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Nutritional deficiencies in iron overloaded patients with hemoglobinopathies

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Abstract

One of the hallmarks of both sickle cell disease (SCD) and thalassemia major (TM) is accelerated oxidative damage. Decreased antioxidant levels and increased oxidant stress biomarkers are found in both diseases. Although isolated vitamin deficiencies have been reported in TM and nontransfused SCD patients, a comprehensive evaluation of vitamin and trace mineral levels has never been performed in chronically transfused SCD or TM patients. As vitamins and trace minerals may be consumed as a result of chronic oxidative stress; we hypothesized that levels of these compounds would correlate with surrogates of iron overload, hemolysis, and inflammation in chronically transfused patients. Using a convenience sample of our group of chronically transfused patients we studied 43 patients with SCD (17 male, 26 female) and 24 patients with TM (13 male and 11 female). The age range for our patients varied from 1.5 to 31.4 years. Levels of vitamins A, thiamin, B6, B12, C, D, E as well as selenium, zinc, copper, and ceruloplasmin were measured. We found that 40–75% of the patients were deficient in A, C, D and selenium and 28–38% of the patients had low levels of B vitamins and folate. There was little association with iron overload, hemolysis, or inflammation. Although the precise mechanism of these deficiencies is unclear, they may contribute to the morbidity of chronically transfused hemoglobinopathy patients.

Introduction

Hemoglobinopathies are characterized by oxidant damage due to increased resting oxygen consumption and circulating prooxidative free hemoglobin (Hgb). In sickle cell disease (SCD), Hgb S is unstable and generates free radicals which damage cellular enzymes and membrane lipids [1]. Previous studies have shown high levels of malondialdehyde (MDA) and other oxidative biomarkers in the plasma of nontransfused SCD patients [2]. A similar finding has been shown in nontransfused thalassemia patients, due to the toxic effect of unpaired globin chains on the membrane [3]. Oxidative stress biomarkers are also elevated in chronically transfused SCD and thalassemia major (TM) patients and correlate most strongly with nontransferrin bound iron (NTBI) levels [4].

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Vitamins and trace minerals represent key buffers against oxidative damage. However, chronic demands on oxidative buffering capacity may produce conditional deficiencies in key amino acids and enzymatic cofactors. In SCD, production of reactive oxygen species and hyperhemolysis have been postulated to be the dominant mechanisms for the consumption of these compounds [5,6]. Non transfused patients with SCD have been demonstrated to have reduced levels of zinc, selenium, and glutathione as well as vitamins A, C, riboflavin, D, and E [7-9]. Chronic transfusions, which decrease hemolysis by suppressing the production of abnormal red cells, could potentially improve the nutrient profile in SCD and TM patients. However, the production of free radicals induced by iron overload in transfused patients may override this effect. In addition, chronic inflammation, a hallmark of SCD, has been demonstrated to reduce levels of some of these nutrients [10,11]. The goal of the present study was to determine the prevalence of vitamin and mineral deficiencies in chronically transfused patients with SCD and TM and their relationship to age, iron-burden, hemolytic rate, and inflammatory markers.

Results

Table I summarizes our patient demographics. The patient populations were similar in age and gender, with a mean age of 14.5 years. In terms of chelation, the majority of the patients were on Deferasirox alone (51). Only 6 patients were on either Defiriprone (1) or Desferoxamine (5). Three patients were on Deferasirox and Desferal together, seven patients were not on chelators. All patients had normal renal function as measured by BUN and creatinine. Some patients had mild proteinuria which was due either to use of Deferasirox or SCD.

Somatic iron burden was significantly higher in SCD patients whether assessed by hepatic iron concentration (HIC, 42%) or ferritin (85%). Despite having greater total body iron, transferrin saturation was nearly 23% lower in SCD patients. SCD patients had higher surrogate values for hemolysis and inflammation. LDH and cell free Hgb were nearly three times larger in SCD patients, indicating that transfusions were incompletely effective in suppressing endogenous RBC production. High sensitivity C-reactive protein (hs-CRP) was also almost 5-fold larger in SCD patients, consistent with greater chronic inflammation [12]. Table II summarizes the mean values and percent of abnormal nutrient values for SCD and TM patients. No gender differences were noted. The magnitude and spectrum of deficiencies was nearly identical for the two groups. Over half of the SCD and TM patients were deficient in vitamins A, C, and D25-OH. Thiamin, B6 and folate were deficient in roughly 1/3 of patients. B12 levels were within population norms. Alpha-tocopherol was abnormal in 29.2% of TM patients but only 10.5% of the SCD patients ($P = 0.09$).

