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Copper Supplementation in Parenteral Nutrition of Cholestatic Infants

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Abstract

Background—Conventional practice is to reduce or eliminate copper supplementation in the parenteral nutrition of infants with cholestasis due to the increased risk of hepatotoxicity. However, there are reports of copper deficiency in cholestatic infants due to copper reduction in their parenteral nutrition.

Objectives—1) To determine the proportion of cholestatic infants who develop elevated serum copper while receiving a non-reduced dose of parenteral copper, 2) To evaluate potential clinical factors that affect serum copper in cholestatic infants, and 3) To evaluate the impact of serum copper on liver disease.

Methods—This is a retrospective review of 28 cholestatic infants receiving 20 mcg/kg/d of copper via parenteral nutrition. Age-adjusted references were used to determine normality of serum copper levels. Multiple linear regression analyses were performed to determine predictors of serum copper and alanine aminotransferase.

Results—Serum copper levels were elevated in 2 infants (7%). On average, infants received 80% of their energy intake from parenteral nutrition for 3 months. Intestinal failure was present in 50% of the patients. Birth weight, gestational age and alanine aminotransferase were identified as predictors of serum copper ($R^2=0.53$; $p=0.0001$). Serum copper, gestational age and total bilirubin were associated with serum alanine aminotransferase ($R^2=0.43$; $p=0.001$).

Conclusion—Supplementation of parenteral copper at 20 mcg/kg/day does not lead to a significant increase in copper toxicity or worsening of liver disease in cholestatic infants.

Keywords

Copper supplementation; infant; copper toxicity; cholestasis; parenteral nutrition

Introduction

There is currently no evidence-based approach to the supplementation of copper (Cu) in the parenteral nutrition of cholestatic infants. Initial reports described Cu deficiency in low birth weight neonates, children recovering from malnutrition, and those receiving Cu-free parenteral

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nutrition (1). These infants had anemia, neutropenia, bone disease (osteopenia and fractures), and growth retardation (1). As a result, Cu supplementation of parenteral nutrition at a dose of 20 mcg/kg/day has been recommended for both preterm and term infants (2). This dose prevents acute deficiencies but is significantly lower than the preterm infant's actual need of 63 mcg/kg/day to achieve in utero accretion rates (3). The conventional clinical practice in most institutions is to omit or reduce Cu in the parenteral nutrition solution in the event of cholestasis in an attempt to reduce the accumulation of Cu in the liver and decrease the risk for hepatotoxicity. Cu deficiency has been reported primarily in premature cholestatic infants with intestinal failure receiving a reduced amount of Cu in their parenteral nutrition (4).

Premature infants have reduced hepatic Cu stores as two-thirds of Cu accumulates during the third trimester of gestation and their requirements are higher due to accelerated growth (5). Patients with intestinal failure have increased Cu losses through the gastrointestinal tract and the American Society for Clinical Nutrition recommends giving these patients an additional 10–15 mcg/kg/d of Cu in their parenteral nutrition (2). Premature infants and those with intestinal failure, often require long-term parenteral nutrition and subsequently develop cholestasis. These children are at risk for Cu deficiency because of reduced Cu stores and increased intestinal losses. An additional risk factor for Cu deficiency is the relatively high enteral intake of zinc and iron which competes with Cu absorption (5). As a result, reducing parenteral Cu supplementation may increase the risk of Cu deficiency in this vulnerable population.

Investigators have not evaluated the prevalence of Cu deficiency in cholestatic infants who have their parenteral Cu supplementation withheld or reduced. The standard of practice at our facility is to continue to supplement the recommended daily dose of 20 mcg/kg/day of Cu in parenteral nutrition despite cholestasis and to monitor serum Cu levels monthly. Our study objectives were to: 1) determine the proportion of cholestatic infants who develop elevated serum copper while receiving a non-reduced dose of parenteral copper, 2) evaluate potential clinical factors that affect serum copper in cholestatic infants, and 3) evaluate the impact of serum copper on liver disease.

Materials and Methods

Study Design

The institutional review boards of Children's Healthcare of Atlanta (CHOA) and Emory University approved the study. A retrospective chart review of infants diagnosed with cholestasis between January 2003 and May 2005 was conducted. Patients were identified from infants on long-term parenteral nutrition followed by the Intestinal Rehabilitation team at CHOA during the period under consideration.

