ZINC AND COPPER STATUS OF THALASSEMIC CHILDREN

Saowanee Kajanachumpol[1], Thanusak Tatu[1], Werasak Sasanakul[1], Ampaiwan Chuansumrit[2] and Phongjan Hathirat[2]

[1]Research Center, Faculty of Medicine, Ramathibodi Hospital, Bangkok 10400, Thailand; [2]Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Bangkok 10400, Thailand

Abstract. We investigated the amount of both zinc and copper in plasma, erythrocytes and hair in 11 patients with hemoglobin H disease, 59 patients with β-thalassemia/HbE disease and 20 patients with homozygous β-thalassemia. Plasma and hair zinc levels were found to be much lower, but erythrocyte zinc levels were higher, in thalassemic patients than in controls. The levels of copper in both plasma and erythrocytes were higher in the patients than in the controls. The mechanism with respect to the increase of the amount of both zinc and copper in erythrocytes was not clear; this result may reflect the impairment of zinc and copper utilization in tissues in the pathogenesis of these thalassemic patients.

INTRODUCTION

The thalassemia (thal) syndromes are characterized by an inherited abnormality in the synthesis of one or more of the abnormal peptide chains of hemoglobin causing hemolytic anemia. One of the most outstanding clinical features of severe thalassemia is growth retardation (Constantoulakis *et al*, 1975).

Zinc is one of the essential trace elements required for various biological processes in humans and animals. Its deficiency results in growth retardation, hypogonadism in males, skin changes and delayed wound healing (Prasad, 1979). These clinical signs are seen in severe thalassemia. Because of these clinical manifestations, it has been inferred that patients with thalassemia could have secondary zinc deficiency.

Many investigators reported that thalassemic patients had low plasma zinc levels (Prasad *et al*, 1965; Arcasoy and Cavdar, 1975; Dogru *et al* 1979; Vatanavicharn *et al*, 1982). It is not known whether zinc deficiency has an important role in the retardation of growth and sexual development seen in the patients with thalassemia. Most investigators have reported the levels of plasma zinc and copper; there are few studies on levels in tissues including erythrocytes and hair. The present study determined the levels of zinc and copper in plasma, erythrocytes and hair in various types of thalassemia.

MATERIALS AND METHODS

This study was conducted on 11 patients with HbH disease (8 males and 3 females, aged 3-18 years), 59 patients with β-thal/HbE (35 males and 24 females, aged 2-18 years) and 20 patients with β-thal major (13 males and 7 females, aged 6-13 years). These patients were selected from the Pediatric Hematology Clinic, Ramathibodi Hospital and informed consent from their parents was obtained. The diagnosis of these thalassemic patients was established by complete blood count, inclusion bodies, electrophoresis of hemoglobin on cellulose acetate strip (Marengo - Row, 1965) and fetal hemoglobin estimation by alkali denaturation method (Betke *et al*, 1959). Splenectomy was done in 22 cases of β-thal/HbE and 7 cases of β-thal major. Subjects receiving blood transfusion less than 90 days (more than 30 days) before taking blood were recorded, 1 case of HbH disease, 3 cases of β-thal/HbE and 10 cases of β-thal major. Fourteen control subjects; 8 males and 6 females, aged 5-14 years, were considered to be healthy on the basis of clinical and blood chemistry.

Zinc and copper in plasma were determined by flame and flameless atomic absorption spectrophotometry, respectively (Smith *et al*, 1975). The erythrocytes were washed two times with an equal volume of 0.9% sodium chloride and centrifuged. After the final wash, packed cells were lysed with deionized water by 1:1 (Steven *et al*, 1977) and analyzed for zinc and copper by the same method used for plasma analysis.

Hair samples were taken (about 50 pieces), approximately 3 cm from the proximal end of the occipital region and washed with detergent before analysis for zinc (McKenzie, 1978).

Statistical analysis

All data were expressed as the mean ± SD. Statistical analysis of the data was performed by using unpaired Student's *t*-test. The level of sigificance was set at $p < 0.05$.

RESULTS

The results in Table 1 show the mean ± SD of age, hematocrit and hemoglobin in the control group, HbH disease, β-thal/HbE and β-thal major. From anthropometry measurement the percentage of subjects with malnutrition based on weight/age (Thai standard Karnjanathiti, 1984) was as follows: 36%, 66% and 74% in HbH disease, β-thal/HbE and β-thal major, respectively.