Trace metal deficiencies were also common. Selenium deficiency was ubiquitous in both populations (67.5% SCD and 75% TM). Mean zinc levels were comparable in SCD and TM, and deficiencies were present in 24.3% and 8.3%, respectively ($P = 0.31$). SCD patients demonstrated mean copper and ceruloplasmin that were at the upper limits of normal and 1/3 of patients had increased copper levels. Both copper and ceruloplasmin levels were higher in SCD than TM. No TM or SCD patient had decreased serum copper although circulating ceruloplasmin levels were decreased in 1/6 of TM patients.

Table III summarizes the predictors of nutrient deficiencies; only statistically significant comparisons are included. Age was the strongest predictor of lower nutrient levels in both SCD and TM patients. Folate, D25-OH, copper and ceruloplasmin levels decreased with increasing age in both groups. Levels of γ -tocopherol increased in SCD patients as they aged. HIC had little association with nutrient levels; although there were weak negative correlations with lower Cu levels in SCD patients and lower D25-OH levels in TM patients. Higher gamma tocopherol levels correlated with higher HIC in TM patients. hs-CRP was positively correlated

with copper and ceruloplasmin levels ($r^2 = 0.37$) and negatively correlated with vitamin A levels ($r^2 = 0.28$) in SCD patients (see Fig. 1).

hs-CRP levels were not elevated in TM patients and were not associated with any nutritional deficiencies. Hemolysis, as measured by LDH and cell-free Hgb, did not predict nutritional deficiencies in either group.

Discussion

These results demonstrate that chronically transfused patients with TM or SCD have significant deficiencies in both fat and water soluble nutrients which are potentially clinically relevant. For example, the levels of Vitamin A found in these patients have been shown to be related to poorer health outcomes in children with and without SCD [10,13]. In addition, Vitamin D levels lower than 20 ng/ml have been associated with multiple problems in the general population [14] and with iron cardiomyopathy in TM [15].

Potential contributors to observed deficiencies are illustrated in Fig. 2, including hemolysis, iron toxicity, ineffective erythropoiesis, inflammation, anemia, diet, and absorption. SCD patients exhibit greater hemolysis and inflammation than TM patients, while TM patients have greater iron toxicity and ineffective erythropoiesis. Despite these phenotypic differences, the spectrum of nutritional deficiencies was remarkably similar between the two diseases (Table II). Intravascular hemolysis, although a potent predictor of vascular disease in SCD, was not correlated with any nutritional deficiencies in both SCD and TM. Inflammation is important to the pathophysiology of SCD [16,17] and markers such as Vitamin A levels decrease in the presence of inflammation while copper levels rise [10,18]. We found that hs-CRP was positively correlated with copper and negatively correlated with Vitamin A in our study (see Fig. 1). Despite these findings, we were unable to demonstrate a correlation between other nutrient deficiencies and inflammation in our patients.

HIC and ferritin values were greater in the SCD than the TM patients, suggesting decreased chelation compliance in this population. Chronically transfused SCD patients have a lower prevalence of iron-mediated endocrine and cardiac mediated dysfunction [19]. Regardless, iron overload offered little overall predictive value for antioxidant status other than mild elevations in γ -tocopherol. The negative relationship between HIC and vitamin D25-OH levels has been previously described in hereditary hemochromatosis, and is thought to reflect inhibition of hepatic 25-hydroxylation by excess iron [20].

