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Effect of Zinc Supplementation on Incidence of Infections and Hospital Admissions in Sickle Cell Disease (SCD)

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Zinc deficiency is a common nutritional problem in adult sickle-cell disease (SCD) patients. Hyperzincuria and increased requirement of zinc due to continued hemolysis in SCD are probable bases for zinc deficiency in these patients. Zinc deficiency affects adversely T-helper$_1$ (TH$_1$) functions and cell mediated immunity and interleukin (IL)-2 production is decreased in zinc deficient subjects. We hypothesized that zinc supplementation will improve T-helper$_1$ function and decrease incidence of infections in patients with SCD. We tested this hypothesis in 32 SCD subjects who were divided in three groups (Grs A, B, and C). Grs A (n = 11) and B (n = 10) were zinc deficient based on cellular zinc criteria and Gr C (n = 11) were zinc sufficient. Gr A subjects were observed for 1 year (baseline), following which they received zinc acetate (50 to 75 mg of elemental zinc orally daily) for 3 years. Gr B subjects were observed for 1 year (baseline), following which they received placebo for 1 year and then switched to zinc supplementation (50 to 75 mg of elemental zinc orally daily) for 2 years. Gr C subjects did not receive any intervention inasmuch as they were zinc sufficient. Prolonged zinc supplementation resulted in an increase in lymphocyte and granulocyte zinc (P = 0.0001), and an increase in interleukin-2 production (P = 0.0001), decreased incidence of documented bacteriologically positive infections (P = 0.0026), decreased number of hospitalizations and decreased number of vaso-occlusive pain crisis (P = 0.0001). The predominant pathogens isolated were staphylococci and streptococci involving the respiratory tract and aerobic gram-negative bacteria, particularly *Escherichia coli,* involving the urinary tract. Further confirmation of our observations will require prospective studies of zinc supplementation in a larger number of SCD patients. Am. J. Hematol. 61:194–202, 1999. © 1999 Wiley-Liss, Inc.

Key words: zinc; sickle cell disease; infection

INTRODUCTION

Zinc deficiency is relatively common in adult sickle-cell disease (SCD) patients, affecting approximately 60–70% of adult subjects in our center [1]. Our diagnosis of zinc deficiency is based on zinc levels in lymphocytes and granulocytes in SCD patients.

Increased hemolysis in SCD patients releases a considerable amount of zinc, which circulates in the plasma pool. This results in an increase in glomerular filtration of zinc, but its reabsorption is hampered by the renal tubular damage caused by repeated vaso-occlusive episodes [2]. The resultant hyperzincuria and a high protein turnover due to increased hemolysis increases the daily requirement for zinc significantly in SCD patients. This increased requirement for zinc is not met by the usual dietary intake. The role of the intestinal absorption in the

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homeostasis of zinc in SCD still remains to be investigated.

Our previous studies have shown that growth delays, hypogonadism in males, abnormal dark adaptation, hyperammonemia, and cell-mediated immune disorders were related to zinc deficiency in SCD patients [3–10]. Response to zinc supplementation trials showed increased growth and gonadal development in males, improvement in dark adaptation, decreased plasma ammonia concentrations, correction of anergy, increased natural killer cell (NK) lytic activity, increased T4 to T8 ratio, increased serum thymulin activity (a thymic hormone involved in T-helper (TH) cells proliferation and differentiation), and increased CD8+CD73+ precytolytic cells [3–11].

Zinc deficiency has been also documented in SCD patients between the ages of 3–18 years. Patients classified as having "poor" growth had a lower serum zinc concentration than those with "normal" growth [12]. Other observers have likewise reported the occurrence of zinc deficiency in SCD patients [13–16].

Infection is the most common cause of death in children with SCD, and is predominantly due to lethal pneumococcal sepsis in children less than 3 years of age [17]. Survival past the age of 3 without prior recognized bacterial infection is associated with a markedly reduced risk of pneumococcal sepsis. Some older SCD subjects have observed an increased incidence of salmonella infection and increased mortality and morbidity due to tuberculosis in patients with SCD. The risk of salmonella osteomyelitis is several hundred times greater in patients with SCD as compared to the normal population [18]. An increased incidence of urinary tract infection due to *Escherichia coli*, with clinical pyelonephritis in up to 25% of adults with SCD has been observed [19,20]. These patients appear to be susceptible to Enterobacter–Klebsiella infection also. Our own clinical impression is that a large number of adult SCD patients who are admitted to the hospital give suggestive evidences for upper or lower respiratory tract infection or urinary tract infection prior to the onset of pain crisis.

Some investigators have observed that patients with SCD are unusually susceptible to infection due to *Mycoplasma pneumoniae*, often manifesting unusual clinical features [21]. Although proven examples of viral hepatitis in SCD patients do not suggest increased incidence of this infection, 20–40% prevalence of macronodular cirrhosis among adult subjects has been reported [20]. It has been shown that primary infection with Parvovirus B19 is the major cause of aplastic crises in patients with SCD [22]. Cryptococcal pneumonia and pneumonia due to cytomegalovirus in patients with SCD have been also reported [23]. Mycoplasma and *Chlamydia pneumoniae* cause the most common infections associated with the acute chest syndrome [20] in SCD patients. It is believed that a much larger percentage of patients with pulmonary infiltrates probably have associated viral infections [20,24].