Zinc and copper status are presented in Table 2. Plasma Zn levels in HbH disease, β-thal/HbE and β-thal major were significantly lower and the levels of zinc in erythrocytes were significantly higher than the controls. Erythrocyte Zn levels in both HbH disease and β-thal/HbE increased significantly compared to β-thal major. Hair zinc concentration tended to be decreased in thalassemia disease and was significantly decreased in HbH disease when compared to the controls.

Plasma copper levels in both β-thal/HbE and β-thal major had a tendency to be increased in com-

Table 1

Mean ± SD of ages, hematocrit and hemoglobin of the subjects.

Parameters	Control (n = 14)	HbH (n = 11)	β-thal/HbE (n = 59)	β-thal major (n = 20)
Age (years)	8.76 ± 2.55 (5-14)	11.00 ± 5.09 (3-18)	9.40 ± 4.60 (2-18)	9.45 ± 5.00 (6-13)
Hematocrit (%)	38.3 ± 2.45	29.1 ± 6.35	20.7 ± 3.59	19.8 ± 4.87
Hemoglobin (g/dl)	13.2 ± 0.89	8.15 ± 1.55	6.56 ± 1.36	6.40 ± 1.59

Table 2

Zinc and copper status in subjects.

Parameters	Control (n = 14)	HbH (n = 11)	β-thal/HbE (n = 59)	β-thal major (n = 20)
Plasma Zn (μmol/l)	10.04 ± 1.26	8.32 ± 1.70[a]	7.98 ± 1.25[a]	8.26 ± 1.24[a]
RBC Zn (μg/g Hb)	28.80 ± 3.99	59.90 ± 14.48[b1b2]	52.99 ± 10.20[b1b2]	46.06 ± 9.45[b1]
Hair Zn (μg/g/hair)	135.20 ± 39.50	98.31 ± 27.1[c]	113.10 ± 44.6	116.20 ± 41.1
Plasma Cu (μmol/l)	12.96 ± 2.38	10.80 ± 2.82[d]	14.38 ± 2.90	13.87 ± 3.49
RBC Cu (μg/g Hb)	1.690 ± 0.16	2.994 ± 0.59[e1e2]	2.631 ± 0.76[e1e2]	1.996 ± 0.60[e1]

a = significantly different from control group at $p < 0.01$
b_1 = significantly different from control group at $p < 0.001$
b_2 = significantly different from β-thal major at $p < 0.025$
c = significantly different from control group at $p < 0.02$
d = significantly different from control, β-thal/HbE and β-thal major at $p < 0.025$
e_1 = significantly different from control group at $p < 0.04$
e_2 = significantly different from β-thal major at $p < 0.005$

parison to the control, but plasma copper values in HbH disase were significantly lower than other groups. Erythrocyte Cu levels in all thalassemia diseases were significantly increased when compared to control levels. Copper content in erythrocytes of both HbH disease and β-thal/HbE were significantly higher than in β-thal major.

DISCUSSION

The results indicated that patients with HbH disease, β-thal/HbE and β-thal major had decreased zinc concentrations in plasma. This finding is in agreement with earlier reports (Prasad *et al*, 1965; Arcasoy and Cavdar, 1975; Dogru *et al*, 1979; Vatanavicharn *et al*, 1982). Conversely, zinc concentrations in erythrocytes were found to be higher. This result is in contrast to the investigation of Dogru *et al* (1979) who found that erythrocyte zince levels in this disease were lower. Surprisingly, the values of zinc in erythrocytes in those control subjects were higher (almost 2 fold) than other reports (Talbot and Ross, 1960; Prasad *et al*, 1975, 1993). Beside thalassemia disease, erythrocyte zinc concentrations were elevated in patients with sickle cell anemia, leukemia and neoplastic disease of the leukopoietic system. The levels of both plasma zinc and erythrocyte zinc did not relate to the severity of the anemia. A significant negative correlation between plasma Zn and erythrocyte Zn was found in β-thal/HbE only ($r = -0.3$ $p < 0.05$).

Hair zinc has been proposed as an index of the chronic state of zinc nutriture. Because of its slower turnover, the zinc content of the hair reflects total body zinc more accurately (McBean *et al*, 1971). Therefore, we have determined zinc levels in hair simultaneously with the levels of zinc in plasma and red cells. Hair zinc concentrations showed a tendency to decrease in all types of thalassemia, but this was not a statistically significant difference.

The mechanisms responsible for decreased plasma zinc levels are not known at present; some investigators suggested that hyperzincuria may have caused zinc deficiency in these patients (Dogru *et al*, 1979). The increase of erythrocyte Zn concentrations may reflect the impairment of zinc utilization in tissues in the pathogenesis of these thalassemic patients.