Despite their many pathophysiologic differences, SCD and TM share some common phenotypes. All patients in this study were chronically anemic. Chronically, anemic patients maintain increased cardiac output to maintain oxygen delivery [21]. This produces a mildly hypercatabolic state, increased resting energy expenditure, and chronic oxidative stress [22-25]. These findings could contribute to increased consumption of nutrients. Malabsorption could also play a role in our findings. Both SCD and TM patients develop siderosis of the exocrine pancreas. In TM pancreatic iron negatively correlates with circulating pancreatic trypsin levels [26] and TM patients have been documented to have significant decreases in stool elastase [27]. While these observations could clearly contribute to malabsorption of fat soluble vitamins, it does not explain the low levels of water soluble vitamins found in these patients.

Most prior work in SCD has focused on nontransfused SCD patients, with deficiencies in both water and fat-soluble vitamins. Although thiamine deficiency has not been reported, deficiencies in riboflavin and pyridoxine have been noted in SCD [28] as well as decreases in serum pyridoxal 5-phosphate concentration [29]. Homocysteine levels are increased in SCD disease [30]. In addition deficiencies in vitamins A, D, and E have been reported in this group

[31-34]. Vitamin D deficiency is associated with decreased bone density in SCD patients [35]. Vitamin A deficiency has been linked to increased hospitalization, poor growth and lower Hgb [10].

Dietary intake has been documented in several studies to be inadequate in SCD patients and to decline further as patients age [36,37]. This is particularly important for folate, which is a nutrient required by patients with increased red cell turnover and which is routinely supplemented in SCD patients despite supplementation in the US diet. As our patients have high red cell turnover, we measured serum folate. This value does not assess long term folate status. Nevertheless we also found nearly one third of our patients were folate deficient. Although we prescribe folic acid to our patients, we did not assess compliance. Thus, the low folate levels seen in this study may be due to non compliance in addition to other factors such as poor diet or malabsorption.

Only one study has examined antioxidant defenses in chronically transfused SCD patients. Alpha-tocopherol levels were lower in transfused than in nontransfused patients and were negatively correlated with number of units transfused [38]. MDA levels, a marker of cellular oxidant damage, was found to be increased 1.8-fold in TM patients compared to controls, but not in SCD patients. MDA levels were positively correlated with HIC, but not NTBI [4].

In contrast with SCD, prior work in thalassemia primarily reflects chronically transfused patients. TM patients have been shown to have low levels of Vitamins E and A as well as decreased RBC superoxide dismutase; these deficiencies were correlated with increased levels of lipid peroxidation [39]. Similar findings were reported in Italian patients where vitamin E and A deficiencies were inversely correlated with liver enzyme levels, suggesting that liver damage may play a role in the extent of depletion of these lipid soluble antioxidants [40]. Vitamin D deficiency has also been reported in several thalassemia cohorts [41].

There are a number of limitations to the present study. We did not have a control group, such as non affected siblings in the same household or non transfused SCD patients. We did not measure dietary intake which could play a role in these findings. In addition, because we do not have age-specific norms in our study, it is possible that our results are artificially skewed. We did not use trace metal free tubes when measuring trace elements such as Zn, Se, and Cu. Nevertheless, only Cu was elevated in this study and was well correlated with ceruloplasmin levels indicating it was not a spurious finding. Finally, because almost all of our patients were on Deferasirox for chelation, we could not meaningfully assess whether the type of chelation had an effect on the findings.

Nevertheless, the breadth and depth of these deficiencies are striking. For example, many authors now consider vitamin D₂₅-OH levels less than 30 ng/ml insufficient for proper bone mineralization and muscle function [14]. Serum PTH and bone density data was incomplete in this study population and further work will be necessary to characterize their relationship to vitamin D stores. Thus, this study is also limited by a lack of measurable functional outcomes which prevent us from determining the degree of harm these deficiencies pose for the patients.

In summary, chronically transfused SCD and TM have broad spectrum nutritional deficiencies of both water and fat soluble nutrients. The precise mechanism of the abnormalities is unclear and is probably multifactorial. In contrast to our underlying hypothesis, iron overload, hemolysis, and inflammatory stress appear to play relative minor roles in these deficiencies. Careful nutritional studies will need to be performed to determine contributions of diet and malabsorption. Regardless of the underlying etiology, these results suggest that all patients with TM and SCD who are chronically transfused should have periodic nutritional evaluation and supplementation as necessary. Further studies of the consequences of these deficiencies are also warranted.

