Plasma Zinc Levels Inversely Correlate with Vascular Cell Adhesion Molecule-1 Concentration in Children with Sickle Cell Disease

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Zinc deficiency has been implicated in impaired cell-mediated immunity of children with sickle cell disease (SCD). However, its influence on the expression of vascular cell-adhesion molecule-1 (VCAM-1) on endothelial cells, a protein involved in vasoocclusion, has not been previously investigated. We therefore measured (soluble) sVCAM-1 and zinc in 76 SCD children and 96 non-SCD children, mean age 7.73 years and 11.24 years, respectively. Although mean zinc levels of both groups were within the normal range (≈14.5 µmol/l), 14.5 % of SCD and 11% of non-SCD children (without inflammation) had levels below normal (10.7 µmol/L). Mean sVCAM-1 concentrations of SCD children (837 µg/l) were significantly higher than those of controls (627 µg/l) ($p<0.001$). Differences persisted after taking into account age, hemoglobin phenotype, and inflammation (alpha-1acid glycoprotein >1g/l and C-reactive protein >10 mg/l). sVCAM-1 negatively correlated with serum ($r=-0.444$) and red blood cells zinc ($r=-0.242$, $p<0.05$) but not with acute-phase proteins. Mean sVCAM-1 tended to be higher in SCD children with than in those without a history of a health problem (infection, pain crisis or were transfused; not significant). Data suggest that zinc may modulate the clinical status of SCD children through VCAM-1 expression, and zinc supplementation may be beneficial in these patients.

Key words: sickle cell anemia ■ zinc ■ C-reactive protein ■ inflammation

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Zinc is an essential trace element in humans and animals. It is a cofactor of >200 enzymes involved in the metabolism of proteins, lipids, carbohydrates, deoxynucleotides and nucleotides.[1,2] This trace element plays a crucial role in preventing lipid peroxidation either via copper/zinc superoxide dismutase, metallothionein or cytochrome P450 systems.[1,2] Zinc deficiency has been implicated in certain abnormalities associated with sickle cell disease (SCD), such as impaired cell-mediated immunity, poor wound healing, increased susceptibility to infection and growth retardation in children.[3-7] Zinc supplementation resulted in improved cell-mediated immunity and growth rate; and reduced the number of hospital admissions, episodes of pain crises and infections compared to the pretreatment period.[4,7]

Previous work by our group and other investigators suggested that SCD children have a low grade of chronic inflammation evidenced by elevated levels of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-8 and acute-phase proteins.[8-13] TNF-alpha activates polymorphonuclear leukocytes leading to generation of high levels of superoxide and hydrogen peroxide.[14] Free radicals have been shown to increase red blood cell (RBC) membrane lipid peroxidation and adherence to endothelium.[15] In vitro studies have shown that RBCs bind to the endothelial system via an interaction of integrin (VLA-4) expressed on the RBCs and vascular cell adhesion molecule-1 (VCAM-1) expressed on the endothelium.[16] Moreover, sickle RBCs adhere to VCAM-1 more efficiently than normal cells.[17,18]

In 1996, Duits et al.[19] reported elevated concentrations of plasma or soluble VCAM-1 (s-VCAM-1) in SCD children compared to control children. However, in addition to the limited sample size, these investigators did not assess factors such as antioxidants that may modulate the expression of sVCAM-1. Considering reports on suboptimal zinc status in children with SCD and the role of zinc in immunity and the antioxidant system, we hypothesize that: a) there is an inverse associa-

tion between circulating levels of sVCAM-1 and those of plasma and RBC zinc; b) sVCAM-1 inversely correlates with poor clinical status (frequency of blood transfusion, infection, pain crisis or general hospitalization). We also wished to determine whether the association between sVCAM-1 and zinc levels, if any, is dependent on the presence of inflammation (elevated levels of acute-phase proteins).

PATIENTS AND METHODS

Patients

Parental and/or patient consent was obtained prior to blood drawing. The study was approved by the institutional review board at Louisiana State University Health Sciences Center and Children's Hospital of New Orleans. The study involved 136 blood samples that were drawn between March 1994 and March 1996 from 76 children with SCD (46 boys and 30 girls), ranging in age from 1–17 years. There were 48 children with hemoglobin SS (HbSS), 18 with HbSC and 10 with HbSß^{thalassemia} (Sß^{thal}). Thirty-nine, 26 and 11 patients were studied once, twice and ≥3 times, respectively. The number of blood samples drawn was a function of the enrollment date. Information on overall health status for the period 1994–1996 was collected by reviewing clinic charts. At the time blood samples were drawn, no participant had symptoms of any disease, was hospitalized, received a blood transfusion or suffered from pain crisis during the preceding month. Blood samples were drawn during regular follow-up at our clinic (Children's Hospital of New Orleans), usually scheduled once every three months. The study also involved 96 frozen plasma samples that were obtained from non-SCD children who were seen at the hospital for routine check-up (children who were previously treated for cancer and who were in remission for ≥4 months, bone marrow transplant patients and 14 healthy children). Non-SCD children were not matched by race or gender, and the age range was 1–22 years. (As explained in the Results section, exclusion of non-SCD children ages >17 years did not affect the data or the conclusion of the study.)

Blood Drawing and Processing

Blood samples were drawn into heparinized vacutainers with low trace elements. They were centrifuged at 400 x g, 4°C, for 10 min within 2 hrs of blood drawing. Plasma was collected, aliquoted in 250–500 µl before freezing at -40°C until used for various measurements.