For copper status, plasma copper concentrations had a tendency to be increased in both β-thal/HbE and β-thal major and erythrocyte Cu concentrations increased significantly in all types of thalassemia. The mechanisms with respect to increased concentration of Cu in erythrocytes were not clear, and may be associated with increase of zinc in erythrocytes. Furthermore, both of them may interact with iron in red cells. To our knowledge, the content of iron in erythrocytes was not evaluated previously. It is well established that thalassemic sera have low zinc levels but increased iron and ferritin levels (Arcasoy and Cavdar, 1975; Fargion *et al*, 1982). This finding implied that zinc and iron levels had some association. Experiments in humans and animals have demonstrated that zinc and iron undergo competitive interactions. Yadrick *et al* (1989) have found that supplementation with zinc in human resulted in a decrease in serum ferritin. Likewise, animal studies revealed that increasing dietary zinc concentration was associated with decreasing levels of liver iron in rats (Lonnerdal *et al*, 1985).

REFERENCES

Arcasoy A, Cavdar AO. Changes of trace minerals (serum iron, copper and magnesium) in thalassemia. *Acta Haematol* 197; 53 : 341-6.

Betke K, Marti HR, Schlicht I. Estimation of small percentages of fetal hemoglobin. *Nature* 1959; 184: 1877-8.

Constantoulakis M, Panagopoulos G, Augoustaki O. Stature and longitudinal growth in thalassemia major. *Clin Pediatr* 1975; 14 : 355-7.

Dogru U, Arcasoy A, Cavdar AO. Zinc levels of plasma, erythrocytes, hair and urine in homozygous beta-thalassemia. *Acta Haematol* 1979; 62 : 41-4.

Fargiion S, Taddei MT, Cappellini MD, Piperno A, Fiorelli G. The iron status of Italian subjects with beta-thalassemia trait. *Acta Haematol* 1982; 68 : 109-14.

Khanjanasthiti P, Watthanakasert S, Junnanondh, *et al*. Growth of adolescence. *J Med Assoc Thai* 1987; 70 : 187-97.

Lonnerdal B, Davidson L, Keen CL. Effect of varying dietary iron and zinc levels on tissue concentrations in the rat. *Nutr Res* (suppl) 1985; 1 : 277-80.

Marengo-Row AJ. Rapid electrophoresis and quantitation of hemoglobins on cellulose acetate. *J Clin Pathol* 1965; 18 : 790-2.

McBean LD, Mahloudji MS, Reinhold JG, Halsted JA. Correlation of zinc concentrations in human plasma and hair. *Am J Clin Nutr* 1971; 24 : 506-9.

McKenzie JM. Alteration of the zinc and copper concentration of hair. *Am J Clin Nutr* 1978; 31 : 470-6.

Prasad AS, Diwany M, Gabr M, Sandstead HH, Mokhtar N. Biochemical studies in thalassemia. *Ann Intern Med* 1965; 62 : 87-96.

Prasad AS, Schoomaker EB, Ortega J, Brewer GJ, Oberleas D, Oelshlegel FJ. Zinc deficiency in sickle cell disease. *Clin Chem* 1975; 21 : 582-7.

Prasad AS. Trace elements : biochemical and clinical effects of zinc and copper. *Am J Haematol* 1979; 6 : 77-87.

Prasad AS, Fitzgerald JT, Hess JW, Kaplan J, Pelen F, Dardenne M. Zinc deficiency in elderly patients. *Nutrition* 1993; 9 : 218-24.

Smith JC, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectrophotometry. *Clin Chem* 1975; 25 : 1487-91.

Steven MD, MacKenzie WF, Anand VD. A simplified method for determination of zinc on whole blood, plasma and erythrocytes by atomic absorption spectrophotometry. *Biochem Med* 1977; 18 : 158-63.

Talbot TR, Ross JF. The zinc content of plasma and erythrocytes of patients with pernicious anemia, sickle cell anemia, polycythemia vera, leukemia and neoplastic disease. *Lab Invest* 1960; 9 : 174-84.

Vatanavicharn S, Pringsulka P, Kritalugsana S, Phuapairoj P, Wasi P. Zinc and copper status in hemoglobin H disease and β-thalassemia/ hemoglobin E disease. *Acta Haematol* 1982; 68 : 317-20.

Yadrick MK, Kenney MA, Winterfeldt EA. Iron, copper and zinc status: response to supplementation with zinc or zinc and iron in adult females. *Am J Clin Nutr* 1989; 49 : 145-50.