Contributing factors to increased susceptibility to infections in patients with SCD appear to include a state of functional asplenia, an opsonophagocytic defect due to an abnormality of the alternative complement pathway, and a deficiency of specific circulating antibodies. The role of abnormalities of cell mediated immunity that have been detected in some SCD patients is less clear.

Our own findings suggest that zinc deficient subjects have impaired IL-2 production and reduced NK activity. Inasmuch as zinc deficiency is known to affect cell mediated immunity adversely, we hypothesized that the cell mediated immune dysfunction in patients with SCD will be corrected and incidence of infections decreased by zinc supplementation. To test this hypothesis we have conducted a sequential treatment design study of zinc supplementation in SCD subjects and the results of this study are presented in this paper.

METHODS

We recruited 32 SCD subjects for this study who were being followed in the hematology-sickle cell adult clinic at the Detroit Medical Center. Twenty-six subjects were homozygous for SCD. Three subjects had SC disease and three were diagnosed to have S β-thalassemia. The group was comprised of 16 males and 16 females. Their ages ranged from 19 to 49 years. Thirty-one subjects were within the age group of 19 to 43 and one woman was 49 years of age. Twenty-three subjects were between 19 and 30 years of age. Subjects who voluntarily consented to participate in our studies were recruited. Exclusion criteria were as follows: (i) nonambulatory; (ii) receiving transfusion more than six per year; (iii) drug dependent; (iv) neurological or psychiatric deficits; (v) subjects on immunosuppressive drugs; (vi) patients who were positive for HIV; and (vii) patients who were positive for hepatitis B.

The clinic subjects had a regular routine physical check-up, hematological evaluation, and other routine laboratory tests. These included a complete history and physical examination, SMA-12, routine urinalysis and cell blood count. History of fever, upper respiratory tract infection, lower respiratory tract infection, urinary tract infection, and episodes of pain crisis were particularly emphasized. Subjects were asked specifically if they were taking any nutritional supplements, especially zinc. Zinc supplemented subjects were not eligible for participation in this study.

SCD patients with mild or moderate deficiency of zinc were selected for a zinc supplementation clinical trial. The criteria for deficiency of zinc were decreased zinc level in either lymphocytes ($<50\ \mu g/10^{10}$ cells) or granu-

locytes (<42 μg 10^{10} cells) or both as established by our previous studies [25]. All other subjects with lymphocyte zinc level >50 μg/10^{10} cells or granulocyte zinc >42 μg/10^{10} cells were considered to have normal zinc status.

Evaluation of Infections

The evaluation of subjects included: (i) routine physical examination and routine hematological tests, urinalysis, and SMA-12 laboratory tests every 4 months; and (ii) evaluation at the time of suspected infection. Historical data included past hospitalizations, frequency of transfusions, sexual history, history of drug abuse, and immunization.

Acutely symptomatic, febrile patients were evaluated by an infectious disease consultant who was a blinded observer. Inpatient and outpatient subjects had a complete history and physical examination, blood culture, complete blood count, multiphasic profile, and chest X-ray. Patients with upper respiratory tract symptoms and signs had throat swabs taken for bacterial culture. Patients with lower respiratory symptoms and signs had respiratory secretions stained and cultured for routine bacteria. In those with pneumonia not responding to routine antibiotics, bronchoscopically obtained alveolar lavage fluid was stained for bacteria/mycobacteria and cultured for bacteria, mycobacteria, fungi, and mycoplasma. Patients with symptoms suggestive of urinary tract infection had centrifuged clean catch urine specimens stained and cultured for bacteria. All febrile patients had blood cultures taken.

Outpatient SCD patients were asked to record their daily oral temperatures in the morning, and twice a day if they were experiencing symptoms suggestive of any infection (upper respiratory tract infection with productive or nonproductive cough and/or chest pain, and burning sensation with urination). They were instructed to visit the Infectious Disease Clinic if their oral temperature was greater than 101°C and persisted for more than 2 days.

Evaluation of Acute Vaso-Occlusive (Painful) Crisis

The clinical team responsible for evaluating acute vaso-occlusive pain crises consisted of two hematologists and one physician assistant. The clinical staff were blinded observers. They diagnosed and managed the patients for vaso-occlusive crisis. A painful crisis was defined as a visit to the Detroit Medical Center facility that lasted more than 6 hrs for acute sickling-related pain affecting several sites, which were treated with a parenterally administered narcotic and parenteral fluids.

Assessment of Zinc Status

Zinc was measured in the plasma, granulocytes, and lymphocytes by established techniques in our laboratory before and after zinc supplementation by methods previously published [25,26]. All precautions to avoid contamination during collection, preparation, and analysis were exercised.

We utilized flameless technique for measurement of zinc in neutrophils, lymphocytes and plasma by using a Varian SpectrAA-4D Zeeman atomic absorption spectrophotometer. The triple distilled water for all solution preparation and sample dilutions were processed through a system consisting of ultrapure "demineralizing" cartridges (Sybron Barnstead Co., Boston, MA). This water was essentially ion-free.

The chemicals used were AR grade (Fisher Scientific Co. Fair Lawn, NJ). Standards were prepared from bovine liver (1577A) obtained from National Bureau of Standards (Gaithersburg, MD). Three pools of internal standards consisting of pooled lymphocytes, pooled granulocytes and pooled platelets were used to check for interassay variation.

