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Original Research Paper

# Evaluation of Oxidative Stress in Malnourished Children at Indore, MP, India

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## ABSTRACT:

**Background:** 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. O**bjective:** The aim of this study was to evaluate the effect of oxidative stress in severe acute malnourished children. **Material and methods:** Serum Malondialdehyde (MDA), vitamin C and serum zinc were determined in 60 severe acute malnourished children (age 6 months - 5 years) and 60 age and gender matched healthy controls. **Results:** s. 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. **Conclusions:** 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, Zinc, Indore***

## 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. Its 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 byproducts. Marker of oxidative stress is MDA, a byproduct 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 micro nutrients 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 tocopheroxy radical which is reduced back to tocopherol by reaction with vitamin C from plasma. Thus vitamin C forms an important antioxidant of aqueous phase.

## MATERIAL AND METHODS:

Present study was conducted in the Department of Biochemistry, Index Medical College Hospital And Research Center, Indore after the approval of Research Advisory Committee (RAC) and Institutional Ethics Committee (IEC). The research protocol was in agreement with the Helsinki declaration. This study consists of malnourished children who were clinically diagnosed in the department of Paediatrics, during the period of 2020 – 22.

## COLLECTION OF BLOOD SAMPLES:

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 [6] serum ascorbic acid by Ayekyaw method [7] and serum zinc by colorimetric method.[8]

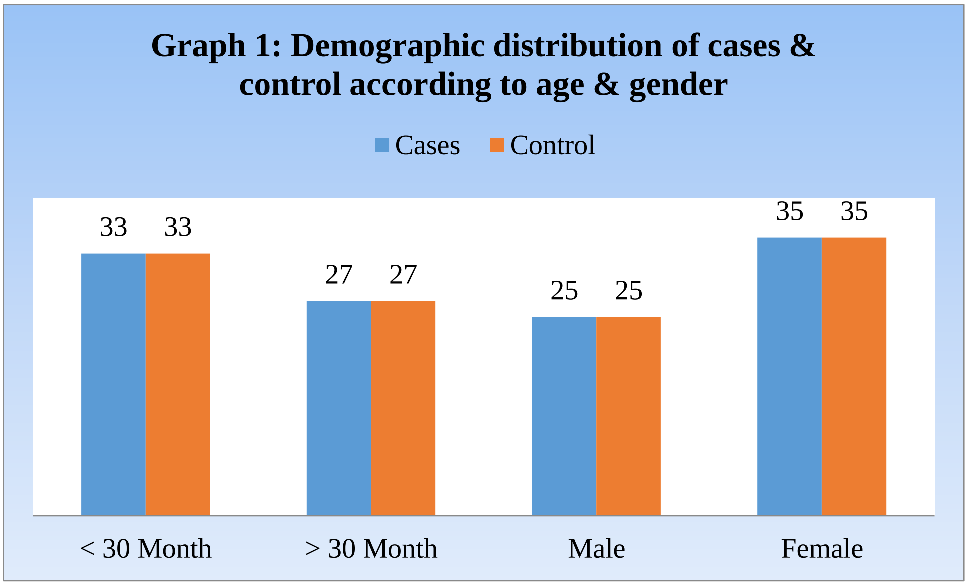
## RESULTS AND OBSERVATIONS:

The present study included a total of 120 samples out of which 60 blood samples were of previously diagnosed severe acute malnourished children and 60 samples were of healthy controls. Controls were age and sex matched to cases with ratio of case to control 1:1.All cases as well as controls belonged to the age group 6 months to 5 years. Comparison between mean values of anthropometric and biochemical parameters of different groups were determined using *student t test*.

**The observations and results obtained from this study were summarized in the following tables:**

**Table: 1. Age wise distribution of study subjects**

| Age Groups | Cases | Controls | Total |
|---|---|---|---|
| **< 30 Months** | 33 | 33 | 66 |
| **> 30 Months** | 27 | 27 | 54 |
| **Total** | 60 | 60 | 120 |




Among 120 subjects, 25 cases + 25 controls are males and 35 cases + 35 controls are females. Incidence of female is 58 % and male is 42 %.

## Table: 2. Distribution of Anthropometric parameters in Cases & Controls

| PARAMETERS | Group | MEAN | SD | Student 't' test | p Value |
|---|---|---|---|---|---|
| Wt. (kg) | Cases n=60 | 8.01 | 2.20 | 82.673 | <0.001 |
| | Controls n=60 | 16.03 | 9.10 | | |
| Ht. (cm) | Cases n=60 | 79.25 | 15.72 | 7.612 | <0.001 |
| | Controls n=60 | 96.59 | 8.00 | | |
| MUAC (cm) | Cases n=60 | 11.84 | 1.29 | 12.903 | <0.001 |
| | Controls n=60 | 14.46 | 0.89 | | |

***Significant, p<0.05, **Very significant, p<0.001 *** Highly significant, p<0.0001**

Table showing the results of Anthropometric parameters measured of malnourished children's and healthy controls. Mean values of various parameters of cases (n= 60) are compared with controls (n=60). Student's t-test was applied at 95% CI and results are expressed as mean ± standard deviation. P values< 0.05 are considered to be significant.