Study Cohort

CHOA at Egleston Hospital is a tertiary care facility with a level IV NICU. Infants with serum Cu measured were included in the analysis if they: 1) were less than 1-year-of-age, 2) had cholestasis defined by a direct bilirubin of > 2 mg/dL, 3) received Cu in their parenteral nutrition at a dose of 20 mcg/kg/day, 4) received more than 50% of their caloric intake from parenteral nutrition, and had been on parenteral nutrition for at least a month. Infants were excluded if: 1) the cholestasis was attributed to causes other than parenteral nutrition such as metabolic disorders and primary liver disease. This was done by reviewing the chart to identify if another diagnosis was identified for cholestasis in these infants, 2) the amount of Cu in the parenteral nutrition was not available for review, e.g., if the patient was transferred from another institution, and 3) the parenteral nutrition was interrupted longer than 7 days for various reasons during the reported total duration of parenteral nutrition for each patient.

Data Collection and Definitions

Demographic information including birth weight, gender, gestational age at birth, and postmenstrual age at the time of serum Cu measurement were abstracted. Postmenstrual age was reported in weeks and represented gestational age plus chronological age (6). Other pertinent data such as a previous episode of sepsis, the duration these infants received parenteral Cu, and the percentage of their energy intake from parenteral nutrition were recorded. Sepsis was defined as a culture-positive infection of the blood, peritoneum, or the urinary tract. Duration of parenteral nutrition was the period of time these infants were receiving parenteral nutrition prior to serum Cu level measurements. The amount of enteral Cu intake was not calculated since the coefficient of absorption of Cu would be affected by the underlying malabsorptive condition of the patients and by the amount of zinc and iron supplementation they received. The enteral losses of Cu could not be accounted for in a retrospective analysis. Serum Cu was chosen as the primary outcome variable. Although direct bilirubin was chosen to define cholestasis, ALT was chosen as a secondary outcome variable to assess the potential hepatotoxic effect of elevated serum Cu. All bilirubin and ALT levels that were included in the analysis were done on the same day of serum Cu measurements.

Infants were categorized as having intestinal failure or not, since this condition is known as an additional risk factor for Cu deficiency (2). Intestinal failure was defined as a critical reduction of functional intestinal mass resulting in long-term parenteral nutrition dependency for more than 90 days (7,8). Short bowel syndrome, chronic enteropathy of infancy (one patient had microvillus inclusion disease and another patient had auto-immune enteropathy), and gastroschisis were the causes of intestinal failure in this group of infants. Patients without intestinal failure had multiple co-morbid conditions that resulted in long-term parenteral nutrition. These conditions included congenital heart disease, severe chronic lung disease, medically treated necrotizing enterocolitis, resection of a short segment of bowel (< 20 cm) secondary to isolated intestinal perforation, or necrotizing enterocolitis.

Statistical Analysis

Descriptive statistics (mean $\pm$ standard deviation) are used to report the demographic and clinical characteristics of our cohort. Age-adjusted references were used to define normality of serum Cu levels. All serum Cu levels were sent in metal free (blue-top) vacutainers to the same laboratory (Arup Laboratories, Salt Lake City, Utah). This laboratory measures serum Cu by inductively coupled plasma-mass spectrometry (ICP-MS). The proportion of patients with elevated serum Cu was calculated. Baseline characteristics of infants with intestinal failure and infants who did not have intestinal failure were compared by a 2-tailed unpaired t-test.

Pearson correlation analysis was used to test the association between serum Cu, ALT, duration of parenteral nutrition, percentage of energy intake from parenteral nutrition, and demographics such as birth weight and post-menstrual age. Multiple linear regression analyses were performed to determine predictors of serum Cu levels and ALT. The regression equation was performed using backward elimination of variables. The independent variables that were included in the model were: gender, birth weight, postmenstrual age, duration of parenteral nutrition, percentage of caloric intake from parenteral nutrition, a previous episode of sepsis, total bilirubin, serum albumin, and serum Cu or ALT, respectively. Bilirubin was not fractionated in all of the infants when serum Cu measurements were obtained; therefore, total bilirubin was included in the model instead of direct bilirubin.

Results

Thirty-one cholestatic infants with a documented serum Cu level were identified during the period under review. Of these, 3 did not meet 1 or more of the inclusion criteria. Consequently,

28 infants were included in the analysis. Ten of the 28 infants had multiple serum Cu evaluations and the total number of observations analyzed was 47. The mean gestational age was 30 ± 4.5 weeks; mean birth weight was 1550 ± 936 grams. Infants were receiving a mean of 80 % of their caloric intake from parenteral nutrition and the mean duration of parenteral nutrition was greater than 3 months with a range between 41 and 261 days (Table 1). In the neonatal intensive care unit, patients routinely received 80–100% of the parenteral nutrition volume containing 20 mcg/kg/d of Cu thus each child had an intake of 16–20 mcg/kg/day of parenteral Cu. Two measurements of serum Cu were elevated out of 47 observations (4.2%). These observations occurred in 2 infants (7%). Seventeen patients (60 %) had a documented septic episode prior to serum Cu measurement. The interval between a septic episode and serum Cu level measurement varied between 2 and 126 days with a mean of 37.5 days. Of the 2 patients with elevated serum Cu, 1 patient had a septic episode 3 weeks prior to serum Cu measurement. This patient was subsequently weaned off parenteral nutrition and a follow-up Cu level was not done. The second patient did not have a documented septic episode and the repeat Cu level was 95 mcg/dl, (normal for age), after receiving a reduced dose for 28 days.

