Possible role of oxidative stress in malnourished children

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Abstract

The present study was undertaken to uncover the possible role of oxidative stress in PEM children. Based on signs and symptoms. Forty five malnourished children around the age group of 1-5 years were divided into 3 groups, – viz Marasmus, Marasmic- Kwashiorkor and Kwashiorkor. Thirty children from same age group were taken as control. Study revealed highly significant increased Levels of Malondialdehyde (MDA), Nitric oxide(NO□) and Erythrocytic glucose 6 phosphate dehydrogenase (RBC-G6PD) activity [p<0.001], where as the levels of vitamin E, Total serum protein , and serum Albumin were significantly decreased in malnourished children as compared with healthy controls. From the present observations, it is evident that, scarcity of principal nutrients, which are part of antioxidant defense were decreased, resulting in oxidative stress.

Key words: peroxide, Protein Energy Malnutrition, Glucose 6 Phosphate Dehydrogenase, Nitric oxide, vitamin E
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Introduction

Protein Energy Malnutrition (PEM) is the most common nutritional disorder in the developing countries. PEM is widely prevalent in the infants and pre-school children[1] the reason for this tragedy are quite clearly poverty under development and inequality[2] the risk of death is directly correlated with the degree of Malnutrition.

The Origin of PEM can be primary when it is the result of inadequate food intake or secondary when it is the result of other diseases, that leads to low food ingestion inade-quate nutrient absorption or utilization increase nutritional requirement or increase nutrient losses [3].

Marasmus involves inadequate intake of proteins and calorie and Kwashiorkor is said to be resulted from in-adequate protein intake. The scarcity of food causes poor supply of antioxidants resulting in imbalance between the pro-oxidants and antioxidants in favour of pro-oxidants, which is called oxidative stress. Now a day's oxidative stress is the major field of attraction among scientist. Therefore the present study was planned to assess the status of oxidative stress in malnourished children, hence following parameters were studied.

MDA is used to assess oxidative stress and free radical damage. Increased oxidative stress occurs in the tissue of PEM. Enhanced lipid peroxidation could be early marker of PEM [1]. NO* is an oxygen and nitrogen containing free radical which like oxygen is double edged sword i.e. essential to life and toxic too [4]. Infection is a main feature of Kwashiorkor which is most important stimuli of NO* Synthase activity. Oxidative stress and nitrosative stress are closely linked [5].

Vitamin E is an alcohol it has a long alkyl tail. Which confers fat solubility? Hydroxyl group can lose a hydrogen atom to provide the vitamin with its characteristic antioxidant property. Vitamin E incorporated in the cell membrane scavenges free radicals produced by lipid peroxidation [6]. The researchers found that muscular dystrophy and

edema can result from free radical mediated mechanisms that occur in vitamin E deficient premature infants stressed by a high diet in polyunsaturated fats [7, 8]. It may be said that the role of vitamin E is very beneficial in malnourished children.

G6PD oxidizes glucose 6 phosphate to 6 phospogluconolactone reducing NADP to NADPH in HMP and is the only source of NADPH in the erythrocytes. Reduced Glutathione (GSH) serves as a substrate for this enzyme and NADPH is required for the reduction of Oxidized glutathione, protein sulfhydryl groups, it is essential factor in the chain reaction that defends the RBC against peroxide, G6PD deficient cells are unable to respond adequately to such an oxidative stress and get hemolysed [9].

Protein is body building food and fundamental basis of cell structure and its function. Albumin is the major constituent of plasma protein, it is vital for regulating osmotic pressure, water balance and stabilizing blood volume. Protein deficiencies lead to Hypoalbuminemia it is considered as one of the hall marks of PEM. Loss of Albumin reduces blood osmolality leading to edema. A low protein diet reproducibly and predictably fail to grow [10] normally and with time because nutritional dwarfs.

The etiology of Kwashiorkor and Marasmus is clearly multifactorial and includes in varying proportions food insecurity, inadequate weaning and other feeding practices, infection and possibly oxidative stress [2].The pathogenesis of edema and anemia commonly found in children with PEM has been suggested to be caused by an imbalance between the production of these toxic radicals their safe disposal and antioxidant potential [1] therefore the present study aimed to explore the status of oxidants and antioxidants in PEM.

Materials and Methods

The present study was carried out in Department of Biochemistry. Dr.V.M. Govt. Medical College and Shree Chhatrapati Shivaji Maharaj, General Hospital Solapur and KBNIMS and KBNTandGH Gulbarga. The present study included 65 subjects 30 were controls and 45 were malnourished children. Further the malnourished children were divided into 3 different groups such as Marasmus, Marasmic Kwashiorkor and Kwashiorkor. The children within the age group of 1-5 years were selected. They were clinically diagnosed for malnourishment. 30 chil-dren from the same socio-economic status having no history of past or concurrent diseases like cancer, obesity, congenital disorders were treated as controls.