Lymphocytes and granulocytes were harvested simultaneously by discontinuous gradient using Histopaques 1077 and 1119 (Sigma). Lymphocytes and granulocytes were prepared for zinc analysis by a method published previous [25,26].

Interleukin (IL)-2 Determination

Peripheral blood mononuclear cells (1×10^6 cells/ml) isolated by Histopaque™ (Sigma) density gradient were cultured in RPMI-1640 supplemented with 10% FBS, 50 μM β-mercaptoethanol, 2 mM L-glutamine and 0.1% gentamicin at 37°C under 95% air/5% CO_2. Cells were incubated with 10 μg PHA-P/ml to generate IL-2. Supernatants were harvested after 24 hr and stored at −20°C until assayed.

IL-2 was assayed by commercially available ELISA kits (Quantikine, R&D Systems, Minneapolis, MN). The interassay variation within the ELISA kits was approximately 7.7–9.2% for IL-2. Proper controls and standards were included with each assay.

Zinc Supplementation Trial Design

We screened adult SCA subjects for cellular zinc levels and immunological parameters. In every case, at least two separate determinations were carried out during baseline. We completed a sequential treatment design study of zinc supplementation in 21 zinc-deficient subjects who were divided into two groups. Gr A zinc deficient subjects ($n = 11$) were observed for 1 year (baseline), following which they received zinc as acetate daily orally for 3 years. Six subjects received 50 mg of elemental zinc (25 mg bid) daily for 3 years. Although we instructed our patients to take zinc supplement 1 hr before each meal, we can not be sure if this was fully complied with. Three subjects received 50 mg of elemental zinc daily orally for 2 years and then they were switched to 75 mg of elemental zinc daily orally in the

TABLE I. Effect of Zinc Supplementation on Laboratory Parameters*

	Base period (mean ± SD)	First year (mean ± SD)	Second year (mean ± SD)	Third year (mean ± SD)	Repeated measures analysis: Time (p)	Repeated measures analysis: Time** Grp (p)
Changes in plasma zinc (μg/dl)						
Gr A (11)	82.87 ± 8.3	96.72 ± 15.0	100.18 ± 82.4	111.45 ± 17.2		
Gr B (10)	83.93 ± 15.0	88.66 ± 13.7	95.76 ± 10.9	112.20 ± 20.5	0.0005	NS
Gr C (11)	91.84 ± 12.0	96.80 ± 16.4	102.20 ± 14.1	—		
Changes in lymphocyte zinc ($\mu g/10^{10}$ cells)						
Gr A (11)	47.45 ± 3.6	51.76 ± 3.1	53.20 ± 2.6	55.50 ± 3.2		
Gr B (10)	47.78 ± 3.2	48.35 ± 3.5	53.38 ± 3.0	54.97 ± 3.9	0.0001	0.011
Gr C (11)	50.22 ± 3.5	51.43 ± 4.4	51.65 ± 5.5	—		
Changes in granulocyte zinc ($\mu g/10^{10}$ cells)						
Gr A (11)	40.76 ± 3.4	44.35 ± 4.8	45.45 ± 4.4	45.90 ± 3.3		
Gr B (10)	39.71 ± 4.4	41.77 ± 4.2	46.16 ± 3.1	46.63 ± 3.8	0.0001	0.03
Gr C (11)	42.71 ± 5.3	41.65 ± 3.5	43.46 ± 5.6	—		
Changes in IL-2 production (pg/ml)						
Gr A (11)	272.7 ± 246.7	598.5 ± 544.8	1014.2 ± 365.8	1538.7 ± 1086.8		
Gr B (10)	255.5 ± 290.1	354.6 ± 336.5	975.3 ± 685.8	1428.3 ± 1067.8	0.0001	<0.0001
Gr C (11)	307.7 ± 253.1	245.1 ± 188.6	279.7 ± 255.7	—		

*Gr A, Subjects were followed for 1 year (baseline period) following which they received zinc for 3 years. Gr B, Subjects were followed for 1 year (baseline period) following which they received placebo for 1 year and then zinc for 2 years. Gr C, Subjects received no intervention and were followed for 3 years. NS = not significant.

third year. The patients were instructed to take the additional pill (25 mg of zinc capsule) at bed time. Two subjects received 50 mg of elemental zinc daily orally for the first year and then 75 mg of elemental zinc daily orally for 2 years. Blood samples were obtained at the end of each 12-month period for analysis.

Gr B zinc deficient subjects ($n = 10$) were first followed for 1 year (baseline), and then were given placebo orally for 1 year. Following placebo, five subjects received 50 mg of elemental zinc daily orally for 2 years. The other five subjects in Gr B, following placebo, received 50 mg of elemental zinc orally daily for 1 year and 75 mg of elemental zinc, the next year. Thus, both Gr A and Gr B subjects were followed for 4 years altogether.

Gr C zinc sufficient subjects ($n = 11$) did not receive any intervention. They were followed for 3 years. This group served as zinc normal controls for Grs A and B.

Statistical Analysis

The initial analyses examined the equivalence of the randomized groups. In order to determine the impact of zinc supplementation, paired *t*-tests were used to examine change in the measures from baseline to year 1. The zinc supplemented group was compared to the placebo group. To determine by group over time and for group crossed with time a repeated measures analysis technique was utilized.