Postmenstrual age did not have any relationship with serum Cu ($r = 0.29$; $p = 0.1$). Serum ALT and birth weight correlated with serum Cu ($r = 0.5$, $p = 0.005$; $r = 0.53$, $p = 0.005$, respectively) in this cohort of cholestatic infants. The association between serum Cu, ALT, and birth weight were further verified by multiple regression analysis. Birth weight and ALT were identified as positive predictors of serum Cu whereas gestational age was a negative predictor of serum Cu (adjusted $R^2 = 0.53$; $p < 0.001$). The duration of parenteral nutrition (Figure 1) and the degree of cholestasis as assessed by direct bilirubin did not explain any of the variance of serum Cu.

Analysis of data in patients with serial Cu measurements did not reveal a significant difference between mean serum Cu levels of patients whose liver disease improved with time ($\text{Cu} = 62 \pm 35$ mg/dl) and patients whose liver disease deteriorated over time ($\text{Cu} = 75 \pm 19.7$ mg/dl, $p = 0.2$). Initial higher serum copper values did not account for progression of liver disease, assessed by the rate of rise of direct bilirubin levels. This was evaluated by paired t-test. The mean initial serum Cu level in patients whose liver disease worsened ($\text{Cu} = 53 \pm 17$) was lower than patients whose liver disease improved with time ($\text{Cu} = 65 \pm 7.4$) ($p = 0.04$).

Half (50%) of the enrolled children in this study had intestinal failure. When compared to infants without intestinal failure, those with intestinal failure were older, had received parenteral nutrition for a longer period of time, and had higher serum Cu and ALT (Table 2). There were no differences in total or direct bilirubin levels between both groups of patients.

There was a significant correlation between ALT and serum Cu ($r = 0.5$, $P = 0.005$). The multiple linear regression analysis with ALT as the dependent variable revealed that serum Cu level, gestational age, and total bilirubin accounted for 43 % of the variation of ALT (adjusted $R^2 = 0.43$; $p = 0.001$) (Table 3).

Discussion

The current clinical practice in most institutions is to reduce or omit parenteral Cu in cholestatic infants due to Cu's potential hepatotoxicity, following recommendations made over 20 years ago and based on limited data (2). The amount of Cu provided should be sufficient to prevent clinical Cu deficiency and not cause liver damage. In the absence of guidelines, Cu supplementation of parenteral nutrition in cholestatic infants continues to vary between institutions.

Cu deficiency has been reported primarily in cholestatic premature infants with intestinal failure who had the Cu dose reduced in their parenteral nutrition and developed neutropenia, anemia, red cell transfusion dependency, and pathologic fractures due to osteopenia (4). In the

neonatal intensive care unit of our institution, a cholestatic preterm infant who became transfusion dependent was diagnosed with Cu deficiency; this infant was not receiving Cu in his parenteral nutrition. As a result, the practice to continue Cu supplementation at a standard dose after the infant develops cholestasis was instituted. All cholestatic infants in this cohort were to receive 20 mcg/kg/d of Cu in their parenteral nutrition. In practice, these infants received at least 16–20 mcg/kg/d of Cu as their feeds were advanced. This is in contrast with the practice of omitting or reducing Cu in parenteral nutrition, which would result in no Cu intake or a dose of < 10 mcg/kg/d. In the current study, we evaluated this practice and report the proportion of cholestatic infants with elevated serum Cu who continue to receive a regular dose of Cu in their parenteral nutrition. Only 2 of 28 infants (7%) developed elevated serum Cu level, a rate considerably less than what might be anticipated. Serum Cu does increase with inflammatory states and previous septic episodes in our patient cohort could have falsely elevated serum Cu levels. In this study, ceruloplasmin levels and free Cu were not measured. Free copper has been proposed as a marker of Cu toxicity by some investigators (9). Therefore, the percentage of patients with Cu toxicity may be underestimated in our cohort since Cu status was assessed with serum copper.

Birth weight, ALT and gestational age had significant associations with serum Cu in cholestatic infants. In healthy infants, serum Cu level at birth is known to correlate with birth weight and gestational age (10). The negative association between Cu and gestational age may be due to the degree of prematurity in this cohort and the presence of other co-morbidities.

