the present study it was found that serum vitamin C status was found to be markedly low in malnourished children. It is an antioxidant vitamin which is used up to convert tocopheroxy radical to tocopherol which breaks the chain of lipid peroxidation and traps free radicals thus preventing oxidative damage. Depleted vitamin C levels have been supported by studies of Khare et al and Ashour et al.

Graph 2: Mean vitamin C levels: A comparison between Cases and Controls.

Zinc is the second most abundantly distributed trace element in the body after iron. Zinc catalyzes enzyme activity, contributes to protein structure, and regulates gene expression. 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.

In the present study, Serum zinc was found to be significantly lowered in malnourished children as compared to that in healthy controls. Similar results have been found in the studies of Ghone et al, Jain et al. The effect of decreased zinc concentration is decreased activity of zinc dependent enzymes like lactate dehydrogenase, glutamate dehydrogenase, alkaline phosphatase, thymidinokinase. Thus zinc has a profound effect on protein, energy and nucleic acid metabolism. So its deficiency can restrict cell proliferation. This could be a cause of stunted growth in malnourished children. Superoxide dismutase is involved in scavenging of free radicals. There are two isoenzymes of SOD-Cu/Zn-SOD and Mn-SOD. Reduced zinc could be partly responsible for the decreased antioxidant activity of this enzyme and consequently increased...
oxidative stress.\(^{2,12}\) Zinc has been shown to play a central role in the functioning of cells mediating non-specific immunity, such as neutrophils and natural killer cells and is needed for specific immune responses, such as T-helper cell function.\(^{21}\) Reduced immunity and increased susceptibility to infections in malnourished children could be the effect of reduced zinc. A recent animal study suggests that supplementation of protein and zinc rich food leads to improved immunity.\(^{21}\)

Significantly lower level of antioxidants like vitamin C, zinc and increased oxidative stress in the form of MDA suggests that failure of antioxidant defense mechanism against oxidative stress may be an important factor in the pathogenesis of malnutrition.

**CONCLUSION:**

Based on this study, there is reasonable evidence for oxidative stress in severe acute malnourished children. The antioxidant levels are decreased in an attempt to combat the increased oxidative stress. Therefore, appropriate use of antioxidants may be helpful in controlling the lesions in the patients of severe acute malnutrition. There is ample scope to conduct further studies for identifying the natural resources, which may be used in the dietary plans of especially malnourished children, through the established chains of public health interventions by government, semi government and private health care providers.

**REFERENCES:**