RESULTS

Table I shows that during the study period, plasma zinc, lymphocyte zinc, granulocyte zinc, and IL-2 production increased significantly in both Grs A and B. Changes in lymphocyte zinc, granulocyte zinc, and IL-2 production also showed group differences over time which were statistically significant. Gr C showed no significant changes in 3 years in the above parameters. Thus, our interpretation is that the laboratory parameters changed with time due to zinc supplementation.

Table II shows the effect of zinc supplementation on hemoglobin, hematocrit and reticulocyte count. Repeated measures analysis showed no changes in hemoglobin and hematocrit, and a marginal decrease in reticulocyte count in Grs A and B with time was observed ($P = 0.1$). Gr C showed no significant change in any of the above parameters during 3 years.

Table III shows that with time, there was a significant decrease in the incidence of documented and clinical infections in Grs A and B. Documented infections represent those where infections were confirmed with positive bacteriological cultures. The clinical infections were those where the cultures were negative but showed clinical evidence of infection such as upper respiratory tract infections, bronchitis, or pneumonia. In the case of incidence of documented infections, there was also a significant difference among groups over the study period ($P = 0.03$). Analysis of Group (Gr) C showed no statistically significant difference over time. Thus, our interpretation is that the changes observed with time in the incidence of documented infections were related to zinc supplementation in both Grs A and B.

Table IV shows that with time, there was a significant change in the number of hospital admissions and vaso-

TABLE II. Effect of Zinc Supplementation on Hemoglobin, Hematocrit, and Reticulocyte Count*

	Base period (mean ± SD)	First year (mean ± SD)	Second year (mean ± SD)	Third year (mean ± SD)	Repeated measures analysis Time (p)	Repeated measures analysis Time** Grp p
Hemoglobin g %						
Gr A (11)	8.97 ± 0.9	8.84 ± 0.5	8.65 ± 0.8	8.80 ± 1.2		
Gr B (10)	10.24 ± 1.8	9.94 ± 1.6	10.04 ± 1.5	9.75 ± 1.6	0.46	0.60
Gr C (11)	8.53 ± 1.9	8.91 ± 2.2	8.53 ± 2.0	—		
Hematocrit %						
Gr A (11)	26.39 ± 2.9	26.0 ± 2.0	25.39 ± 3.1	26.05 ± 4.4		
Gr B (10)	29.85 ± 5.1	29.17 ± 4.7	29.48 ± 4.2	29.46 ± 4.7	0.76	0.89
Gr C (11)	24.89 ± 5.9	25.46 ± 7.1	25.69 ± 7.3	—		
Reticulocyte %						
Gr A (11)	12.16 ± 7.0	12.66 ± 7.1	11.90 ± 7.7	10.00 ± 5.2		
Gr B (10)	8.96 ± 2.8	9.3 ± 4.2	10.44 ± 6.1	8.18 ± 2.6	0.10	0.54
Gr C (11)	11.44 ± 4.1	10.95 ± 7.6	10.72 ± 7.0	—		

*Gr A, Subjects were followed for 1 year (baseline), following which they received zinc for 3 years. Gr B, Subjects were followed for 1 year (baseline), following which they received placebo for 1 year and then zinc for 2 years. Gr C, Subjects received no intervention and were followed for 3 years.

TABLE III. Effect of Zinc Supplementation on the Incidence of Infections*

	Base period (mean ± SD)	First year (mean ± SD)	Second year (mean ± SD)	Third year (mean ± SD)	Repeated measures analysis Time (p)	Repeated measures analysis Time** Grp (p)
Incidence of documented infections						
Gr A (11)	1.73 ± 1.2	0.36 ± 0.5	0.36 ± 0.5	0.09 ± 0.5		
Gr B (10)	1.70 ± 1.5	1.30 ± 1.4	0.50 ± 0.7	0.30 ± 0.06	0.0026	0.03
Gr C (11)	0.73 ± 0.8	0.64 ± 0.7	0.55 ± 0.5	—		
Incidence of clinical infections						
Gr A (11)	1.27 ± 1.1	0.36 ± 0.6	0.00 ± 0.0	0.00 ± 0.0		
Gr B (10)	0.90 ± 0.9	0.60 ± 0.8	0.10 ± 0.3	0.00 ± 0.0	0.0006	0.09
Gr C (11)	0.27 ± 0.4	0.36 ± 0.5	0.18 ± 0.4	—		

*Gr A, Subjects were followed for 1 year (baseline), following which they received zinc for 3 years. Gr B, Subjects were followed for 1 year (baseline), following which they received placebo for 1 year and then zinc for 2 years. Gr C, Subjects received no intervention and were followed for 3 years.