After obtaining prior consent, venous blood was collected from the subjects under aseptic condition by venipuncture using 5ml sterile disposable syringe and needle. About 4ml of blood was collected, 2ml was poured in to sterile heparinised bulb and remaining blood was taken in a sterile plain bulb and was allowed to clot. Serum was separated by centrifugation at 3000rpm for 10 min. at room temperature. The sample was stored at 4oc before analysis and all the samples were analyzes on the same day of collection.

All the methods were standardized first and standard graphs were obtained. Serum Lipid peroxide was measured by precipitating lipoproteins with trichloroacetic acid and boiled with thiobarbituric acid which reacts with Malondialdehyde to give pink colour as per Kei satoh's method [11]. For Nitric oxide serum was deproteinized first and then nitrate the stable product of nitric oxide, present in filtrate is reduced to nitrite, which is measured by diazotization of sulphonilamide and coupling with Naphthylethylene Diamines as in Najawa and cortas method [12]. Vitamin E was estimated by Baker and Frank method. Serum tocopherol was determined by re-duction of ferric to ferrous ions, which forms a red complex with dipyridyl [13].

RBC G6PD by Kornberg and Horecker method which is based on rate of formation of NADPH is measure of the enzyme activity it can be followed by means of the increase in extinction at 340 or 360 nm [14]. Serum protein was estimated by Biuret method. Alkaline copper sulphate solution reacts with peptide linkage of protein give blue purple colour. Serum albumin is estimated by Bromocresol Green dye method. Albumin has an affinity for anionic forms of many indicators at PH below 5 BCG an anionic dye bind tightly to albumin [15].

Results

All the results were expressed in mean ± SD. Statistical analysis was done by using student't' test. Correlation coefficient was determined between Lipid peroxide $p<0.005$ was considered a significant where as $p<0.001$ was considered as highly significant.

Table 1: Levels of oxidants in controls and different Groups of malnourished children.

Groups	Number of Subjects	MDA (nmol/dl)	NO• (µmol/L)
Control	30	1.2 ± 0.05	32.96 ± 1.98
Malnourished Children			
Marasmus	15	9.37±0.08 ***	37.96±1.65 ***
Marasmic - Kwashiorkor	15	5.82±0.21*** ###	43.48±1.65 *** ###
Kwashiorkor	15	2.36±0.12 *** ### $$$	45.95±1.64 *** ### $$$

Table No. 2: Levels of biochemical parameters in controls and different Groups of malnourished children

Groups	Vitamin E (mg/l)	G6PD (µ/mg of Hg)	Total Protein (g/dl)	Albumin(g/dl)
Control	7.02 ± 0.28	11.97 ± 1.09	7.06± 0.5	4.25±0.44
Malnourished Children				
Marasmus	3.43±0.24 ***	12.93±0.91 **	4.73±0.48***	3.44±0.38***
Marasmic-Kwashiorkor	3.01±0.22*** ###	13.58±0.90***#	3.47±0.44***###	2.69±0.44***###
Kwashiorkor	4.13±0.64***###$$$	14.41±0.95***#$$	2.89±0.41***###$$$	1.88±0.37***###$$$

Comparison

P>0.01 – Non Significant (*, #, $)
p<0.05 – Significant (**, ##, $$)
p<0.001 – Highly significant (***, ###, $$$)
* Marasmus as compared with control
* Marasmic Kwashiorkor as compared with control
* Kwashiorkor as compared with control
#Marasmic Kwashiorkor as compared with Marasmic
#Kwashiorkor as compared with Marasmic
$ Kwashiorkor as compared with Marasmic Kwashiorkor

Significantly higher levels of serum Lipid peroxide in the form of MDA [p<0.001] and serum NO*. [p<0.001] were observed in malnourished children when compared with healthy controls. Maximum rise in MDA is observed in marasmus, while maximum rise in NO* was observed in kwashiorkor [Table-1] decreased serum vitamin E levels [p<0.001] was observed in malnourished children com-pared with healthy controls maximum decrease in Maras-mickwashiorkor[Table 2]. Significantly higher activity of RBC G6PD [p<0.001] was observed in malnourished children compared to healthy controls. Serum Albumin and protein levels were decrease by [p<0.001] in malnourished children compared to healthy controls with maximum decrease in kwashiorkor. [Table 2]

Discussion

Increase levels of MDA indicate Lipid peroxidation inadequate protein in the diet and increase the accumulation of free radicals leads to oxidative stress. Lipid peroxida-tion can cause the cellular damage tissue modification and increase many pathological events, skin lesions and bleached hairs [8]. Oxidative damage of protein and Lipoprotein is a possible pathogenic mechanism for liver injury in Malnutrition [16] (Fig1)

NO* is a weak radical produced in various cells under both physiological and pathophysiological conditions. Infection a main feature of kwashiorkor represents most important stimuli of inducible NO* synthase activity [17]. Oxidative stress and nitrosative stress are closely linked and NO* as a vasoactive and potentially toxic metabolite may contribute to the pathophysiology of kwashiorkor in malnutrition [5].