Methods

Vitamin and micronutrient panels were obtained as part of annual routine clinical screening. Permission for review of medical records and waiver of consent was obtained from the Committee on Clinical Investigation at Children's Hospital Los Angeles. A convenience sample of 43 chronically transfused patients with SCD and 24 patients with TM were recruited from our population of chronically transfused hemoglobinopathy patients, and had nutrition panels drawn at the time of a regularly scheduled transfusion. All patients were requested to fast over night and hold their iron chelator for 24 hr prior to having blood drawn for the study. Levels of the following vitamins were obtained: A, thiamin, B6, B12, C, D 25OH, D1-25, and E (alpha and gamma tocopherol). Levels of folate, selenium, zinc, copper, and ceruloplasmin were also obtained. Trace element-free tubes were not used in this study. Samples were collected and processed according to clinical laboratory specifications, including rapid collection and freezing of samples for measurement of Vitamin C. (Quest Nichols Institute, San Juan Capistrano, CA).

Results were expressed as absolute values as well as the percent of patients outside the reference range for the referral laboratory. Reference ranges were not age-specific. Serum LDH and hs-CRP were measured as part of regular clinical care, and used as surrogate markers of hemolysis and inflammation, respectively. Liver iron was measured by MRI using established techniques [42]. These measurements were collected annually or biannually as part of routine chelation monitoring; only MRI liver iron estimates within 12 months of the laboratory draw were accepted.

All TM patients received simple transfusions of 10–15 cc/kg every 3 or 4 weeks to maintain a pretransfusion Hgb between 9–10 g/dl. Six patients with SCD received exchange transfusions and the rest received ~10 cc/kg of packed cells at the same intervals as the TM patients. Folate 1 mg/day and vitamin E 400U/day are routinely supplemented in our chronically transfused patients; none were receiving vitamin D or calcium replacement. All patients had normal BUN and creatinine levels. Spot urinary protein/creatinine ratios were rarely and intermittently elevated in patients on deferasirox but no patient had required dose modification or discontinuation because of proteinuria.

Parameter differences between genders and between diseases were evaluated using unpaired *t*-Test or Wilcoxin-Signed rank test when appropriate (JMP5.1, SAS Cary, NC). Predictors of abnormal vitamin levels were evaluated using linear regression with respect to age, HIC, LDH, and hs-CRP. Bonferroni correction was applied to correct for multiple comparisons.