TABLE IV. Effect of Zinc Supplementation on Hospital* Admission and Vaso-occlusive Pain Crisis

	Base period (Mean ± SD)	First year (Mean ± SD)	Second year (Mean ± SD)	Third year (Mean ± SD)	Repeated measures analysis Time (p)	Repeated measures analysis Time**Group (p)
Number of hospital admissions						
Gr A (11)	7.18 ± 3.4	6.90 ± 3.6	4.72 ± 3.4	3.36 ± 3.0		
Gr B (10)	7.20 ± 6.3	8.00 ± 5.2	5.10 ± 4.5	3.20 ± 2.9	0.0001	0.07
Gr C (11)	4.54 ± 4.4	5.36 ± 5.3	4.54 ± 4.3	—		
Vaso-occlusive pain critis						
Gr A (11)	7.00 ± 3.3	6.72 ± 3.6	4.46 ± 3.4	3.27 ± 2.9		
Gr B (10)	6.70 ± 6.5	7.60 ± 5.4	4.90 ± 4.3	2.80 ± 2.7	0.0001	0.09
Gr C (11)	4.45 ± 4.5	5.18 ± 4.8	4.36 ± 4.5	—		

*Gr A, Subjects were followed for one year (baseline), following which they received zinc for 3 years. Gr B, Subjects were followed for 1 year (baseline), following which they received placebo for 1 year and then zinc for 2 years. Gr C, Subjects received no intervention and were followed for 3 years.

TABLE V. Effect of Zinc Supplementation on Various Parameters Comparison of Zinc vs. Placebo During the First Year*

	Base period	First year	Paired *t*-test (*p*)
Lymphocyte zinc			
Gr A (11)	47.45 ± 3.6	51.76 ± 3.1	0.03
Gr B (10)	47.78 ± 3.2	48.35 ± 3.6	NS
Gr C (11)	50.22 ± 3.6	51.43 ± 4.4	NS
Incidence of documented infections			
Gr A (11)	1.73 ± 1.2	0.36 ± 0.5	0.0038
Gr B (10)	1.70 ± 1.5	1.30 ± 1.4	NS
Gr C (11)	0.73 ± 0.8	0.64 ± 0.7	NS
Incidence of clinical infections			
Gr A (11)	1.27 ± 1.1	0.36 ± 0.7	0.024
Gr B (10)	0.90 ± 0.9	0.60 ± 0.8	NS
Gr C (11)	0.27 ± 0.5	0.36 ± 0.5	NS
Number of hospital admissions			
Gr A (11)	7.18 ± 3.4	6.90 ± 3.6	NS
Gr B (10)	7.20 ± 6.3	8.00 ± 5.2	NS
Gr C (11)	4.54 ± 4.4	5.36 ± 5.3	NS
Vaso-occlusive pain crisis			
Gr A (11)	7.00 ± 3.3	6.72 ± 3.6	NS
Gr B (10)	6.70 ± 6.6	7.60 ± 5.4	NS
Gr C (11)	4.45 ± 4.5	5.18 ± 4.8	NS

*Gr A, Subjects were followed for 1 year (baseline period) following which they received zinc for 1 year. Gr B, Subjects were followed for 1 year (baseline period) following which they received placebo for 1 year. Gr C, Subjects received no intervention.

TABLE VI. Documented Pathogens and Sites of Infection in Different Groups

Gr A		Gr B		Gr C	
Baseline	1 yr Zinc	Baseline	1 yr Placebo	Baseline	1 yr Control
8 *S. aureus* [7-Pneumonia (blood culture positive in 2 cases)] [1-Tonsillitis]	3 *S. aureus* [3-Pneumonia]	4 *S. aureus* [2-Tonsillitis] [2-Bacteremia]	9 *S. aureus* [3-Pneumonia (blood culture positive in 2 cases)] [6-Tonsillitis]	3 *S. aureus* [3-Pneumonia]	2 *S. aureus* [1-Pneumonia] [1-Tonsillitis]
2 *Strep. pneumoniae* [2-Tonsillitis]		4 *Strep. pneumonia* [4-Pneumonia]	3 *Strep. pyogenes* [3-Tonsillitis]	1 *Strep. pneumoniae* [1-Pneumonia]	1 *Strep. pneumoniae* [1-Pneumonia]
5 *E. Coli* [5-Urinary tract inf.]	1 *Strep. agalactia* [1-Urinary tract inf.]	3 *E. Coli* [3-Urinary tract inf.]	1 *N. gonorrhea*	3 *E. Coli* [3-*Urinary tract inf.]*	3 *E. Coli* [3-Urinary tract inf.]
1 *K. pneumoniae* [1-Urinary tract inf.]		1 *P. mirabilis* [1-Urinary tract inf.]			1 *K. pneumoniae* [1-Leg wound infection]
2 *Candida albicans* [2-Pharyngitis]		3 *Strep. pyrogenes* [3-Tonsillitis]		1 *P. aeroginosa* [1-Leg wound infection]	
1 *Chicken pox*		2 *H. influenza* [1-Tonsillitis] [1-Sinusitis]			

occlusive pain crisis in Grs A and B. The group differences over time with respect to hospital admission and vaso-occlusive pain crisis were of borderline significance ($P = 0.07$ and $P = 0.09$, respectively).

Gr A subjects ($n = 11$) received 50 mg of elemental zinc daily orally for 1 year following 1 year of baseline observation, whereas Gr B subjects ($n = 10$) received placebo daily orally for 1 year. At the end of 1 year of supplementation, the data were compared and analyzed by paired *t*-test. Statistically significant differences were observed for lymphocyte zinc and incidence of documented and clinical infections but no differences were seen with respect to number of hospital admissions or number of vaso-occlusive pain crisis (Table V). We interpret this to mean that whereas changes in the incidence of infections and lymphocyte zinc were observed with 50 mg of elemental zinc supplementation per day for 1 year, this dosage and duration were not sufficient to show any effect on vaso-occlusive pain crisis.