## Table: 3. Distribution of Oxidant & Antioxidants in Cases & Controls

| PARAMETERS | Group | MEAN | SD | Student 't' test | p Value |
|---|---|---|---|---|---|
| MDA (nmol/ml) | Cases | 3.07 | 0.33 | 27.644 | <0.001 |
| | Controls | 1.59 | 0.24 | | |
| Vit.C (mg/dl) | Cases | 0.46 | 0.04 | 27.266 | <0.001 |
| | Controls | 1.61 | 0.32 | | |
| Zn (µg/dl) | Cases | 53.10 | 4.33 | 32.674 | <0.001 |
| | Controls | 105.20 | 11.56 | | |

Table showing mean lipid peroxidation (MDA) levels significantly higher while Vitamin C, Zinc levels are significantly lower in malnourished children when compared with controls (p < 0.001).

## Table: 4. Gender-wise distribution of Oxidant & Antioxidant in Cases

| Variables | Group | MEAN | SD | Student 't' test | p Value |
|---|---|---|---|---|---|
| Vit.C (mg/dl) | Male | 0.47 | 0.04 | 0.546 | 0.587 |
| | Female | 0.46 | 0.04 | | |
| MDA (nmol/dl) | Male | 3.06 | 0.25 | 0.211 | 0.834 |
| | Female | 3.08 | 0.39 | | |
| Zn (µg/dl) | Male | 53.32 | 4.23 | 0.331 | 0.742 |
| | Female | 52.94 | 4.45 | | |

Table showing no significant difference of mean values of lipid peroxidation (MDA), Vitamin C & Zinc between male and female malnourished children.

## DISCUSSION:

In the present work, we examined the status of both antioxidant and oxidant activities. Malnourished children were found to have more oxidant damage products and less antioxidant levels. Alternatively, the control group consisting of healthy children had comparatively less oxidant damage product and more antioxidant level. ROS degrades polyunsaturated lipids, forming MDA. Raised levels of lipid peroxidation products in the serum are used as a marker for tissue damage and MDA is regarded as one of the most stable products of lipid peroxidation. In the present study, mean MDA level in malnourished children is 3.07±0.33 nmol/ml and in healthy controls is 1.59±0.24 nmol/ml. The difference is statistically significant. A significant increase in the level of MDA in malnourished children as compared to controls indicates the occurrence of lipid peroxidation. These findings are consistent with Jain et.al [2008][9], Bosnak et al [2010][10], Perampalli [2010][11], Ghone et al [2013][12]and Khare [2014][13.] Bosnak et al [2010] has proposed that several mechanisms may lead to oxidative stress in malnourished children. The most important one is the subnormal intake of nutrients such as carbohydrates, proteins and vitamins leading eventually to accumulation of ROS. The second mechanism for oxidative stress in malnutrition may be a non-specific chronic activation of the immune system due to chronic inflammation.[9]Khare et al [2014] have proposed that increased MDA levels in children with different grades of PEM may cause accelerated protein carbonyl (PC) formation in plasma proteins especially in albumin. Ascorbic acid is a water soluble antioxidant which can scavenge a number of reactive oxygen species e.g. hydroxyl, alkoxyl, peroxyl, peroxide anion, hydroperoxyl radicals and reactive nitrogen radicals like nitrogen dioxide, nitroxide, peroxynitrite at very low concentrations. Ascorbic acid is highly efficient in neutralizing the free radicals released from activated polymorphonuclear leukocytes. It protects the lipids in plasma and LDL from damage by free radicals.[14, 15, 16] In the present study, mean ascorbic acid level in malnourished children is 0.46±0.04 mg/dl and that in healthy control is 1.61±0.32mg/dl. Level of ascorbic acid is significantly decreased in malnourished children as compared to controls (p<0.001) Similar findings were reported by Ashour et al [1999][17]and Une et al [2013][18]. The data suggested that the mechanisms for low serum albumin in these patients may be related to the role of ascorbic acid as a plasma antioxidant. In contrast to our study, Akinyanju et al [1983] found no significant difference in the mean leucocyte ascorbic acid levels in protein energy malnourished children as compared to age and sex matched healthy controls.[19] Antioxidant dependent defences play an important role in scavenging of free radicals. Organism's susceptibility to free radical stress and peroxidative damage is related to the balance between the free radical load and the adequacy of antioxidant defences. Abnormally high levels of free radicals and the simultaneous decline in antioxidant defence mechanisms can lead to damage of cell membrane, cellular organelles and cell compounds such as DNA, proteins, sugar & lipids. These consequences of oxidative stress can promote development of complications in severe acute malnourished children. Cause of decreased level of vitamin C in such children is due to reduced dietary intake as well as increased consumption to counteract the oxidative stress induced by malnutrition. Trace mineral deficiencies underlying protein-energy malnutrition (PEM) are now being increasingly recognized. One of the essential trace elements is zinc. Previous studies in animals and human suggest that Zinc requirements are increased during rapid growth.[20, 21] Zinc has significant antioxidant property thereby protecting cells from damage due to free radicals. Golden et al has demonstrated that inadequate Zinc intake may be a limiting factor for growth in malnourished infants.[22] In the present study, it was observed that mean serum zinc levels was significantly decreased in malnourished children's 53.10±4.33 µg/dl as compared to healthy controls 105.20±11.56 µg/dl (p<0.001) Similar results were reported by various researchers like Singla et al [1996][23], Thakur et.al [2004][24], Ugwuga et al [2007][25], Jain et al [2008][26]and Kumar GR et al [2011][27].Zinc deficiency is primarily caused by zinc deficient diet consumed by the malnourished children. Secondary cause could be an inadequate absorption from the gut. Fiber, phytates and other minerals such as Cu have been demonstrated to decrease zinc bioavailability.

## 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.

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