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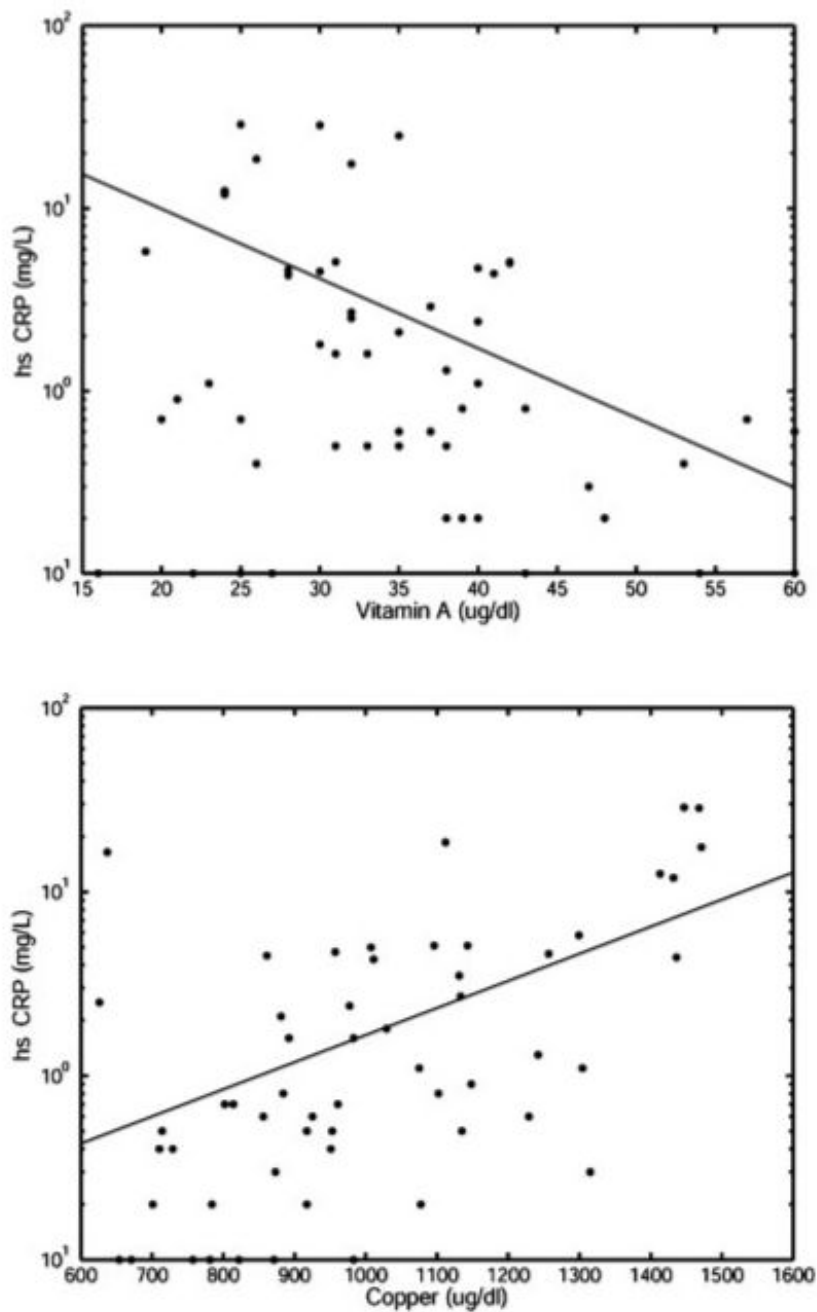


Figure 1. Graphs of the relationship between hs-CRP and vitamin A (top) ($r^2 = 0.37$, $P < 0.0027$, and negatively correlated), and hs CRP and copper (bottom) ($r^2 = 0.28$, $P < 0.0014$, and positively correlated). Vitamin A and copper are on a linear scale, whereas hs CRP is on a log scale. The appropriate fits are shown.