The mean incidence of documented and clinical infec-

TABLE VII. Effect of Different Doses of Zinc Supplementation on Number of Hospital Admissions and Vaso-Occlusive Pain Crisis*

	Base period (mean ± SD)	First year (mean ± SD)	Second year (mean ± SD)	Third year (mean ± SD)	Repeated measures analysis: Time (p)	Repeated measures analysis: Time**Group (p)
Number of hospital admissions						
Doses*						
0/0/50/50 (5)	11.2 ± 6.8	11.8 ± 4.3	8.8 ± 3.3	5.4 ± 2.4		
0/0/50/75 (5)	2.2 ± 1.7	4.2 ± 2.2	1.4 ± 0.8	1.4 ± 1.2	0.0003	0.001
0/50/50/50 (6)	6.0 ± 3.8	6.3 ± 3.6	4.5 ± 2.9	4.5 ± 3.4		
Number of vaso-occlusive pain crises						
0/0/50/50 (5)	10.8 ± 7.1	11.8 ± 4.3	8.4 ± 3.1	5.0 ± 2.1		
0/0/50/75 (5)	2.6 ± 2.0	3.4 ± 1.5	5.1 ± 0.8	0.6 ± 0.8	<0.0001	<0.03
0/50/50/50 (6)	6.0 ± 3.8	6.3 ± 3.6	4.3 ± 4.2	3.8 ± 3.4		

*0/0/50/50, following base period placebo the first year, 50 mg of elemental zinc the other 2 years. 0/0/50/75, following base period placebo the first year, 50 mg of elemental zinc the second year and 75 mg elemental zinc the third year. 0/50/50/50, following base period 50 mg of elemental zinc all 3 years.

tions, hospital admission and vaso-occlusive pain crisis was not changed at the end of the first year in Gr C subjects of observation.

Table VI shows the patterns of documented infections. Respiratory tract and urinary tract were most commonly affected. The predominant pathogens isolated were staphylococci and streptococci involving the respiratory tract and aerobic gram-negative bacteria, particularly *E. coli*, involving the urinary tract.

Table VII shows the effect of different doses of zinc supplementation on the number of hospital admissions and vaso-occlusive pain crisis. Over time, there was a significant change in the number of hospital admissions and the incidence of vaso-occlusive pain crisis in SCD patients. There were also significant group differences over time with respect to the above parameters. Our interpretation is that with 50 mg of elemental zinc supplementation for one year and 75 mg of elemental zinc supplementation for an additional year and 3 years of 50 mg of elemental zinc supplementation to SCD patients resulted in a significant reduction in the incidence of vaso-occlusive pain crisis in the last year of observation. The reduction in the incidence of vaso-occlusive pain crisis following 3 years of 50 mg of elemental zinc daily supplementation in the last year was 12 to 40% compared to the previous year, whereas following the supplementation of 50 mg of elemental zinc daily for 1 year and then increasing the dose of 75 mg of elemental zinc daily for an additional year, the incidence of vaso-occlusive pain crisis decreased almost 80% in SCD patients in comparison to the incidence in the previous year. In the repeated measures analysis, subjects who received 50 mg of elemental zinc for 2 years and 75 mg of elemental zinc in the third year, ($n = 3$) and subjects who received 50 mg the first year and 75 mg of elemental zinc for two years, ($n = 2$) were excluded because of small numbers.

We encountered no side-effects due to zinc supplementation in this study. Plasma copper levels showed no decrease due to zinc supplementation (data not shown).

DISCUSSION

Zinc deficiency was first recognized in humans in 1963 [27], and is now known to be fairly widespread throughout the world [28]. In the Middle East, we observed that most of the zinc deficient dwarfs did not survive beyond the age of 25 and we were told by local physicians that pneumonia, parasitic diseases, or viral infections were responsible for their deaths. Several abnormalities of cell-mediated immunity have been observed in zinc-deficient humans [28–30]. An extreme example of the effects of zinc deficiency on the human immune system is acrodermatitis enteropathica, a genetic disorder of zinc malabsorption [28]. This condition is characterized by mucocutaneous lesions, diarrhea, failure to thrive, and frequent severe infections with fungi, viruses, and bacteria. Affected subjects have decreased thymic hormone activity. All these changes are corrected by zinc supplementation [28].

A mild deficiency of zinc which is widespread and prevalent throughout the world is associated with decreased thymulin activity, reduced ratio of CD4+/CD8+ T-cells, decreased TH_1 function and decreased production of IL-2 [10]. All these abnormalities are corrected by zinc repletion [10]. Inasmuch as IL-2 plays a central role in the expansion and maintenance of thymocytes and peripheral T-cell populations, the generation of anti-viral and anti-tumor specific cytotoxic T-cell, delayed type hypersensitivity responses and up-regulation of NK lytic activity implies that mild zinc deficiency could also lead to increased susceptibility to infections.

