Antioxidant micronutrients in children with thalassaemia in Egypt

M.R. Nasr, S. Ali, M. Shaker and E. Elgabry

ABSTRACT Repeated blood transfusions in patients with thalassaemia subject them to peroxidative tissue injury by secondary iron overload. To study the relationship between iron overload and antioxidant micronutrient status among children with thalassaemia, we measured serum levels of vitamins A and E, zinc, selenium, and copper in 64 children with β-thalassaemia major and 63 age- and sex-matched controls. All of these elements were significantly lower in the thalassaemic children compared with controls. There was a highly significant inverse correlation between serum ferritin and serum retinol levels, and significant inverse correlations between serum iron and retinol and between serum iron and selenium. Serum ferritin showed a significant positive correlation with duration of chelation and transfusion treatments. Ways are needed to counteract this oxidative damage and its deleterious effect on the prognosis of thalassaemia.

Les micronutriments antioxydants chez des enfants atteints de thalassémie en Egypte

RESUME Les transfusions sanguines répétées chez les patients atteints de thalassémie les exposent à une atteinte péroxydatrice des tissus par surcharge en fer secondaire. Afin d’étudier la relation entre la surcharge en fer et la statut en micronutriments antioxydants chez les enfants thalassémiques, nous avons mesuré les taux de vitamine A et E, zinc, sélénium et cuivre sériques chez 64 enfants atteints de β-thalassémie majeure et 63 témoins appariés selon l’âge et le sexe. Tous ces éléments étaient significativement plus faibles chez les enfants thalassémiques que chez les témoins. Il y avait une corrélation inverse très significative entre les taux de ferritine et de rétinol sériques, et des corrélations inverses significatives entre le fer et le rétinol sériques ainsi qu’entre le fer et le sélénium sériques. La ferritine sérique présentait une corrélation positive significative avec la durée de la chélation et les traitements par transfusion. Des moyens sont nécessaires pour contrer cette atteinte oxydative et son influence sur le pronostic de la thalassémie.

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Introduction

Thalassaemia genes are remarkably widespread, and are believed to be the most prevalent of all human genetic diseases [1]. In Egypt, thalassaemia represents the commonest cause of haemolytic anaemia. In multicentre studies, the carrier rate has been reported in the range of 9%-10% [2].

It has been reported that iron overload in β-thalassaemia due to repeated blood transfusion leads to an enhanced generation of reactive oxygen species and to oxidative stress [3]. The long-term clinical consequences are heart failure, liver fibrosis or cirrhosis and endocrinopathies. An increased consumption of antioxidants may result from these disorders [4].

Antioxidants are a complex and diverse group of molecules that protect key biological sites from oxidative damage. They scavenge free radicals and other reactive oxygen intermediaries [5]. This antioxidant system depends firstly on the integrity of an enzymatic system that requires adequate intake of trace minerals such as selenium, copper, zinc and manganese, and secondly, on adequate concentrations of vitamins E, A, and C in the cytoplasm and lipid membrane of the cell [6]. We aimed to study the state of antioxidant micronutrients in Egyptian children with thalassaemia.

Methods

The current study included 64 thalassaemic children (as determined by clinical picture and laboratory investigations, including haemoglobin electrophoresis), attending the outpatient clinic in the Department of Paediatrics, Ahmed Maher Teaching Hospital, Cairo during the period May 2000–May 2001. The 64 cases comprised 34 females and 30 males (age range 2–18 years). All were receiving regular blood transfusion and desferrioxamine chelation therapy. As controls, 63 age- and sex-matched healthy children were enrolled. None of the controls were receiving multivitamins or mineral preparations.

After obtaining verbal consent from the parents, each of the patients and controls had a full history taken (including, for the cases, duration of transfusion and chelation treatment), and each received a thorough clinical examination, including anthropometric measurements. Laboratory investigations were carried out to determine levels of serum iron, ferritin, serum zinc, selenium, copper, serum retinol and α-tocopherol.

Serum retinol and α-tocopherol were estimated by high pressure liquid chromatography (HPLC) according to the method of Bieri et al. [7]. Serum trace elements were determined by flame atomic absorption spectrophotometry (Unicam 929), according to the method of Falchuk et al. [8]. Data were analysed in the computer unit of the National Nutrition Institute (NNI) using SPSS version 5.0.1.

Results

The mean age and standard deviation of the children with thalassaemia was 9.36 ± 4.62 years, and of the controls 9.08 ± 4.12 years (no significant difference, $P = 0.5$). The mean height of the thalassaemic group was $121.16 \pm 23.35$ cm, and of the controls $131.49 \pm 24.64$ cm ($P = 0.8$). The mean weight of the thalassaemic children was $26.94 \pm 12.83$ kg, and of the controls $31.32 \pm 14.06$ kg ($P = 0.9$).

The mean duration of transfusion treatment was $7.28 \pm 3.75$ years and of duration chelation treatment was $4.58 \pm 3.25$ years.
The serum levels of zinc, selenium, copper and retinol were highly significantly lower in the thalassaemic patients compared with the controls ($P < 0.001$). Serum α-tocopherol was also significantly lower ($P < 0.005$) (Table 1).