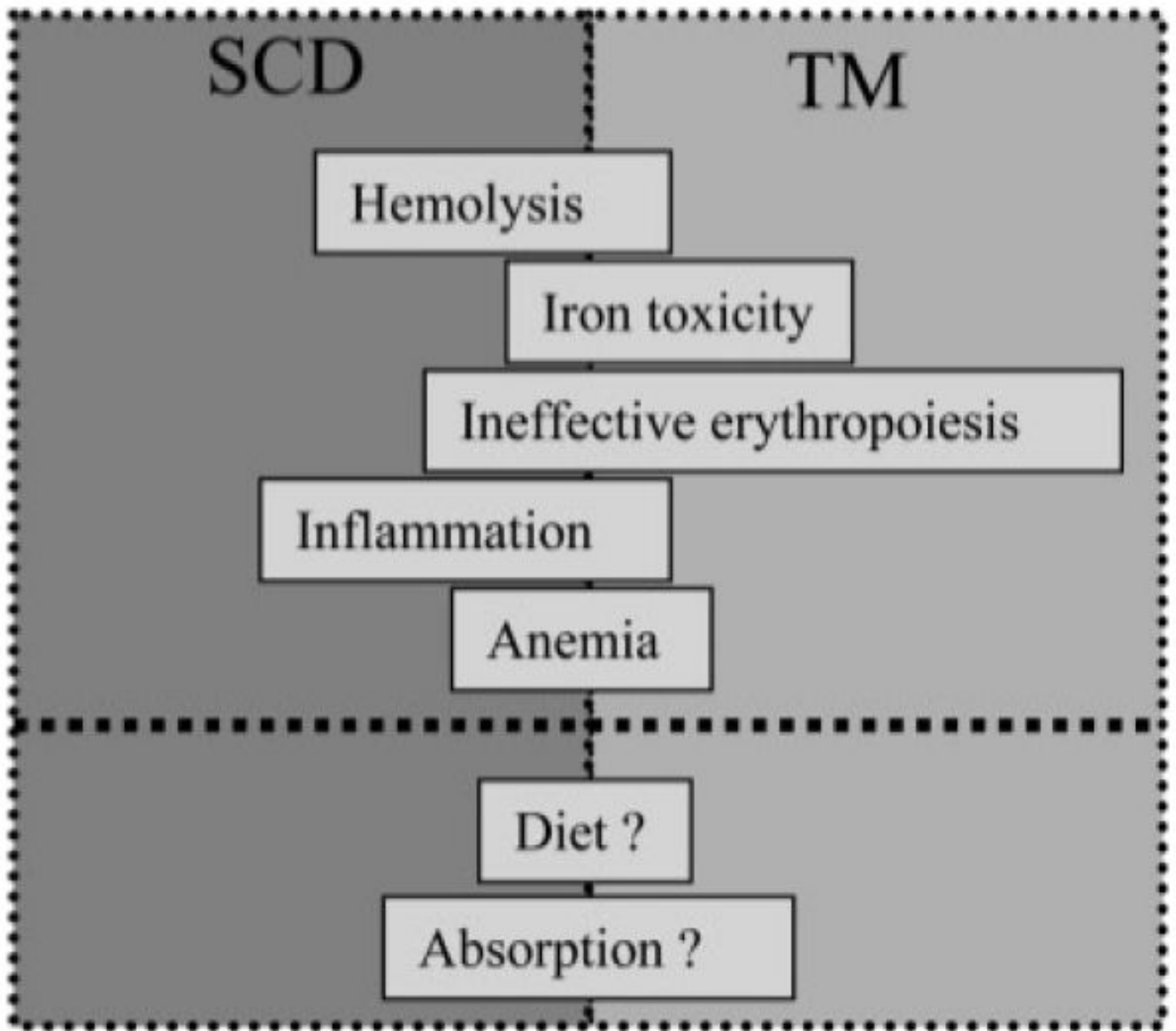


Figure 2. Schematic representing potential contributors to vitamin and mineral deficiencies in chronically transfused TM and SCD patients, including hemolysis, iron toxicity, ineffective erythropoiesis, inflammation, chronic anemia, diet, and malabsorption. Left–right position of the bar signifies the relative importance in a particular disease state. Little is known regarding the actors below the bold dotted line, thus, they are indicated by question marks and are centered with respect to the disease states.

TABLE I
Demographics and Pertinent Lab Values

	Reference range	Sickle cell N= 43	Thalassemia N= 24	P-value
Male:female	–	17:26	13:11	NS
Age (yr)	–	14.4 ± 6.6 (1.5–31.4)	14.7 ± 7.6 (1.9–25.8)	NS
HIC ^a (mg/g dry wt)	<1.6 mg/g dry wt	19.5 ± 13.8 (2.2–70.2)	13.7 ± 11.4 (2–39.5)	<0.03
Ferritin (ng/mL)	5 months to 15 years: 10–140 Males > 15 years: 30–300 Females > 15 years: 10–150	3874 ± 4451 (81–27200)	2089 ± 1920 (246–8230)	<0.02
% Saturation (Fe)	15–50%	68 ± 24 (11–103)	84 ± 18 (36–106)	<0.02
LDH	315–618 U/l	1263 ± 717 (288–4209)	445 ± 136 (292–717)	<0.0001
Plasma hemoglobin	0–10 mg/dl	50 ± 60 (3–330)	16 ± 10 (3.5–49)	<0.005
hs CRP	<1.0 mg/l	5.8 ± 8 (0.2–28.2)	1.2 ± 3.4 (0.1–16.4)	<0.004

Demographic and other lab values of the patients studied. Laboratory results are expressed as mean ± SD. *P*-values are indicated as shown. HIC, hepatic iron concentration (as determined by MRI); Fe, iron; LDH, lactate dehydrogenase; hs-CRP, high sensitivity C reactive protein.