RBCs were separated from mononuclear cells by density gradient centrifugation on Ficoll-Hypaque. Mononuclear cells were used for lymphocyte proliferation studies, and results will be reported elsewhere. RBCs were washed twice with phosphate-buffered saline (PBS, pH 7.4) at 400 x g, 4°C, for 10 min, and packed cells were resuspended in two volumes of PBS. Hemoglobin was measured by the cyanmethemoglobin method.[20] RBCs were then aliquoted and frozen at -40°C until used for zinc assay.

Measurement of Plasma Zinc

In order to precipitate proteins, 100 µl of plasma were mixed with 400 µl 25% trichloroacetic acid (TCA) in a 1.5-ml capacity Eppendorf tubes. Ultra-pure water obtained from Millipore filters (Milli-Q50) was used to prepare the 25% TCA solution. Tubes were incubated at room temperature for 10 min. After centrifugation at 400 x g for 5 min, the supernatant was used for zinc measurement in an atomic absorption spectrophotometer (Model 3030B, Perkin Elmer; Norwalk, CT) as described in the literature.[21]

Measurement of Red Blood Cell Zinc

RBC zinc was measured only in 59 samples from patients. One-hundred microliters of RBCs were added to 400 µl nitric acid. After mixing with a vortex, the samples were heated for 10 min in a special microwave (Model MDS200, CEM Innovators in Microwave Technology, Matthews, NC) to digest RBCs. After cooling the samples to room temperature, they were centrifuged at 400 x g for 10 min. Supernatant was aspirated and immediately frozen at -80°C until used for zinc measurement by atomic absorption spectrophotometry. While results of plasma zinc were expressed as µmol/l, those of RBCs were expressed as µmol/g hemoglobin.

Table 1. Mean age and concentrations of serum acute-phase proteins in children with and without sickle cell disease

	HbSS	HbSC	HbSß^{thal}	All SCD Children	Controls
Age (Years)	7.76 ± 0.52	6.87 ± 1.08	8.55 ± 1.52	7.7 ± 0.46^{b}	11.2 ± 0.85^{a}
AGP, g/l	0.74 ± 0.03	0.76 ± 0.06	0.8 ± 0.09	0.75 ± 0.03 (0.72)^{b}	1.43 ± 0.11 (1.11)*^{a}
CRP, mg/l	6.18 ± 1.51	1.72 ± 0.73	1.98 ± 0.87	4.72 ± 1.03 (0.24)^{b}	17.22 ± 17.27 (0.24)*

Hb: hemoglobin, AGP: alpha-1-acid glycoprotein, CRP: C-reactive protein. Values are mean ± SEM. For age, n=48, 18, 10, 76 and 96 children with HbSS, HbSC, HbSß^{thal}, all SCD, and non-SCD subjects, respectively. For acute-phase protein concentrations, each blood sample was treated as a subject: 90 HbSS, 26 SC, 20 HbSß^{thal} and 96 controls. For each row, means followed by different superscript letters are significantly different: a>b, p<0.001; control children compared to those with SCD. * Values in parentheses are medians. When four CRP values that were between 61 mg and 520 mg/l were excluded from the non-SCD group, the mean was only: 5.95 ± 1.43 mg/l.

Measurement of sVCAM-1 and Acute-Phase Proteins

sVCAM-1 was measured by enzyme immunoassay with kits that were purchased from R&D (Minneapolis, MN). The method provided with the kit was carefully followed. To rule out inflammation, C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) were measured by radial immunodiffusion with polyclonal antibodies (produced in the goat and rabbit), standards, and controls purchased from Sigma (St. Louis, MO) as described in the literature.[22] Inflammation was defined as CRP >10 mg/l and/or AGP >1 g/l. All test samples, standard and controls were run in duplicate.

Statistical Analysis

Data were analyzed in three ways: a) Each blood sample was treated as a subject, b) The mean of multiple samples for each SCD child was first calculated and used in the analysis, and c) Only the first blood sample from each SCD child was used in the analysis. In regard to the first type of analysis, the assumption was that data from multiple blood samples from each child were independent from each other and were a true reflection of the health status at the time of blood drawing. The higher sample size (n=136 samples) would provide a higher power than the use of 76 SCD children. In the second type of analysis (use of the means of multiple samples), the assumption was that the measurements under study are stable over a period of several months and are a true reflection of the actual concentration for the subject. This assumption may not be true because the concentration of zinc and acute-phase proteins may change depending on health status. As further described under results, because the three types of analyses gave the same trend of differences between SCD and non-SCD subjects, only the graphs of sVCAM-1 and zinc derived with the first type of analysis (each blood sample is considered as a subject) are reported.

Descriptive analysis (mean, SEM), analysis of variance (ANOVA) and simple Pearson's correlation coefficients were calculated by the use of Microstatistical Program (Ecosoft Inc., Indianapolis, IN) as described in the literature.[23] Multiple regression analysis with plasma levels of sVCAM-1 as the dependent variable, acute-phase proteins, age, hemoglobin phenotype, frequency of pain crisis, infection or hospitalization due to any health problem, and number of blood units received as independent variables was also performed. When ANOVA detected significant differences between means, Scheffé's test was performed to determine which pairs of means were statistically significant. In some instances, Student's t test was also performed. The level of significance was set at $p<0.05$.

