Antioxidant micronutrients in children with thalassaemia in Egypt

M.R. Nasr,[1] S. Ali,[2] M. Shaker[1] and E. Elgabry[2]

المغذيات الزهيدة المقدار المضادة للأكسدة عند الأطفال المصابين بالثلاسيمية في مصر

منى رمضان نصر، سحر علي، مريم شاكر، إيمان الجابري

الخلاصة: لقد ثبت أن تكرار نقل الدم للمرضى المصابين بالثلاسيمية يعرِّضهم لإصابة الأنسجة بالأكسدة الفائقة نتيجة التحميل الثانوي المفرط بالحديد. وقد قمنا بقياس المستويات المصلية من الفيتامينات A وE ومن الزنك والسيلينيوم والنحاس في 64 طفلاً مصابين بالثلاسيمية، فضلاً عن 63 حالة مقارنة مع شواهد مقابلة على أساس السن والجنس، وذلك بغية فهم العلاقة بين حالة التحميل المفرط للحديد والمغذيات الزهيدة المقدار المضادة للأكسدة في الأطفال المصابين بالثلاسيمية. وقد ثبت انخفاض كل هذه العناصر في الأطفال المصابين بالثلاسيمية بالمقارنة بالشواهد المقابلة. كما وجد ترابط عكسي بين مستويات فيريتين المصل وريتينول المصل، وكذا ترابط عكسي واضح بين حديد وريتينول المصل وحديد وسيلينيوم المصل. وأظهر مستوى فيريتين المصل ترابطاً إيجابياً مع مدة العلاج بالـخَلْب ونقل الدم. ومن ثَـمَّ فإننا نحتاج إلى سبل لمناهضة هذا التلف التأكسدي الفائق والآثار الضارّة التي يحدثها على مآل الثلاسيمية.

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 peroxydative des tissus par surcharge en fer secondaire. Afin d'étudier la relation entre la surcharge en fer et le 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.

[1]Department of Paediatrics, Ahmed Maher Teaching Hospital, Cairo, Egypt.
[2]National Nutrition Institute, Cairo, Egypt.
Received: 27/12/01; accepted: 06/03/02

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 biologic 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 ± 23.35 cm, and of the controls 131.49 ± 24.64 cm ($P = 0.8$). The mean weight of the thalassaemic children was 26.94 ± 12.83 kg, and of the controls 31.32 ± 14.06 kg ($P = 0.9$).

The mean duration of transfusion treatment was 7.28 ± 3.75 years and of duration chelation treatment was 4.58 ± 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*].

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

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

s = *standard deviation.*

Table 2 **Correlation between serum levels of ferritin and certain micronutrients, and between iron and micronutrients, in Egyptian children with thalassaemia**

Comparison	Correlation coefficient *r*	*P*-value
Ferritin versus zinc	0.2149	NS
Ferritin versus selenium	–0.0611	NS
Ferritin versus copper	0.0593	NS
Ferritin versus retinol	–0.3807	< 0.001
Ferritin versus α-tocopherol	–0.0139	NS
Ferritin versus iron	0.4756	< 0.001
Iron versus zinc	–0.1565	NS
Iron versus selenium	–0.3561	< 0.05
Iron versus copper	0.0232	NS
Iron versus retinol	–0.3608	<0.05
Iron versus α-tocopherol	–0.0007	NS

NS = not significant.

Plasma zinc and thymaline enzyme activity in patients with thalassaemia major was significantly decreased in the study by Consolini et al. [*13*]. Al-Refaie et al. found that desferrioxamine treatment was associated with zinc loss [*14*].

In contrast to our results with regard to serum zinc and copper, Bashir [*15*] found significantly increased levels of serum zinc and copper in patients with β thalassaemia. Suthipark et al. reported different levels of trace elements (zinc and copper) in thalassaemic patients compared with non-thalassaemic controls due to abnormal trace element metabolism in thalassaemic patients [*16*]. These antioxidant trace elements are important cofactors for several antioxidant enzyme systems: copper-zinc superoxide dismutase (Cu-Zn SOD), which is found in the extracellular fluid [*17*] and GPx, a selenium-dependent enzyme found mainly in the cytosol and mitochondria of animal cells [*18*].