With the exception of serum retinol, there was no significant correlation between serum ferritin and the studied micronutrients. A highly significant inverse correlation was found between serum ferritin and serum retinol levels ($P < 0.001$). Analysis of the relationship between serum iron and the studied micronutrients (Table 2) showed a significant inverse correlation with serum retinol and selenium ($P < 0.05$).

No significant correlation was found between the serum levels of the studied micronutrients and the duration of transfusion treatment, or with the duration of chelation treatment, in spite of the highly significant positive correlation between serum ferritin levels and both the duration of transfusion and duration of chelation (Table 3).

**Discussion**

The present study showed significantly lower serum levels of all the studied antioxidant micronutrients—zinc, selenium, copper, retinol and vitamin E (α-tocopherol)—in thalassaemic children compared with the matched healthy controls. These results agree with those of Fuchs et al. [9], which showed significantly lower levels of serum zinc and vitamin E in thalassaemic patients, and also with those of Chan et al. [10]. This latter study showed significantly lower levels of several antioxidants (selenium, zinc, vitamins E, C and carotenoid) in patients with thalassaemia, sickle-cell anaemia and glucose-6-phosphate dehydrogenase deficiency that result in clinical manifestation of mild to severe haemolysis in patients with these disorders.

Bender and Bender reported that vitamin E deficiency manifests as a shortened half-life of erythrocytes, which can progress to increased intravascular haemolysis and haemolytic anaemia, and that selenium has a sparing effect on vitamin E and delays the onset of deficiency syndrome [11]. Plasma selenium was significantly decreased, as was plasma glutathione peroxidase (GPx) activity in patients with thalassaemia major, whether on the oral iron chelator L1, or on subcutaneous desferrioxamine therapy, as in the study by Bartfay and Bartfay [12].

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**Table 1** Comparison of serum levels of certain micronutrients in Egyptian children with thalassaemia and a control group

<table>
<thead>
<tr>
<th>Micronutrient</th>
<th>Mean serum levels ± s (μg/dL)</th>
<th>Controls ($n = 63$)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with thalassaemia ($n = 64$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>$12.45 ± 5.44$</td>
<td>$95.18 ± 10.35$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Selenium</td>
<td>$23.43 ± 5.30$</td>
<td>$33.05 ± 10.9$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Copper</td>
<td>$60.60 ± 15.71$</td>
<td>$162.72 ± 23.83$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Retinol</td>
<td>$23.03 ± 7.53$</td>
<td>$55.05 ± 15.09$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>$513.57 ± 141.5$</td>
<td>$599.64 ± 164.2$</td>
<td>&lt; 0.005</td>
</tr>
</tbody>
</table>

$s = standard\ deviation.$
Fat-soluble vitamins A and E are important non-enzymatic antioxidants [6]. Our results revealed that both vitamins were significantly decreased in the thalassaemic children compared with the matched controls. These results agree with other studies [4,19,20], two of which [4,20] explained the significantly lower levels of vitamins A and E as due to an excessive iron fraction that generates a lipid peroxidation process, with subsequent consumption of antioxidants. These results were confirmed by those of Gerster [21] and De Luca et al. [3].

In our results there was a highly significant inverse correlation between serum retinol and serum ferritin, and a mildly significant inverse correlation between serum retinol and serum iron. There was no significant correlation between serum α-tocopherol (vitamin E) and serum ferritin, or with serum iron. These results contrast with those of Livrea et al. [20], where a significant inverse correlation between serum vitamin E and serum ferritin was found. De Luca et al. [3] found a significant positive correlation between serum vitamin E and non-transferrin-bound iron ($P < 0.001$).

The significant positive correlation between serum ferritin and duration of chelation in our patients may reflect factors such as suboptimal dosage or, more probably, poor compliance of the patients, as the only available iron chelator for these patients is desferrioxamine, which requires prolonged subcutaneous infusion three to seven times per week.

**Conclusion**

Due to deeply disturbed iron metabolism, intense production of oxygen free radicals occurs in β-thalassaemia, with subsequent consumption of antioxidants. The adminis-
Table 3 Correlation between duration of transfusion treatment and serum levels of certain micronutrients, and between duration of chelation treatment and micronutrients, in Egyptian children with thalassaemia

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Correlation coefficient r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of transfusion versus zinc</td>
<td>-0.0395</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of transfusion versus selenium</td>
<td>0.0516</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of transfusion versus copper</td>
<td>-0.0322</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of transfusion versus retinol</td>
<td>-0.2447</td>
<td>0.05</td>
</tr>
<tr>
<td>Duration of transfusion versus α-tocopherol</td>
<td>-0.0762</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of transfusion versus iron</td>
<td>0.0388</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of transfusion versus ferritin</td>
<td>0.6962</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Duration of chelation versus zinc</td>
<td>-0.0792</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of chelation versus selenium</td>
<td>0.1308</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of chelation versus copper</td>
<td>0.0657</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of chelation versus retinol</td>
<td>-0.2061</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of chelation versus α-tocopherol</td>
<td>-0.1081</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of chelation versus iron</td>
<td>-0.0195</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of chelation versus ferritin</td>
<td>0.6471</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

NS = not significant.

The administration of selective antioxidants, along with an appropriate diet, might represent a promising way of counteracting oxidative damage and its deleterious effect on the prognosis of the disease.

References


6. Allard JP et al. Oxidative stress and plasma antioxidant micronutrients in hu-