RESULTS

General Information

Approximately two-thirds of patients were homozygous (HbSS); 23% and 13% of children had the SC and Sβ^{thal} phenotypes, respectively. Four of the ten children with HbSβ^{thal} (10 blood samples) and six children (also 10 blood samples) had the β^{thal-0} and β^{thal+} phenotypes, respectively. There was no significant difference in the mean age among children with different hemoglobin phenotypes (Table 1). Although non-SCD children were older than SCD children ($p<0.01$), only 11 children were aged >17 years (the upper limit for children with SCD). Information on the number of episodes of pain crisis, infections and/or number of blood units received between 1994 and 1996 was obtained from 61 patients. Although patients were under stable conditions, 13, 21 and 12 patients had ≥1 episode of infection, suffered from pain crisis and received blood transfusion during the two-year follow-up period, respectively.

Acute-Phase Proteins

Although the mean concentrations of AGP and CRP were higher in non-SCD children than in SCD children

Table 2. Concentrations of zinc (µmol/l) in SCD and non-SCD children without and with inflammation.

	HbSS	HbSC	HbSβ^{thal}	All SCD	Non-SCD	P†
All subjects	14.7 ± 0.33(90)	14.6 ± 0.87(26)	13.9 ± 0.097(20)	14.6 ± 0.31(136)	14.55 ± 0.78(82)*	NS
AGP ≤1g/l	14.8 ± 0.37(76)	14.1 ± 0.97(20)	14.73 ± 1.22(15)	14.66 ± 0.35(111)	17.56 ± 1.46(28)	<0.05
AGP >1 g/l	14.19 ± 0.78(13)	16.02 ± 2.03(6)	11.50 ± 0.66(5)	13.08 ± 0.72(24)	13.5 ± 0.93(46)	NS
CRP ≤10 mg/l	14.63 ± 0.37(77)	14.79 ± 0.86(23)	13.94 ± 1.03(19)	14.55 ± 0.33(119)	14.65 ± 0.99(53)	NS
CRP >10 mg/l	15.36 ± 0.82(13)	8.63 ± 2.49(2)	13.46 ± 0 (1)	14.40 ± 0.87(16)	16.51 ± 1.82(17)	NS
No inflammation	14.60 ± 0.39(68)	14.71 ± 0.96(18)	14.73 ± 1.22(15)	14.64 ± 0.36(101)	16.67 ± 1.60(23)	<0.05
1 APP >normal	15.50 ± 0.75(16)	14.19 ± 1.97(8)	10.9 ± 0.63(4)	14.48 ± 0.75(28)	13.62 ± 1.06(43)	NS
2 APP >normal	13.61 ± 0.94(5)	N/A	13.46 ± 0(1)	13.58 ± 0.77(6)	15.87 ± 2.0(10)	NS
RBC Zn µg/g Hb	0.42 ± 0.02(42)	0.43 ± 0.04(11)	0.32 ± 0.03(6)	0.41 ± 0.02(59)	ND	–

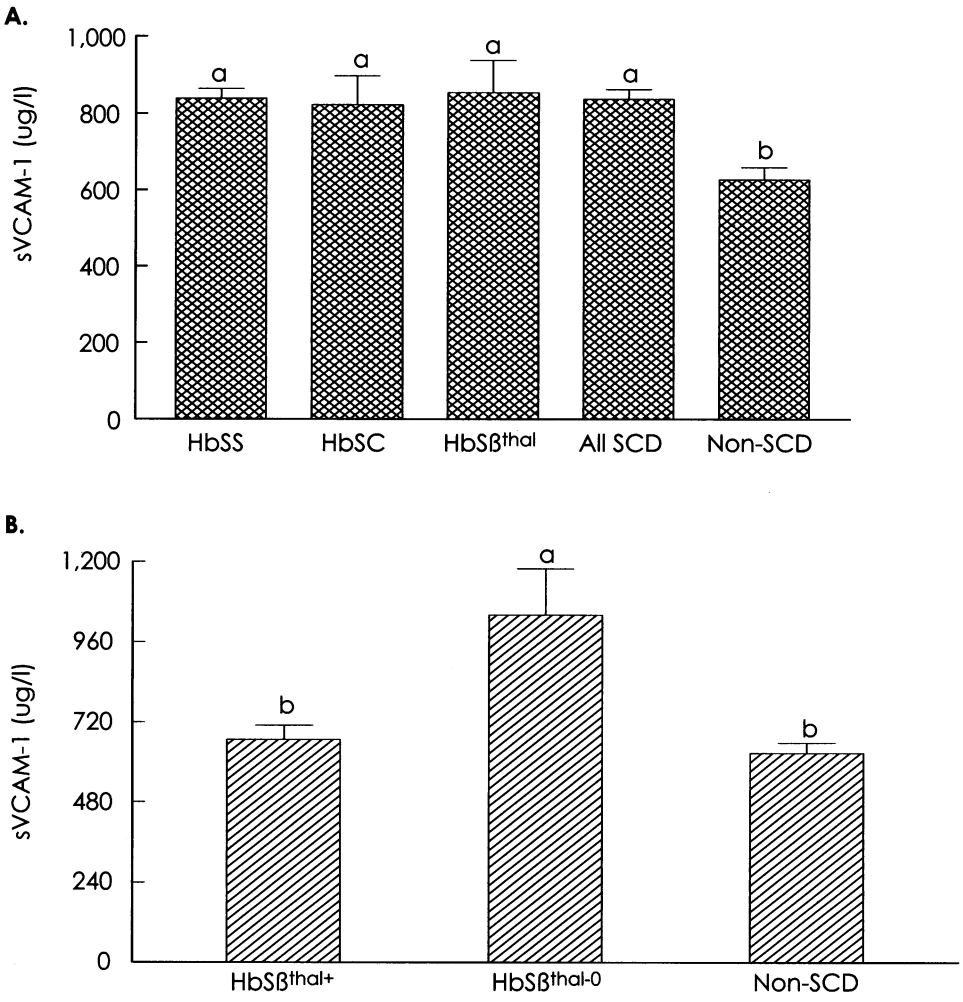
Values are means ± SEM. AGP: alpha-1-acid glycoprotein, APP: acute-phase proteins, CRP: C-reactive protein, Hb: hemoglobin, N/A: not applicable (because no child with HbSC phenotype had two acute-phase proteins above normal), ND: not determined, SCD: sickle cell disease, RBC: red blood cells; * Numbers in parentheses are sample sizes. † p values are based on all SCD children versus non-SCD children (ANOVA). When the analysis was repeated by using the means of data of multiple blood samples before performing ANOVA, or by using only the first blood sample from each SCD child, the same trend was observed (data not shown).