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**

Comparison	Correlation coefficient *r*	*P*-value
Duration of transfusion versus zinc	–0.0395	NS
Duration of transfusion versus selenium	0.0516	NS
Duration of transfusion versus copper	–0.0322	NS
Duration of transfusion versus retinol	–0.2447	= 0.05
Duration of transfusion versus α-tocopherol	–0.0762	NS
Duration of transfusion versus iron	0.0388	NS
Duration of transfusion versus ferritin	0.6962	< 0.001
Duration of chelation versus zinc	–0.0792	NS
Duration of chelation versus selenium	0.1308	NS
Duration of chelation versus copper	0.0657	NS
Duration of chelation versus retinol	–0.2061	NS
Duration of chelation versus α-tocopherol	–0.1081	NS
Duration of chelation versus iron	–0.0195	NS
Duration of chelation versus ferritin	0.6471	< 0.001

NS = not significant.

tration 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

1. Honig GR. Hemoglobin disorders. In: Behrman RE, Kliegman RM, Jenson HB, eds. *Nelson textbook of pediatrics*, 16th ed. Philadelphia, WB Saunders Company, 2000.
2. El-Beshlawy A et al. *Thalassemic prevalence and status in Egypt.* Abstract presented at the Annual Meeting of the American Pediatric Society, San Francisco, California, 1–4 May 1999.
3. De Luca C et al. Blood antioxidant status and urinary levels of catecholamine metabolites in beta-thalassemia. *Free radical research*, 1999, 30:453–62.
4. Reller K et al. Iron overload and antioxidant status in patients with beta-thalassemia major. *Annals of the New York Academy of Sciences*, 1998, 850:463–5.
5. Baumgartener TG. Vitamins. In: Van Way CW, ed. *Nutrition secrets.* Philadelphia, Hanley and Belfus, 1999:13–20.
6. Allard JP et al. Oxidative stress and plasma antioxidant micronutrients in hu-

mans with HIV infection. *American journal of clinical nutrition*, 1998, 67:143–7.

7. Bieri JG, Tolliver TJ, Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *American journal of clinical nutrition*, 1979, 32:2143–9.

8. Falchuk KH, Hilt KL, Vallee BL. Determination of zinc in biological samples by atomic absorption spectrometry. *Methods in enzymology*, 1988, 158:422–34.

9. Fuchs GJ et al. Nutritional factors and thalassaemia major. *Archives of disease in childhood*, 1996, 74:224–7.

10. Chan AC, Chow CK, Chiu D. Interaction of antioxidants and their implication in genetic anemia. *Proceedings of the Society for Experimental Biology and Medicine. Society for Experimental Biology and Medicine (New York, NY)*, 1999, 222:274–82.

11. Bender DA, Bender AE. Vitamin E and selenium. In: Bender DA, Bender AE. *Nutrition: a reference handbook*. Oxford, Oxford University Press, 1997:261–426.

12. Bartfay WJ, Bartfay E. Selenium and glutathione peroxidase with beta-thalassemia major. *Nursing research*, 2001, 50:178–83.

13. Consolini R et al. Immunological evaluation of patients with beta-thalassemia major. *Acta haematologica*, 2001, 105: 7–12.

14. Al-Refaie FN et al. Zinc concentration in patients with iron overload receiving oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one or desferrioxamine. *Journal of clinical pathology*, 1994, 47:657–60.

15. Bashir NA. Serum zinc and copper levels in sickle cell anaemia and beta-thalassaemia in North Jordan. *Annals of tropical paediatrics*, 1995, 15:291–3.

16. Suthipark KU et al. Red cell and plasma calcium, copper and zinc in beta-thalassemia/hemoglobin E. *Southeast Asian journal of tropical medicine and public health*, 1991, 22:171–5.

17. Bunker VW. Free radicals, antioxidants and aging. *Medical laboratory sciences*, 1992, 49:299–312.

18. Neve J, Vertongen F, Molle L. Selenium deficiency. *Clinics in endocrinology and metabolism*, 1985, 14:629–56.

19. Kassem M. *Study of antioxidants in patients with β-thalassemia major* [M.Sc. thesis]. Cairo, Egypt, Ain Shams University, 1999.

20. Livrea MA et al. Oxidative stress and antioxidant status in beta-thalassemia major: iron overload and depletion of lipid-soluble antioxidants. *Blood*, 1996, 88:3608–14.

21. Gerster H. High-dose vitamin C: a risk for persons with high iron stores? *International journal for vitamin and nutrition research*, 1999, 69:67–82.