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RESEARCH ARTICLE

Micronutrients and Antioxidant status in children with protein energy malnutrition

Lalit Une, Sheenu Gupta*

Department of Paediatrics, L.N.Medical College & RC, Kolar road, Bhopal

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ABSTRACT

Introduction: Protein energy malnutrition (PEM) is one of the most important public health problems in many developing countries including India, South East Asia and Africa. It is a wide-spread deficiency disease among children of low socio-economic groups. Oxidative stress might plays an important role in conversion of marasmus to kwashiorkor.

Aim: Hence the aim of this present study is to assess serum levels of micronutrients which directly plays an important role as antioxidants so that, the cause of oxidative stress can be identified.

Materials and Methods: For this study total 54 children (both male and female) of age between 1-5 years who were suffering from different degrees of PEM were selected and grouped into Marasmus (n=31) and kwashiorkor (n= 23). 15 children who had no signs of PEM were selected as control group. In all the participants we measured plasma vitamin A, C, E plasma zinc, selenium and antioxidant enzyme Glutathione Peroxidase (GSH-Px) and its co-substrate Glutathione (GSH).

Observation: though we observed lower levels of vitamin A, C, E in both groups of PEM when compared to control, very least levels were observed in kwashiorkor when compared between three groups ($P<0.01$). And the same lowest levels of GSH-Px and its co-substrate GSH in kwashiorkor was observed ($p<0.01$).

Conclusion: from the above results we demonstrate that oxidative stress might plays as important role in conversion of marasmus to kwashiorkor.

Keywords: kwashiorkor; marasmus; PEM; oxidative stress; β-carotenes.

1. INTRODUCTION

Protein energy malnutrition (PEM) is one of the most important public health problems in many developing countries including India, South East Asia and Africa. It is a wide-spread deficiency disease among children of low socio-economic groups. According to UNICEF in India, around 46 percent of all children below the age of three are malnourished and underweight and of all the states Madhya Pradesh stands first with 55%. 1 in 3 of the world's malnourished children lives in India[1]. Clinically severe malnutrition presents in the form of marasmus, kwashiorkor and marasmic kwashiorkor. Marasmus is relatively easy to treat with low mortality, but because of edema, altered serum electrolytes, impaired immune response, neurological abnormalities and hepatomegaly due to fatty infiltration, it is difficult to treat and slower recovery and rate of mortality [2]. Of many hypothesis for development of kwashiorkor syndrome, free radical theory postulated by Golden and Ramdath is most accepted one. According to them various noxae produces lipid peroxidation, hydroperoxides and carbonyl compounds which creates an imbalance between free radical production and scavenging mechanism, thus creating oxidative stress like environment which might plays an important role in development of kwashiorkor syndrome in malnutrition [3]. The oxidative damage caused by free radicals is might be due to decrease in antioxidant defense system which extensively comprises of vitamins like vitamin C, A, E and enzymatic antioxidants like glutathione peroxidase and non enzymatic metabolites like glutathione. Along with these Zinc and Selenium also plays an important role in scavenging free radicals which are produced continuously in our body and in greater amount in PEM. Hence the present study is undertaken to assess the intensity of oxidative stress like

*Corresponding author: Sheenu Gupta, | Department of Paediatrics, L.N.Medical College & RC, Kolar road, Bhopal|
Email: sheenu.gupta@yahoo.com

environment in PEM by estimating plasma antioxidant enzyme Glutathione Peroxide (GSH-Px) activity, its co-substrate glutathione (GSH) levels and serum micronutrients like zinc, selenium, vitamin A, C, E.

2. METHODS AND MATERIALS:

This cross sectional study was done in department of Paediatrics, J. K. Hospital & L.N.Medical College-Bhopal, India and is approved by institutional ethical committee and written consent was taken from parents of participants. For this study total 54 children (both male and female) of age between 1-5 years living in slums around kolar region of Bhopal, Madhya Pradesh-India, who were suffering from different degrees of PEM were selected and grouped into Marasmus (n=31) and kwashiorkor (n= 23). These children were admitted in Paediatrics department of our tertiary care hospital for treatment. 15 children who had no signs of PEM were selected as control group.

From both control and study group, 2 ml of venous blood was taken with heparinized syringe and centrifuged at 4°C and was stored at -80°C till the biochemical investigations were done. Serum Zinc and Selenium were measured with readily available commercial kits by using fully auto clinical chemistry analyzer Biosystems A25. Serum Glutathione was estimated by Hissin and Milf method [4]. Pro-vitamin form of vitamin A, β-carotene levels was estimated by Neeld and Pearson method [5]. Plasma vitamin C was estimated by Roe and Kuether method [6] and vitamin E by Fabianek et al method [7]. Glutathione peroxidase was estimated by Paglia D.M method [8].

Statistical analysis:

All the values were expressed in mean ± SD. One way ANOVA was applied to see the statistical difference in the mean values of serum biological parameters between control, marasmus and kwashiorkor groups. Simple student t test was applied to assess the statistical difference of the above said biochemical parameters between marasmus and kwashiorkor children groups. P<0.05 was considered as statistically significant and p<0.01 considered as highly significant. All the data were analyzed using statistical software SPSS version 19.

3. RESULTS:

In this study from table-1 we observed very low levels of plasma antioxidant vitamins like vitamin A, C, E in both marasmus and kwashiorkor groups when compared to control group with p<0.01. of serum zinc and selenium, zinc showed very low levels in both groups of PEM with p<0.01 when compared to controls, where as in selenium levels there was only marginal difference between study groups (p<0.05). In GSH-Px and its co-substrate levels we observed same statistically highly significant lower levels in PEM group (P<0.01). From table-2 to assess the statistical difference in the levels of above said parameters between marasmus and kwashiorkor we observed no difference in serum selenium levels between two groups of PEM with p>0.05.