(Table 1, $p<0.05$), the median levels of CRP were identical in both study groups and were within normal range. When statistical analysis was repeated after calculating the means of serial measurements from SCD children, or when only the first blood samples of SCD children were used, the same trend was observed (no table shown). The higher mean levels of acute-phase proteins in non-SCD children are very likely related to the previous diseases and/or treatment. However, when the four samples with CRP levels between 61 mg/l and 520 mg/l were excluded, the mean (± SEM) of the non-SCD children was only 5.95 ± 1.43 mg/l, and it was not significantly different from that of SCD children.

Zinc Concentration

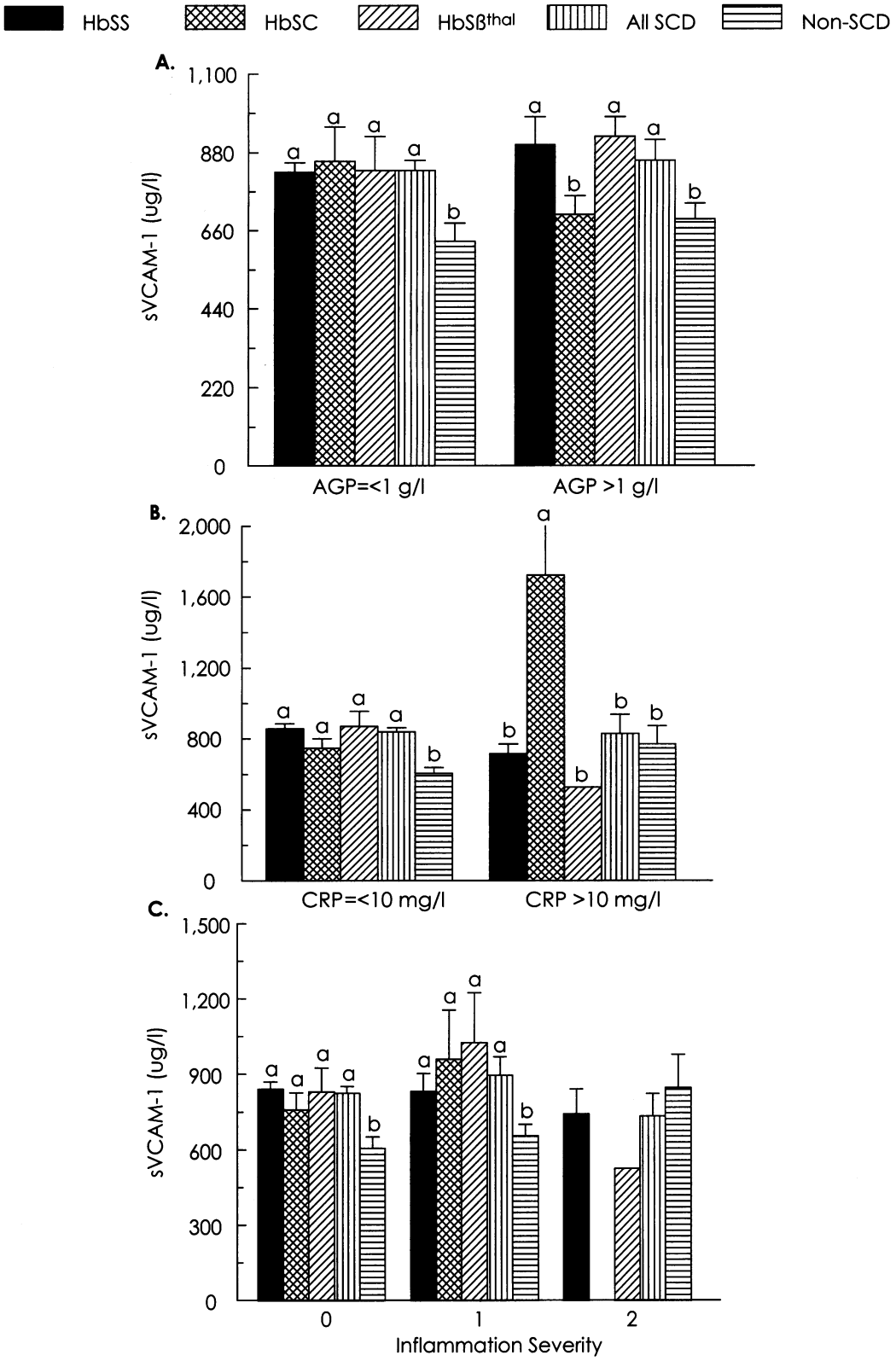
The mean plasma zinc concentrations of SCD and non-SCD were within the normal range (≥10.7 μmol/l). There were no significant differences in mean plasma zinc levels among children with different hemoglobin phenotypes, or between SCD children and non-SCD children (Table 2). When either the means of zinc concentration of multiple samples (14.25 ± 0.34 μmol/l) or only the first blood sample for each SCD patient (13.95 ± 0.36 μmol/l) was used instead of the 136 individual samples, we also found no significant differences between SCD and non-SCD children (14.55 ± 0.78 μmol/l). However, when children with inflammation were excluded, the mean zinc levels of SCD children were significantly lower than those of the non-SCD children (Table 2, $p<0.05$). In the subgroups of children with inflammation, mean zinc levels were not different between SCD and non-SCD children. Eleven of the 76 SCD children (14.5%) had plasma zinc levels below normal (<10.7 μmol/l) at least once, and 17 others (22.4%) had plasma zinc only slightly above the cut-off point (10.7–12.2 μmol/l). In the non-SCD group, although 25 samples had zinc below normal, inflammation contributed to the abnormal concentration. In fact,

