Zinc and antioxidant vitamin deficiency in patients with severe sickle cell anemia

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BACKGROUND: Patients with severe sickle cell anemia (SCA) have a higher potential for oxidative damage due to chronic redox imbalance in red blood cells that often leads to hemolysis, endothelial injury and recurrent vaso-occlusive episodes. This study evaluated the plasma levels of vitamins A, C and E as indicators of antioxidant status. In addition, serum levels of zinc and copper were also estimated.

PATIENTS AND METHODS: Twenty-five adult patients with severe sickle cell anemia (12 males and 13 females aged 29.72±12.94 years) and 25 matched controls were studied. Plasma levels of vitamins A, C and E were measured by HPLC technique. Serum zinc and copper levels were measured by atomic absorption spectrometry.

RESULTS: There was a significant decrease in plasma levels of vitamins A, C and E and in serum levels of zinc in patients with SCA as compared with controls ($P<0.0001$). Serum copper levels were signficantly elevated compared with controls ($P<0.0001$).

CONCLUSION: These findings emphasize the significant deficiencies of the antioxidant vitamins A, C and E and the trace element zinc along with the significant elevation of serum copper in patients with severe sickle cell disease. Further studies are needed to find out whether supplementation of antioxidant vitamins and zinc may ameliorate some sickle cell disease complications.

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Patients with sickle cell anemia suffer from many complications, including growth retardation (decreased height and weight compared to their peers), chronic hemolytic anemia, recurrent and painful vaso-occlusive episodes, acute chest syndrome and impaired immune function. Although the exact reasons are not well established, the literature indicates that low levels of zinc, folic acid, and vitamins A, C and E could be contributing factors.[1-6]

Sickle cell anemia is a hereditary disorder with a high potential for oxidative damage due to a chronic redox imbalance in red cells that often results in continuous generation of reactive oxygen species (ROS) and clinical manifestations of mild to severe hemolysis.[7-8] The production of ROS can be grossly amplified in response to a variety of pathophysiological conditions such as hypoxia, inflammation, infection, dehydration and deficiency in antioxidant vitamins.[9,10]

Our study was designed to evaluate levels of vitamins A, C and E as indicators of antioxidants status in patients with sickle cell anemia. In addition, levels of the trace elements zinc and copper were determined since zinc deficiency is known to occur in patients with sickle cell anemia and may contribute to immune impairment and growth retardation. The copper level is commonly reciprocal to the zinc level (i.e. a

Table 1. Characteristics of severe sickle cell patients and normal controls.

Parameter	Patients (Mean ± SD)	Controls (Mean ± SD)
N	25	25
- Males	12	17
- Females	13	8
Age (year)	29.72 ± 12.9	29.0 ± 7.56
WBCs (× 10^9/L)	10.9 ±4.5	5.5 ±1.3
RBCs (× 10^{12}/L)	3.14 ± 0.7	5.0 ± 0.5
Hemoglobin level (g/L)	88 ± 12	145 ± 15
- Hb A (%)	0	96.1 ± 0.6
- Hb S (%)	83.8 % ± 7.5	0
- Hb F (%)	12.2 % ± 7.4	1.4 ± 0.6
- Hb A_2 (%)	3.0 % ± 1.2	2.6 ± 0.4
MCV (fL)	85.8 ± 11.7	86.3 ± 4.1
MCH (pg)	28.4 ± 4.5	29.8 ±1.5
Platelets (× 10^9/L)	364 ± 153	239 ± 68.2
Reticulocytes (%)	6.2 ± 2.6	1.1 ± 0.5
Bilirubin (µmol/L)	50.8 ± 29	11.4 ± 2.8
Painful episodes/year	6.0 ± 4	-
Infections/year	4.2 ± 2.9	1.2 ± 0.4
Acute chest syndrome/year	3 ± 2	-

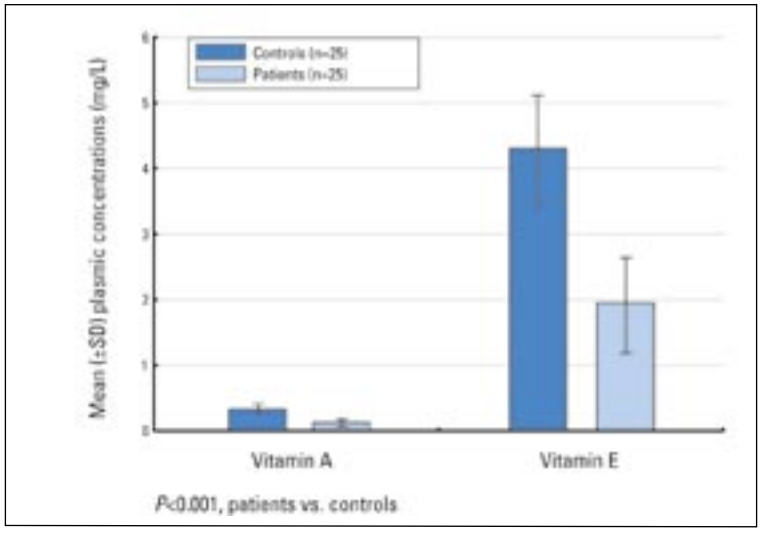

Figure 1. Plasma vitamin A and E concentrations in sickle cell disease patients vs. controls.