Parameters	Control	Marasmus	Kwashiorkor	P value
β-carotene (µg/L)	647 ± 94	426 ± 81	53.2 ± 10.9	<0.01#
Vitamin-C (mg/dl)	1.2 ± 0.3	0.63 ± 0.5	0.51 ± 0.1	<0.01#
Vitamin-E (mg/L)	11.6 ± 0.1	7.4 ± 0.3	6.3 ± 0.5	<0.01#
Zinc (µg/dl)	103.5 ± 12.4	67.3 ± 9.1	375 ± 36	<0.01#
Selenium (µg/L)	63.8 ± 8.1	51.4 ± 6.3	50.7 ± 3.9	<0.05*
Glutathione (mg/L)	10.5 ± 0.2	6.8 ± 0.7	6.01 ± 0.1	<0.01#
Glutathione-peroxidase(U/g Hb	96.3 ± 4.7	73.6 ± 6.1	58.9 ± 7.2	<0.01#

# P<0.01; **P>0.05.

Table-1: Showing One-way ANOVA (analysis of variance) of biological parameters between three groups.

Parameters	Marasmus	Kwashiorkor	P value
β-carotene (µg/L)	426 ± 81	53.2 ± 10.9	<0.01#
Vitamin-C (mg/dl)	0.63 ± 0.5	0.51 ± 0.1	<0.01#
Vitamin-E (mg/L)	7.4 ± 0.3	6.3 ± 0.5	<0.01#
Zinc (µg/dl)	67.3 ± 9.1	375 ± 36	<0.01#
Selenium (µg/L)	51.4 ± 6.3	50.7 ± 3.9	<0.05*
Glutathione (mg/L)	6.8 ± 0.7	6.01 ± 0.1	<0.01#
Glutathione-peroxidase(U/g Hb	73.6 ± 6.1	58.9 ± 7.2	<0.01#

# P<0.01; **P>0.05.

Table-2: Showing statistical difference in the levels of biological parameters between marasmus and kwashiorkor groups.

4. DISCUSSION:

In the present study when compare with healthy control group we observed low levels of micronutrients like vitamin A, C, E and microminerals like Zinc, Selenium, antioxidant enzyme like GSH-Px and its co-substrate GSH in marasmus and kwashiorkor groups. As infections runs parallel with malnutrition [9] might causes activation of macrophages respiratory burst mechanism for the release of Super oxide (O^-_2) H_2O_2 and HOCl for bactericidal activity. As such O^-_2 is not very reactive radical specie but, much of toxicity is thought to be due to conversion into more damaging species like $ONOO^-$ and $OH^·$ Radicals. Superoxide's protonated form, $H_2O_2^·$ is more reactive and can oxidize poly unsaturated fatty acids (PUFA). The same high lipid peroxidation rate in kwashiorkor was observed by lenhartz H et al [10] . SOD produces H_2O_2 that can exert some direct toxic effect and can be precursor of $OH^{\cdot}$ by Fenton and Haber-Weiss reaction with iron.

$$H_2O_2 + Fe(II) \longrightarrow Fe(III) + OH^{\cdot} + OH^{-} \quad (I)$$

Fenton reaction.

$$H_2O_2 + O2^{\cdot -} \longrightarrow O2 + OH^{\cdot} + OH^{-} \quad (II)$$

Haber-Weiss reaction.

Hydroxyl Radical ($OH^{\cdot}$) that is formed by Fenton and Haber-Weiss reactions, because of its extreme reactivity it is so-called as main factor of oxygen toxicity. It reacts with all biological materials, oxidatively by hydrogen withdrawal, double bond addition, electron transfer and radical formation, and initiates autoxidation, polymerization and fragmentation.

In this study we observed low levels of Zinc both in marasmus but least in kwashiorkor. This might be because of the consumption of Zn in counter reacting ROS which are generated due to tobacco or high copper of areca quid metabolism. Many experimental studies demonstrated the inhibitory effect of Zn on transition metal mediated site specific oxidative injury by inhibiting metal induced $OH^{\cdot}$ generation [11]. In the present study we observed very low levels of ascorbic acid in marasmus and kwashiorkor groups. As ascorbic acid is potent water soluble antioxidant the biological system might has utilized it in scavenging/neutralizing an array of ROS species which were produced at very high level because of increased activity of NADPH oxidase of immune cells.

In the present study though we observed lower levels of serum selenium in PEM group when compared to control group, but no statistical difference between marasmus and kwashiorkor. Even no statistical difference in serum selenium between marasmus and kwashiorkor, we observed very low activity of plasma GSH-Px activity (which is selenium dependent) in kwashiorkor when compared to marasmus. This is well explained by the fact that the serum glutathione (GSH) levels are lowest in kwashiorkor when compared to marasmus. Our results were positively correlates with Reid M et al [12]. Being co-substrate of GSH-Px GSH plays a major role in the maintenance of the intracellular redox state and is the most essential and powerful antioxidant which enables other antioxidants like vitamins A and C, to continuously perform their antioxidant activities effectively. The depletion of GSH indicates the oxidative stress like environment in PEM. The decrease in plasma vitamin C and glutathione makes the loss of vitamin E and β-carotenes, as they were lost in the form of α-tocopheroxyl radical and β-carotenes on binding with free radicals. Though excess free radical production might play an important role in depletion of these micronutrients, incomplete intake might also aggravate this status in kwashiorkor.

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Conflict of Interest: None Declared

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