Figure 1. Concentration of sVCAM-1 in controls and in SCD children as a function of hemoglobin phenotype (A), and in SCD children with Hbß$^{thal+}$ and Hbß$^{thal-0}$ phenotype (B)



Values are mean ± SEM. For each graph, bars labeled with different superscript letters are significantly different: a>b, $p<0.001$.

Figure 2. Concentration of sVCAM-1 in SCD and non-SCD children as a function of AGP (A), CRP (B) or both acute-phase proteins (C)



Sample sizes and abbreviations are as described in Table 2. For each graph, bars labeled with different superscript letters are significantly different ($p < 0.05$).

only nine (11%) plasma samples with zinc levels below normal had both AGP and CRP in the normal range.

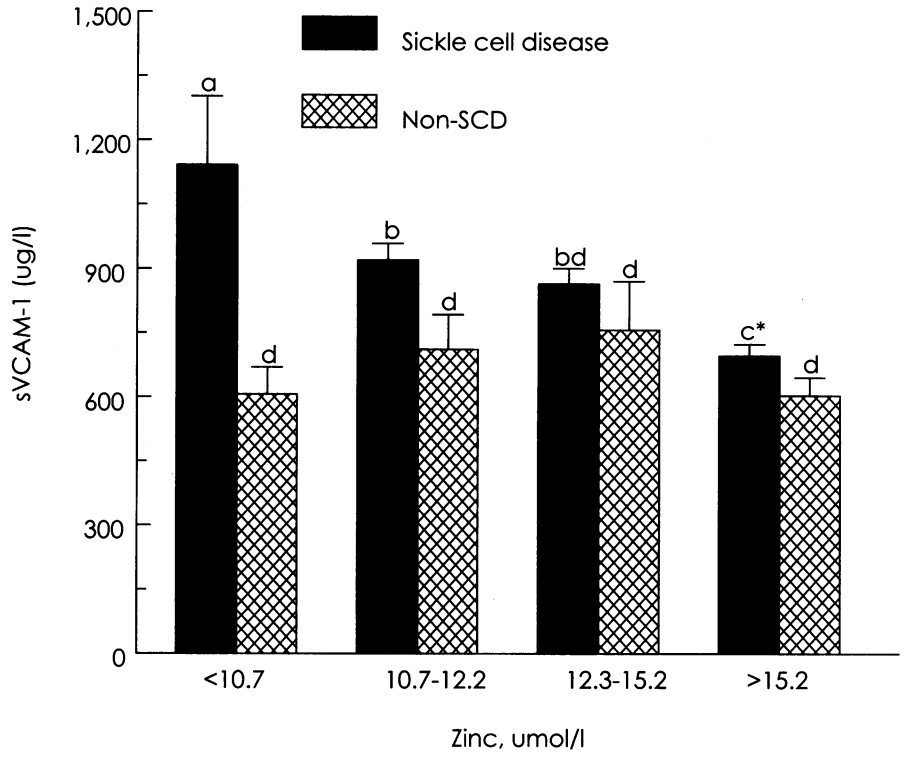
sVCAM-1 Concentration

We observed no significant differences in mean levels of sVCAM-1 among subgroups of children with different hemoglobin phenotypes (Figure 1A). In contrast, the mean sVCAM-1 concentrations of SCD children (each hemoglobin phenotype and combined subgroups) were 33.7% to 36.2% higher than those of non-SCD children (Figure 1A, $p<0.001$). Interestingly, plasma samples from SCD children with HbSß$^{thal-0}$ phenotype had mean sVCAM-1 levels that were significantly higher than those obtained from patients with the Sß$^{thal+}$ phenotype (Figure 1B, $p<0.05$). Additionally, the mean (± SEM) zinc levels of the former group tended to be lower (12.79 ± 1.32 µmol/l) than those of the later group (15.04 ± 1.41 µmol/l), but the difference was not significant. Moreover, SCD children with HbSß$^{thal+}$ phenotype had mean sVCAM-1 levels that were not significantly different from those of non-SCD children, whereas they were significantly higher than those of children with HbSß$^{thal-0}$ phenotype ($p<0.05$, Figure 1B).

When the means of multiple samples for each SCD child were first calculated and then used in ANOVA (mean ± SEM: SCD 843 ± 30 µg/l vs. 627 ± 31 µg/l for non-SCD children) or only the first blood sample for each patient was used (851 ± 32 µg/l), SCD children still had significantly higher mean sVCAM-1 than controls ($p<0.001$). The differences between patients and controls also persisted when the 11 non-SCD children who were aged >17 years were excluded (622 ± 33 µg/l, $p<0.001$) and when the analysis was limited to children without evidence of inflammation (Figure 2A–C, $p<0.05$). Although the same trend was observed in children with inflammation, the difference between SCD and non-SCD children was significant only with AGP (Figure 2A) and in the subgroup with one acute-phase protein above normal range (Figure 2B, $p<0.05$). In the subgroup of children with both AGP and CRP above the normal range, there was no significant difference between SCD and non-SCD children in plasma levels of sVCAM-1. In non-SCD children, sVCAM concentration increased by 8.3% and 39.8% with inflammation, with one and two acute-phase proteins above normal, respectively, (Figure 2C, $p<0.05$). In SCD children, we observed no linear increase in sVCAM-1 levels with the number of acute-phase proteins above normal.