In this study microbial culture-validated infection data

were obtained for bacterial infections but not for viral infections. Our results showed a highly significant effect of zinc supplementation on reduction of incidence of bacterial infections in SCD patients. This may be because zinc affects multiple aspects of the immune system. Zinc deficiency affects adversely TH_1 cytokine production, B lymphocyte development, and antibody production, as well as immunological functions of macrophage [31]. In addition to its effects on bacterial infections, we also observed a decrease in the incidence of clinical infections, mainly representing episodes of acute bronchitis, upper respiratory tract infections, and pneumonia, with negative culture reports, suggesting that these infections may represent viral etiology. Viral infections may trigger acute episodes of pain, bone marrow aplasia, and splenic sequestrations of red cells in SCD patients [20,22,24].

Zinc supplementation in zinc deficient subjects (Grs A and B), resulted in an increase in zinc concentration in the plasma and cells, an increase in IL-2 production, and a decrease in the incidence of infections. Gr C represented normal zinc status SCD subjects and in these subjects the incidence of infections, number of hospital admissions and number of vaso-occlusive pain crises were much less in comparison to the subjects in Grs A and B. Thus, our results of zinc supplementation and a follow up of zinc normal SCD subjects suggest that it is clinically beneficial to maintain a normal zinc status in SCD patients.

Several reports have shown that in addition to erythrocytes, endothelial cells and leucocytes play important roles in the onset and maintenance of vaso-occlusion in sickle cell disease [32,33]. Cytokines, coagulation factors, and atherogenic factors such as homocysteine also seem to play roles in vaso-occlusive events [32].

Soluble adhesion molecules are released by endothelial cells upon pro-inflammatory cytokine stimulation [34,35]. The observation that asymptomatic Hb SS and Hb SC patients have increased levels of soluble vascular cell adhesion molecule-1 is, therefore, an indication of a persistent activation of endothelial cells. When activated, endothelial cells increase their surface expression of adhesion molecules and, thereby, promote sickle red blood cell and leucocyte adherence [34,35]. Inasmuch as zinc is known to decrease the production of IL-1β, a pro-inflammatory cytokine in humans [29], we speculate that zinc supplementation may result in decreased levels of soluble vascular cell adhesion molecule-1 leading to a decrease in the incidence of vaso-occlusive pain crisis in SCD patients.

It is possible that some of the clinically beneficial results of zinc supplementation observed here in SCD patients may have been due to pharmacological effects of zinc rather than to the effects of simply correcting intracellular zinc deficiency. For example, in this study we observed that administration of 75 mg of elemental zinc daily, a dose 5 times the RDA, induced an increase in IL-2 production in Grs A and B which was considerably greater than which we observed in normal non-zinc-supplemented control subjects. This raises the possibility that supranormal zinc supplementation induces greater than normal cytokine production.

Brewer et al. [36] observed that zinc supplementation in higher dosage (25 mg of elemental zinc every 4 hrs) decreased significantly the number of irreversible sickle cells and the incidence of vaso-occlusive pain crisis was decreased in patients with SCD. They related this effect of zinc to an inhibition of calmodulin activity and calcium leakage inside the sickle cells.

The only toxicity of high level of zinc supplementation known is that it results in copper deficiency but this is easily correctable by oral copper administration in RDA amount. We have monitored plasma copper levels and have not observed any decrease in our subjects due to zinc supplementation. We monitored both plasma and cellular zinc levels, which increased significantly in zinc-supplemented subjects, indicating that patients were indeed taking their zinc capsules. However, a lack of reciprocal effect on copper levels suggests that our patients probably took zinc with their meals in spite of our directions. Zinc can be administered to pregnant women also inasmuch as it is not mutagenic.

We conclude from the results of the pilot study described here that long term zinc supplementation is a safe therapeutic modality for treatment of adult SCD patients which may decrease the incidence of infections, vaso-occlusive pain crises, and hospital admissions. Confirmation of this conclusion will require further prospective studies of zinc supplementation is SCD patients by using larger numbers of test subjects.

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REFERENCES

1. Prasad AS, Schoomaker EB, Ortega J, Brewer GJ, Oberleas D, Oelshlegel FJ. Zinc deficiency in sickle cell disease. Clin Chem 1975; 21:582–587.
2. Yuzbasiyan-Gurkan VA, Brewer GJ, Vander AJ, Guenther MJ, Prasad AS. Net renal tubular reabsorption of zinc in healthy man and impaired handling in sickle cell anemia. Am J Hematol 1989;31:3187–3190.
3. Prasad AS, Cossack ZT. Zinc supplementation and growth in sickle cell disease. Ann Intern Med 1984;100:367–371.
4. Prasad AS, Abbasi AA, Rabbani P, DuMouchelle E: Effect of zinc supplementation on serum testosterone level in adult male sickle cell anemia subjects. Am J Hematol 1981;10:119–127.