zinc deficiency is associated with a copper excess), which may contribute to free radical production and oxidative damage.[11,13]

Patients and Methods

Twenty-five adult patients with severe sickle cell anemia (12 males and 13 females aged 29.72±12.94 years) and 25 normal healthy adults (age- and sex-matched, 17 males and 8 females aged 29.0±7.56 years) (Table 1) were enrolled in the study. Exclusion criteria were age younger than 15 years, the presence of β- or α-thalassemia trait, G6PD deficiency, regular blood transfusion, treatment with hydroxyurea, use of vitamin and trace element supplements other than folic acid, illness other than sickle cell manifestations, or pregnancy.

The severity index of sickle cell anemia was considered high if the patient was having four or more severe painful episodes per year that required hospital admission, in addition to a history of other major sickle cell complications such as severe anemia (Hb < 7.0 g/dL), avascular necrosis of the hip joint, acute chest syndrome, priapism or hepatosplenic sequestration.

The patients were recruited from the Hematology Clinic at King Khalid University Hospital and informed consent was taken from each patient. A careful history and thorough clinical examination were carried out. Fasting venous blood samples were collected under complete aseptic conditions for complete blood count. A peripheral blood film examination confirmed the presence of sickle cells. Confirmation of diagnosis was by hemoglobin electrophoresis with HbSS > 95% in the absence of HbA and a normal HbA_2 (<3.5%). Blood samples for determination of the antioxidant vitamins A, C and E and trace elements zinc and copper were collected as follows: 5 mL of whole blood were collected into EDTA-containing, light-protected tubes for vitamins A and E measurement; 5 mL of whole blood were collected into lithium heparin tubes for vitamin C measurement; 5 mL of whole blood were collected into plain tubes for zinc and copper measurement. The EDTA tubes, the heparinized tubes and the plain tubes were centrifuged at 5400g and the plasma and serum were separated into clean, properly labeled tubes. The plasma samples were immediately used for the measurement of vitamins A, C and E using HPLC technique. The serum samples were used for zinc and copper measurement. Determination of vitamins A and E in plasma was performed according to the method described by Comstock et al (1993)[14]

and the determination of vitamin C was performed according to the method of Rumelin et al (1999).[15] Serum copper and zinc were measured by atomic absorption spectrometry according to the method described by Evenson (1994).[16]

The data were analyzed using SPSS programme version 10.1. Student t test was used to compare the results between patients with sickle cell anemia and the normal control group.

Results

Plasma levels of vitamin A, C and E and serum zinc level were significantly lower in patients with severe sickle cell anemia as compared with the normal control group (P<0.0001) (Figures 1, 2, 3). Serum copper on the other hand, was significantly higher in patients with sickle cell anemia as compared with the normal controls (P<0.0001) (Figure 3).

Discussion

Our results indicate that there are significantly lower plasma levels of the antioxidant vitamins (A, C and E), lower serum levels of zinc and significantly higher serum levels of copper in patients with severe sickle cell anemia. Since patients with sickle cell anemia are under continuous oxidative stress due to sickle cell redox imbalance,[17] a deficiency in antioxidant vitamins and trace elements may contribute to the severity of sickle cell manifestations, which could be aggravated further by elevated copper, which is a well known pro-oxidant.[18,19]

Despite a normal diet, patients with sickle cell disease may have inadequate intake of certain elements such as zinc because of chronic pain, reduced appetite and hemolysis. Another reason for reduced zinc and antioxidant vitamin levels could be increased demand and consumption. A third reason is increased urinary excretion due to impaired renal concentration and hypoxanthinuria.[22-24]

Other data in the literature suggest that regular supplementation of sickle cell patients with these vitamins and trace elements may ameliorate some of the sickle cell manifestations such as vaso-occlusive crises, acute chest syndrome, recurrent infection and growth retardation.[1-6] Our findings are in agreement with those of several other studies.[20,21] Essien found that the plasma concentrations of the antioxidant vitamins A (retinol), C (ascorbic acid) and E (Alpha tocopherol) were significantly low in patients with sickle cell anemia and he suggested that the deficiency of these antioxidant vitamins could account for some of the observed manifestations of sickle cell disease such as increased susceptibility to infection and hemolysis.[23-25] Gray et al, analyzed the dietary intake and serum level of vitamin A, folic acid, iron, red cell folate and red cell zinc of 9 patients with sickle cell anemia and 19 controls. They found that despite adequate dietary intake of proteins, vitamins and trace elements, patients with sickle cell anemia had less body weight and a significantly lower red cell zinc and serum vitamin A levels compared with controls.[26] Adelekan et al found that plasma levels of the antioxidant vitamins tocopherol, retinol, carotenes and ascorbic acid were significantly lower in 22 patients with sickle cell anemia in steady state as compared with nine controls.[27] Westerman et al

