Micronutrients and Antioxidant status in children with protein energy malnutrition

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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.
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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Marasmus</th>
<th>Kwashiorkor</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-carotene (µg/L)</td>
<td>426 ± 81</td>
<td>53.2 ± 10.9</td>
<td>53.2 ± 10.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Vitamin-C (mg/dl)</td>
<td>0.63 ± 0.5</td>
<td>0.51 ± 0.1</td>
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<td>&lt;0.01</td>
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<tr>
<td>Vitamin-E (mg/L)</td>
<td>7.4 ± 0.3</td>
<td>6.3 ± 0.5</td>
<td>6.3 ± 0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Zinc (µg/dl)</td>
<td>67.3 ± 9.1</td>
<td>375 ± 36</td>
<td>375 ± 36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Selenium (µg/L)</td>
<td>51.4 ± 6.3</td>
<td>50.7 ± 3.9</td>
<td>50.7 ± 3.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Glutathione</td>
<td>6.8 ± 0.7</td>
<td>6.01 ± 0.1</td>
<td>6.01 ± 0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glutathione</td>
<td>73.6 ± 6.1</td>
<td>58.9 ± 7.2</td>
<td>58.9 ± 7.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Peroxidase(U/g Hb)</td>
<td>96.3 ± 4.7</td>
<td>73.6 ± 6.1</td>
<td>73.6 ± 6.1</td>
<td>&lt;0.01</td>
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Table-1: Showing One-way ANOVA (analysis of variance) of biological parameters between three groups.

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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 (O2-) H2O2 and HOCl for bactericidal activity. As such O2- 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, H2O2 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 H2O2 that can exert some direct toxic effect and can be precursor of OH· by Fenton and Haber-Weiss reaction with iron.

\[
\begin{align*}
H_2O_2 + Fe(II) & \rightarrow Fe(III) + OH^- + OH^- \quad \text{(I)} \\
H_2O_2 + O_2^- & \rightarrow O_2 + OH^- + OH^- \quad \text{(II)}
\end{align*}
\]

Fenton reaction.

Haber-Weiss reaction.

Hydroxyl Radical (OH·) 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 area quid metabolism. Many experimental studies demonstrated the inhibitory effect of Zn on transition metal mediated site specific oxidative injury by inhibiting metal induced OH· 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.

5. REFERENCES:
6. Roe JH, Kuether CA. the determination of ascorbic acid in whole blood and urine through 4-2 dinitrophenylhydrazine derivative of dihydroascorbic acid J Biol Chem 1943; 147: 399-407.