The concentrations of sVCAM-1 of children with SCD varied with plasma zinc levels (Figure 3). They

Figure 3. Concentration of sVCAM-1 as a function of plasma zinc levels in SCD and non-SCD children



Values are mean ± SEM; N=12, 25, 51 and 48 for SCD children, and 25, 9, 14 and 34 non-SCD children with plasma zinc <10.7, 10.7–12.2, 12.3–15.2, and >15.2 µmol/l, respectively. Zinc levels were not available in 14 plasma samples from non-SCD children. Within the SCD children group, sVCAM-1 levels decreased with increased plasma zinc levels: a>b>c, $p<0.05$. Within each range of zinc, bars with different superscript letters are significantly different: a> d, b>d, $p<0.05$; and c*>d, $p=0.053$

were higher in patients with plasma zinc below normal (<10.7 μmol/l) and lowest in those with plasma zinc >15.2 μmol/l. ANOVA detected significant differences in sVCAM-1 concentrations among the four ranges of plasma zinc (<10.7, 10.7–12.2, 12.3–15.2, >15.2 μmol/l, $p<0.01$). In non-SCD children, sVCAM-1 concentrations did not significantly vary with zinc status. For each range of zinc concentration, the mean sVCAM-1 levels of SCD children tended to be higher than those of non-SCD children ($p<0.05$). However, the differences between both groups decreased with increased zinc levels (88% for zinc <10.7 μmol/l vs. 15% for zinc >15.2 μmol/l). Although children with SCD with plasma zinc >15.2 μmol/l had the lowest mean sVCAM-1 concentrations, they still tended to have higher mean levels than non-SCD children ($p=0.053$), which suggests an influence of the disease itself in addition to zinc status. As for the overall SCD population, sVCAM-1 varied with zinc status in each hemoglobin phenotype group, being highest in blood samples with the lowest zinc levels and lowest in those with the highest zinc levels ($p<0.05$, no figure shown). Mean sVCAM-1 concentrations also varied with RBC zinc levels (Figure 4). They were higher in SCD children with the lowest RBC zinc levels (<0.30 μmol/g hemoglobin) and lowest in those with RBC zinc ≥0.50 μmol/g hemoglobin ($p<0.05$).

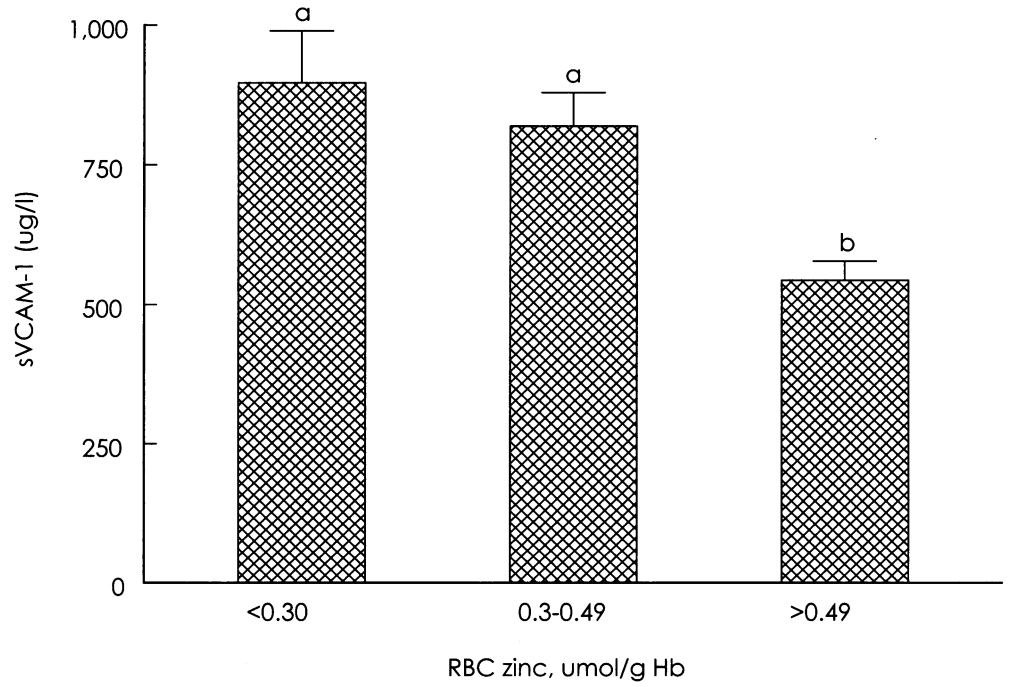
Plasma Zinc and sVCAM-1 as a Function of Clinical Status

Plasma concentrations of zinc and sVCAM-1 were also analyzed as a function of infection, pain crisis, blood transfusion or a combination of these problems (Figure 5). We found no significant difference in mean levels of sVCAM-1 between SCD children who suffered and those who did not suffer from pain crisis and/or infection, or between children who received and those who did not receive a blood transfusion. However, SCD children who suffered from any health problem once or ≥2 tended to have higher mean sVCAM-1 than those who did not, but the difference was not statistically significant.