^aHIC values were calculated by MRI R2 and R2* methods.

TABLE II
Nutrient Levels in Chronically Transfused Sickle Cell Patients (Top) and Thalassemia Patients (Bottom)

Nutrient	Reference range	Sickle cell patients		Thalassemia patients	
		Mean ± S.D.	%abn	Mean ± S.D.	%abn
A (μ/dl)	38–98	34.4 ± 8.5	73.70%	34.6 ± 12.2	52.40%
Thiamin (μ/l)	2.4–11.7	3.3 ± 21.6	38.50%	4.1 ± 4.0	37.50%
B6 (ng/ml)	3.3–26	8.1 ± 27.7	34.20%	7.0 ± 5.9	34.80%
B12 (pg/ml)	200–1100	664.4 ± 399.2	4.70%	528 ± 152.0	0
Folate (ng/ml)	>8	13.5 ± 6.0	28.60%	11.8 ± 7.7	37.50%
C (mg/dl)	0.2–1.9	0.28 ± 0.29	56.70%	0.32 ± 0.40	66.70%
D25 (pg/ml)	20–100	16.3 ± 6.2	74.40%	17.1 ± 8.5	50.00%
D1-25 (pg/ml)	1.5–60	61.1 ± 18.2	48.60%	59.9 ± 19.5	39.10%
E-alpha (mg/dl)	5.7–19.9	8.7 ± 4.7	10.50%	7.5 ± 7.5	29.20%
E-gamma (mg/dl)	<4.3	2.3 ± 1.3	7.90%	3.0 ± 5.0	4.20%
Selenium (μ/l)	110–160	107.1 ± 13.3	67.50%	99.5 ± 20.7	75.00%
Zinc (μ/dl)	65–124	81.9 ± 17.6	24.30%	83.0 ± 15.6	8.30%
Copper (μ/l)	590–1180	1092.1 ± 221.1*	34.20%	851 ± 155.0*	0
Ceruloplasmin (mg/dl)	24–71	33.7 ± 7.5*	4.70%	26.2 ± 4.8*	16.70%

The vitamins and trace minerals measured are listed with their normal ranges. The patients' mean values plus or minus the standard deviation are in the second column. The percent of patients whose measured values fell outside the normal range are listed as percent abnormal (third column). All abnormal values were indicative of deficiencies with the exception of D1, 25-OH, and Cu which were elevated in SCD. The values where there is a difference between SCD and TM patients are indicated with a ** symbol. Blood samples were drawn while patients were fasting overnight and their usual iron chelator was held for 24 hr.

All abnormal results, with the exception of copper, represent deficiencies.

TABLE III
Significant Relationships among Vitamin/Mineral Levels, Age, Hepatic Iron Concentration, and High Sensitivity CRP

	Age		HIC		Transf %		hs-CRP	
	SCD	TM	SCD	TM	SCD	TM	SCD	TM
Vitamin A	r^2	r^2	r^2	r^2	-	-	r^2	r^2
Folate	↓ 0.37	↓ 0.21	-	-	↓ 0.30	-	↓ 0.28	-
D25-OH	↓ 0.23	↓ 0.45	-	↓ 0.16	-	-	-	-
D1-25	↓ 0.37	-	-	-	-	-	-	-
γ tocopherol	↑ 0.30	-	-	↑ 0.22	-	-	-	-
Copper	↓ 0.25	↓ 0.25	↓ 0.15	-	-	-	↑ 0.35	-
Ceruloplasmin	↓ 0.15	↓ 0.37	-	-	-	-	↑ 0.25	-

This table is restricted to vitamin/mineral levels demonstrating physiologically relevant correlations ($r^2 > 0.15$). These include Vitamin A, Folate, Vitamin D25-OH, Vitamin D 1-25, γ -tocopherol, Copper, and Ceruloplasmin. Negative and positive correlations are indicated by respective arrows as shown beneath the table. Multivariate model was limited to the independent variables analyzed in TABLE III. All $P < 0.0125$. Bold type indicates retained significance < 0.05 in multivariate analysis.

↑ indicates positive correlation.

↓ indicates negative correlation.