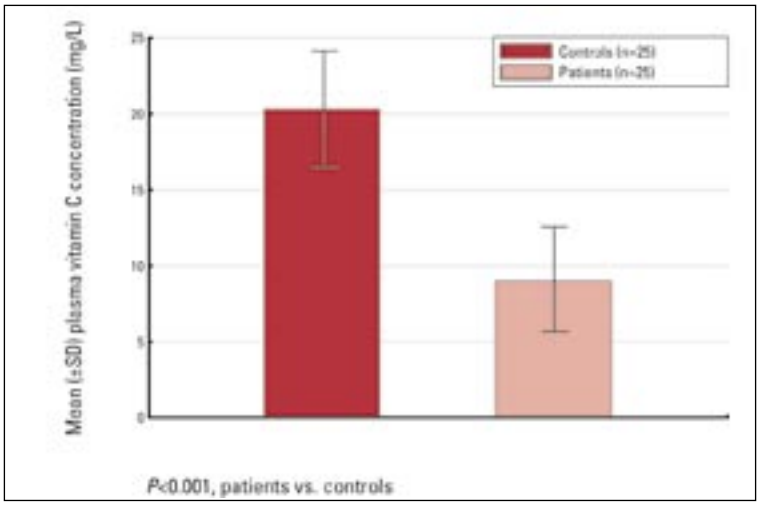

Figure 2. Plasma vitamin C concentrations in sickle cell disease patients vs. controls.

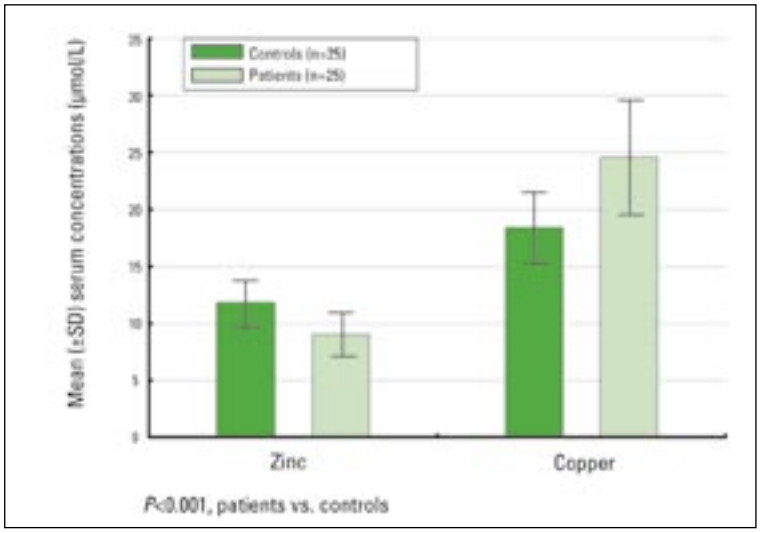

Figure 3. Serum zinc and copper concentrations in sickle cell disease patients vs. controls.

found that serum ascorbic acid levels were significantly lower with a 36% increase in urinary excretion as compared with controls.[28] Zinc deficiency in patients with sickle cell anemia (probably due to continuous hemolysis and hyperzincuria) has been documented by several studies with strong indications that zinc deficiency is associated with impaired T-helper (TH_1) functions, cell-mediated immunity and reduced interleukin-2 (IL-2) production and an increased rate of bacterial infection, vasoocclusive crises, frequent hospital admissions and growth retardation.[29-32] These complications were reduced with zinc supplements.[33] Complications of low levels of zinc, which is a known antioxidant, are associated with elevated levels of copper, which is a well known pro-oxidant and may potentiate oxidative stress in patients with sickle cell disease.[34] Kiline et al found that patients with sickle cell anemia have lower serum zinc and higher copper levels while urine showed increased zinc excretion and very low copper excretion.[35] Other studies have indicated that patients with sickle cell anemia suffer from multiple deficiencies in antioxidant vitamins and trace elements, which could be attributed to several factors, including inadequate intake in the face of increased demand, consumption and excretion.[36-40] Dietary supplementation with various antioxidant vitamins and trace elements has been shown to ameliorate some of the sickle cell manifestations.[41-44] The implications of our study along with other studies indicate that patients with sickle cell anemia require regular supplements with antioxidant vitamins and trace elements with proper monitoring. Our study shows that plasma levels of the antioxidant vitamins (A, C and E) and serum levels of zinc are significantly lower in patients with sickle cell disease as compared with a control group. In addition, serum levels of copper were significantly higher in sickle cell anemia patients. Therefore, supplementation with antioxidant vitamins and certain trace elements such as zinc may ameliorate some of the sickle cell symptoms and improve quality of life. Further studies are needed to assess the effect of antioxidant vitamins and trace elements supplementation on these clinical manifestations of sickle cell anemia.

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