Vitamin E or α tocopherol is fat soluble powerful antioxidants, due to inadequate food intake micronutrient deficiency occurs and its utilization in nullifying effect of free radicals. Vit E deficiency can lead to muscular dystrophy and increase abnormal lysis of red blood cells. Due to this Anemia and muscular wasting occurs in malnourished children [18, 19] (Fig1).

G6PD is the only means of providing NADPH in erythrocytes. Decreased activity of G6PD impairs the synthesis of NADH in RBC [20]. Increased erythro- cytic G6PD activity is to provide additional NADPH. When the rate of oxidation of glutathione exceeds the capacity to Supply reducing equivalent theNADPH level can fall despite the increase in rate of its supply [21].

Dietary deficiency of protein is a primary cause for decrease in serum protein and Albumin level. Due to deficiency of protein, lipoprotein synthesis can not take place leading to fat accumulation in liver which finally result in fatty liver. In fatty liver albumin synthesis decreases and decrease in serum protein and Albumin decreases the plasma colloidal osmotic pressure, leading to edema [16] (Fig 1).

The study has shown the possible pathogenetic stimuli for free radical generation in malnourished children. On the basis of experimental data it can be concluded that imbal-ance in oxidants and antioxidants has been developed in these children in favour of oxidations. As main cause of malnourishment is scarcity of food, finally help these children by making public aware of the consequences. Still study provides suggestion that if food is supplied to these children, antioxidant rich diet may achieve better recovery.

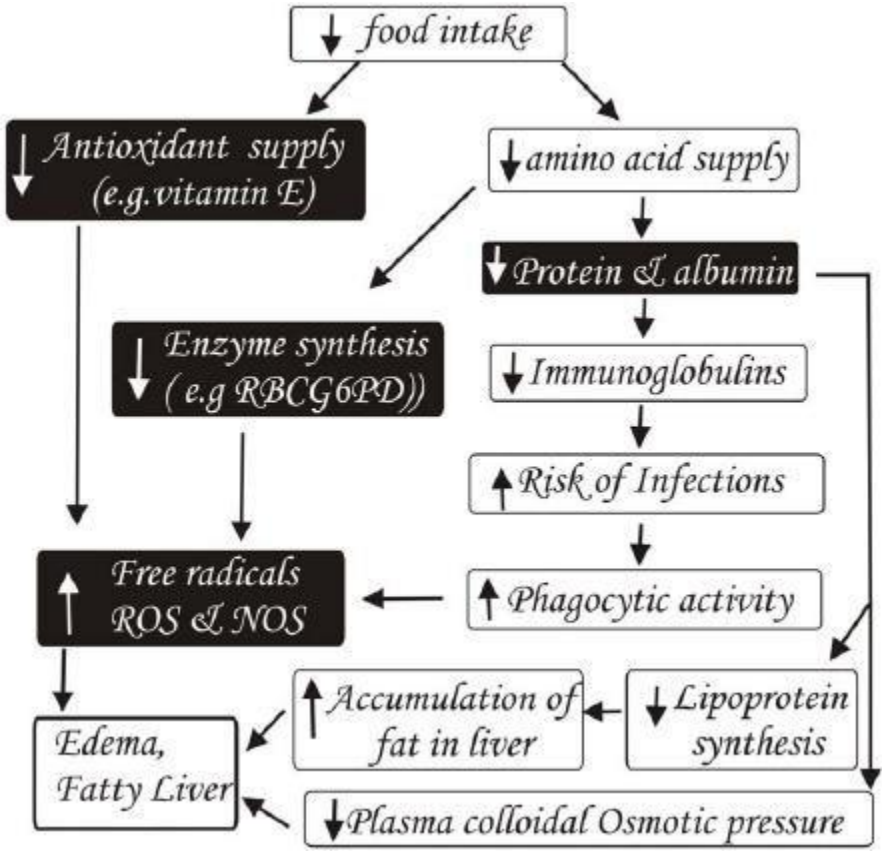

Figure 1: Showing possible mechanism involved to induce oxidative stress and its consequences in malnourished children.

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