Correlation Coefficients

In SCD patients, plasma ($r=-0.444$) and RBC ($r=-0.242$) zinc negatively and significantly correlated with sVCAM-1 ($p<0.05$). Significant correlations were also observed between plasma zinc and sVCAM-1 concentrations in each hemoglobin phenotype group ($r=-0.34$, $r=-0.58$, $r=-0.78$, for SS, SC and $S\beta^{thal}$, respectively; $p<0.05$). The higher correlation between zinc and sVCAM-1 in children with $HbS\beta^{thal}$ phenotype was in part due to 10 blood samples that were obtained from children with $S\beta^{thal\text{-}0}$. RBC zinc did not significantly correlate with the frequency of infection, units of blood

Figure 4. Concentration of sVCAM-1 as a function of red blood cell zinc in children with sickle cell disease



RBC: red blood cell, Hb: hemoglobin. Values are mean ± SEM. Sample sizes are: 12, 33, and 14, for RBC zinc <0.3, 0.3–0.49, and >0.49 μmol/g Hb, respectively. Bars followed by different superscript letters are significantly different; a>b, $p<0.05$

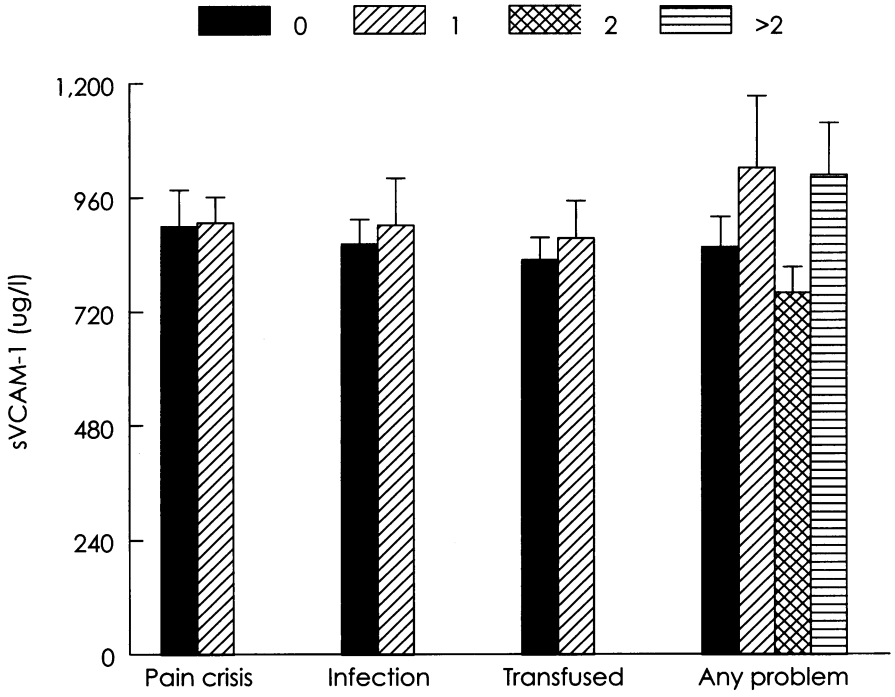
transfusion or plasma zinc, but it slightly negatively, though not significantly, correlated with pain crisis (r= -0.22). Although sVCAM-1 concentration did not significantly correlate with frequency of pain crisis, it weakly but positively correlated with episodes of infection (r=0.3, p<0.05).

To determine whether the inverse association between zinc and sVCAM-1 levels was simply due to general inflammation, we also calculated correlation coefficients between plasma levels of acute-phase proteins and those of sVCAM-1. In SCD children, no significant correlations between acute-phase proteins and either sVCAM-1, plasma zinc or RBC zinc were noted (r=-0.125–0.0004). In non-SCD children, while plasma zinc did not correlate with sVCAM-1, it negatively, though weakly, correlated with AGP (r=-0.296, p<0.05). However, sVCAM-1 levels positively and significantly correlated with those of CRP (r=0.329, p<0.05) and nonsignificantly with those of AGP (r=0.190), suggesting an influence of inflammation on sVCAM-1 levels in non-SCD children. In multiple regression analysis that included zinc, CRP, AGP, hemoglobin phenotype, gender, age, frequency of pain crisis, infection and number of blood units received as independent variables, plasma zinc levels explained 23–36% of sVCAN-1 variation (p<0.005). When hemoglobin, hematocrit and white blood cell counts were included in the model, zinc explained up to 42.53% (p<0.005) of sVCAM-1 levels, compared with 8.48%, 8.08%, 6.36% and <3% (not significant) for age, number of blood units received, hematocrit and acute-phase proteins, respectively.

DISCUSSION

There are two main hypotheses that we tested in the current study. The first is that, in SCD children, there is a negative association between plasma zinc and sVCAM-1 concentration. Data summarized in Figure 3 and multiple regression analysis support our hypothesis. The second hypothesis is that there is a negative association between sVCAM-1 and clinical status. Although sVCAM-1 levels were not significantly associated with frequency of pain crisis, infection or number of blood units received, they tended to be higher in those who had a history of a health problem (either pain crisis, infection, blood transfusion requirement or frequency of hospitalization compared with none of these problems). Additionally, despite the small sample size, children with HbSß$^{thal-0}$ had significantly higher mean sVCAM-1 levels than those with HbSß$^{thal+}$ and who usually have a less severe disease. At least in this subgroup of SCD children, data support our hypothesis.