5. Warth JA, Prasad AS, Zwas F, Frank RN. Abnormal dark adaptation in sickle cell anemia. J Lab Clin Med 1981;98:189–194.
6. Prasad AS, Rabbani P, Warth JA. Effect of zinc on hyperammonemia in sickle cell anemia subjects. Am J Hematol 1979;7:323–327.
7. Tapazoglou E, Prasad AS, Hill G, Brewer GJ, Kaplan J. Decreased natural killer cell activity in patients with zinc deficiency with sickle cell disease. J Lab Clin Med 1985;105:19–22.
8. Ballester OF, Abdallah JM, Prasad AS. Impaired IgM antibody responses to an influenza virus vaccine in adults with sickle cell anemia. Am J Hematol 1985;20:409–412.
9. Ballester OF, Abdallah JM, Prasad AS. Lymphocyte subpopulation abnormalities in sickle cell anemia. Am J Hematol 1986;21:23–27.
10. Prasad AS, Meftah S, Abdallah J, Kaplan J, Brewer GJ, Bach JF, Dardenne M. Serum thymulin in human zinc deficiency. J Clin Invest 1988;82:1202–1210.
11. Beck FWJ, Kaplan J, Fine N, Handscheu W, Prasad AS. Decreased expression of CD73 (Ecto-5′-nucleotidase) in the CD8+ subset is associated with zinc deficiency in humans. J Lab Clin Med 1997;130: 147–156.
12. Phebus CK, Maciak BJ, Gloninger MF, Paul HS. Zinc status of children with sickle cell disease: Relationship to poor growth. Am J Hematol 1998;29:67–73.
13. Niell HB, Leach BE, Kraus AP. Zinc metabolism in sickle cell anemia. JAMA 1979;242:2686–2687.
14. Karayalcin G, Lanzkowsky P, Kazai AB. Zinc deficiency in children with sickle cell disease. Am J Pediatr Hematol Oncol 1979;1:283–284.
15. Russell MO, Finan A, Moskowitz SR, Elmer MA, Gill FM. Nutrition and poor growth in pre-adolescent children with sickling disorders. Pediatr Res 1981;15:586.
16. Daeschner CW, Matusik MC, Carpentieri U, Haggard ME. Zinc and growth in patients with sickle cell disease. J Pediatr 1981;98:778–780.
17. Barrett-Conner E. Bacterial infection and sickle cell anemia. Medicine 1971;50:97–112.
18. Hook EW, Campbell CG, Weens HS, Cooper GR. Salmonella osteomyelitis in patients with sickle cell anemia. N Engl J Med 1957;257: 403–407.
19. Onwubalili JK. Sickle cell disease and infection. J Infect 1983;7:2–20.
20. Buchanan GR. Infection. In Sickle Cell Disease. Embury SH, Hebbel RP, Mohandas N, Steinberg MH, editors. New York: Raven Press; 1994. pp. 567–588.
21. Shulman ST, Barlett J, Clyde WA, Ayoub EM. The unusual severity of mycoplasma pneumonia in children with sickle cell disease. N Engl J Med 1972;287:164.
22. Sergeant GR, Sergeant BE, Thomas PW, Anderson MJ, Patou G, Pattison JR: Human parvo-virus infection in homozygous sickle cell disease. Lancet 1993;341:1237–1240.
23. Hardy RE, Cummings C, Thomas F, Harrison D. Cryptococcal pneumonia in a patient with sickle cell disease. Chest 1986;89:892–895.
24. Haddad JD, John JF, Pappas AA: Cytomegalovirus pneumonia in sickle cell disease. Chest 86:265–266, 1984.
25. Meftah S, Prasad AS, Lee D, Brewer GJ. Ecto 5′ nucleotidase (5′NT) as a sensitive indicator of human zinc deficiency. J Lab Clin Med 1991;118:309–319.
26. Wang H, Prasad AS, DuMouchelle E. Zinc in platelets, lymphocytes, and granulocytes by flameless atomic absorption spectrophotometry (AAS). J Micro Nutr Anal 1989;5:181–190.
27. Prasad AS, Miale A Jr., Farid Z, Sandstead H, Schulert A. Zinc metabolism in patients with syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism and hypogonadism. J Lab Clin Med 1963; 61:537–549.
28. Prasad AS: Clinical spectrum of human zinc deficiency. In Prasad AS. Biochemistry of zinc. New York: Plenum; 1993. pp. 219–258.
29. Beck FWJ, Prasad AS, Kaplan J, Fitzgerald JT, Brewer GJ. Changes in cytokine production and T-cell subpopulations in experimentally induced zinc deficient humans. Am J Physiol 1997;272:E1002–E1007.
30. Ballester OF, Prasad AS. Anergy, zinc deficiency and decreased nucleoside phosphorylase activity in patients with sickle cell anemia. Ann Intern Med 1983;98:180–182.
31. Shankar AH, Prasad AS. Zinc and immune function: The biological basis of altered resistance to infection. Am J Clin Nutr 1998;68(suppl): 4475–635.
32. Schnog JB, Lard LR, Rojer RA, Vander Dijs FPL, Muskiet FAJ, Duits AJ. New concepts in assessing sickle cell disease severity. Am J Hematol 1996;58:61–66.
33. Smolinski PA, Offermann MK, Eckman JR, Wick TM. Double-stranded RNA induces sickle erythrocyte adherence to endothelium: A potential role for viral infection in vaso-occlusive pain episodes in sickle cell anemia. Blood 1995;85:2945–2950.
34. Hebbel RP, Vercellotti GM. The endothelial biology of sickle cell disease. J Lab Clin Med 1997;129:288–293.
35. Solovey A, Gui L, Key NS, Hebbel RP. Tissue factor expression by endothelial cells in sickle cell anemia. J Clin Invest 1998;101:1899–1904.
36. Brewer GJ, Brewer LF, Prasad AS. Suppression of irreversible sickled erythrocytes by zinc therapy in sickle cell anemia. J Lab Clin Med 1977;90:549–554.