Figure 5. Concentration of sVCAM-1 in children with sickle cell disease as a function of health status



Values are mean ± SEM. Sample sizes are: 22 and 15, 30 and 13, 32 and 10 for children without and with pain crisis, without and with infection, or did not receive and received blood transfusion, respectively. For health status, 0 = no health problem (n=19), 1 = either pain crisis, infection, received blood transfusion or was hospitalized (n = 12); 2 = any two health problems (n = 12), >2 = any combination of the above problems (n=9). Either partial or no information was available in some of the children. No significant difference was observed between SCD children who suffered and those who did not suffer from theses problems in part due to small sample sizes.

The lack of association between sVCAM-1 and frequency of pain crisis is very likely due to the low overall rate of pain crisis (21 episodes) in this population and excellent medical care. The third goal of our study was to determine whether elevated concentration of sVCAM-1, if any, is due to general inflammation. As described in the last paragraph of the section on Results, in SCD children, sVCAM-1 did not positively correlate with AGP and CRP, and these acute-phase proteins explained <3% of sVCAM-1 variation, suggesting that they are not very sensitive in detecting mild inflammation in this population.

Previous work by some,[3,5] though not all, investigators[24,25] has shown low concentrations of plasma, RBC and neutrophil zinc, and impaired neutrophil function in children with SCD compared to age-matched controls. The low plasma zinc levels were attributed to increased urinary losses.[25] Zinc supplementation improved zinc status, natural killer cell activity and neutrophil functions.[26,27] Although mean plasma zinc levels of SCD children that were included in the current study were within the normal range, 14.5% of patients had plasma zinc below normal and another 22% of patients had levels only slightly above normal (10.7–12.2 μmol/l). The suboptimal levels were not due to acute or severe inflammation as suggested by the levels of AGP and CRP. In fact, the mean levels of AGP (0.73 mg/l) and CRP (4.15 mg/l) of the 37 plasma samples with zinc levels ≤12.2 μmol/l were not significantly different from those with higher plasma zinc levels (AGP 0.76 g/l, CRP 4.94 mg/l). Although 85.5% of children with SCD had plasma zinc levels in the normal range, 63% had sVCAM-1 levels that were 1.5-fold above the median of non-SCD children. SCD children with plasma zinc levels >15.2 μmol/l had the lowest sVCAM-1 levels that were still higher than those found in non-SCD children, suggesting that there is an interaction between the disease and zinc status.

The significance of elevated circulating levels of sVCAM-1 in children with SCD is uncertain. However, we know from other diseases, such as diabetes, that elevated levels are associated with a high incidence of complications.[28] In such patients, sVCAM-1 was considered a marker of vascular dysfunction and disease progression. It has been observed by Gladwin et al. that treatment of patients with SCD with hydroxyurea, a drug that improves clinical status, was associated with lower blood levels of sVCAM-1 compared to patients not receiving this drug.[29] Additionally, the same group also observed an inverse correlation between sVCAM-1 and fetal hemoglobin concentration, a key factor that determines disease severity. Our results, therefore, suggest that zinc supplementation may downregulate VCAM-1 expression and may contribute to the positive effects of zinc in previous studies.[4,7]

There are several mechanisms by which zinc may modulate the expression and/or release of sVCAM-1: a) by decreasing the secretion of proinflammatory cytokines; b) by maintaining optimal activity of copper/zinc superoxide dismutase, an enzyme involved in the reduction of hydrogen peroxide to water, thus reducing the risk of free-radical formation; and c) by inhibition of cytochrome P450 system.[30] The generation of inflammatory cytokines (TNF-alpha, IL-1beta and IL-8, previously shown to be elevated in SCD subjects) and activation of endothelial cell adhesion molecules (VCAM-1, intercellular adhesion molecule-1 and E-selectin) are due to reactive oxygen species (ROS) and a variety of ligands such as TNF receptors and IL-1 receptors via NF-kappaB activation.[11-13,31] Zinc decreases ROS by several mechanisms. It competes with iron and copper for binding to cell membranes and some proteins, displacing these redox-active metals; secondly, it binds to SH groups protecting them from oxidation.[31] Zinc induces the production of metallothionein, which is an excellent scavenger of hydroxyl ions, and zinc is also an inhibitor of NADPH oxidase, which in turn, results in decreased ROS.[31,32] Additionally, zinc upregulates the expression of A20 (a zinc finger protein) leading to inhibition of NF-kappaB activation via inhibiting TNF-receptor-associated factor (TRAF)-induced phosphorylation of IKK.[31] Lack of NF-kappaB activation (by blocking IKK phosphorylation) leads to a decrease in the expression of activated endothelial cell adhesion molecules and, hence, low sVCAM-1 levels. Suboptimal zinc status alone or together with elevated body iron stores resulting from blood transfusion may therefore favor endothelial cell activation by one or several of these mechanisms, leading to increased VCAM-1 expression and plasma sVCAM-1 levels.

In summary, our data suggest that there is an inverse association between plasma zinc and sVCAM-1 levels in SCD children under stable condition. Although suboptimal zinc status in some children with SCD and increased sVCAM-1 in patients with SCD have been previously reported,[1,2,17,26] [and Moore C., 1996 (personal communication)], the one new piece of information from our study is the strong negative association between zinc status and sVCAM-1 levels. We propose that zinc supplementation may be an adjuvant alternative drug for the management of sickle cell anemia in children who do not tolerate the cytotoxic drug hydroxyurea. We also propose that measurement of serum sVCAM-1 be included in the assessment of inflammation in children with SCD because it appears to be more sensitive than AGP and CRP.

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