In the infants with intestinal failure, serum Cu level and ALT were significantly higher than in those infants without intestinal failure ($p = 0.001$). Patients with chronic diarrhea have increased fecal Cu losses and other factors appear to counteract this effect (1). Patients with abnormalities of liver excretory function have decreased gastrointestinal Cu losses, which can account for the elevated serum Cu in patients with intestinal failure and liver disease (11). Infants with intestinal failure were older than infants without intestinal failure. Therefore, age may seem a potential confounder that could account for a higher serum Cu in patients with intestinal failure. However, an increase in serum Cu with age was not observed in cholestatic infants ($r = 0.32$; $p = 0.1$). Infants with intestinal failure received parenteral nutrition for a longer period of time, but duration of parenteral nutrition was not associated with serum Cu or ALT by multiple linear regression analysis.

Initial higher serum copper values were not associated with the progression of liver disease, assessed by the rate of rise of direct bilirubin levels in the group of patients with serial Cu measurements. Copper toxicity is known to cause hepatotoxic cell injury, manifested mainly with increased ALT. Serum and hepatic Cu have been observed to be elevated in adult patients with liver diseases (12,13). However, increased hepatic Cu content is not always equivalent to Cu-induced liver toxicity which is factor of sub cellular compartmentalization and molecular association (14). Total Cu content of the liver of term infants is 10 times that of the adult and is similar to that found in patients with Wilson's disease and biliary cirrhosis (15). This increased neonatal liver Cu content is physiologic and necessary to overcome the negative Cu balance in the early postnatal period (14). Premature infants have decreased hepatic Cu stores and remain in negative Cu balance even when they develop liver disease. This could potentially explain the Cu deficiency that develops when preterm infants receive reduced doses of Cu in their parenteral nutrition.

In this study, factors that affect the amount of Cu delivered to cholestatic infants such as the duration of parenteral nutrition and the percentage of parenteral caloric intake did not influence serum Cu level (Figure 1). Therefore, reducing the amount of parenteral Cu in these infants may not be necessary and could be harmful because it increases their risk for Cu deficiency.

This study evaluated the effect of parenteral Cu supplementation on Cu status and liver disease. Moreover, a different approach to Cu supplementation was evaluated, challenging the current clinical practice of omitting or reducing parenteral Cu dosage in the event of cholestasis in premature infants. This cohort included patients who have been on parenteral nutrition for at least 30 days. Therefore, the analysis did not include sick preterm infants with cholestasis who died in the first 30 days of life. This may have contributed to the lack of elevated serum Cu in our cohort. Our study had a small sample size and lacked a control group. However, we targeted a population of infants to evaluate an area that is not well investigated. This study will serve as the basis for future prospective research studies that can answer more specifically and more rigorously the parenteral Cu requirements of cholestatic infants.

Our cohort of patients received most of their caloric intake from parenteral nutrition (mean 80%) for longer than 1 month. Therefore, the results obtained in this study reflect the effects of parenteral Cu on Cu status. We controlled for confounders to the association between serum Cu and ALT. These confounders include sepsis, age, duration of parenteral nutrition, and percentage of energy intake from parenteral nutrition.

Summary

Our study findings suggest that supplementation of parenteral Cu at a regular dose does not lead to a significant increase in Cu toxicity or worsening of liver disease in cholestatic infants. Scientific data about the optimal parenteral Cu dosage in the event of cholestasis are lacking. Further studies are needed to investigate Cu homeostasis in this particular population and determine the optimal protocol to manage Cu in the parenteral nutrition of cholestatic infants.

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Figure 1.
Serum Cu variability with duration of parenteral nutrition in patients with and without intestinal failure.

Table 1

Demographics and Clinical Characteristics of Study Infants

	N = 28
*Birth weight (grams)	1550 ± 936
*Gestational age at birth (weeks)	30 ± 4.5
^a Postmenstrual age (weeks)	46 ± 9.9
% of parenteral nutrition calories	81 ± 18
*Duration of parenteral nutrition (days)	113 ± 57
Intestinal failure (%)	14/28 (50%)

* Values are mean ± standard deviation, unless otherwise specified

^a Postmenstrual age = gestational age plus chronological age.

Table 2

Clinical Characteristics of Infants with and without Intestinal Failure

	Intestinal failure	No intestinal failure	p- value
N	14	14	
Birth weight (grams)*	1832.5 ± 1043.3	1267.5 ± 747.9	0.1
Gestational age (weeks)*	30.9 ± 4.9	29.4 ± 4.2	0.39
Gender/Male	71 %	64%	0.69
Postmenstrual age (weeks)*	50.1 ± 11.5	42.3 ± 6	0.03
Duration of parenteral nutrition (days)*	136 ± 68.2	90.4 ± 33.7	0.03
Parenteral nutrition calories (%)*	76.1 ± 17.5	87.2 ± 17.3	0.1
Cu level (mcg/dl)*	88.4 ± 29.5	55.8 ± 18.9	0.001
Alanine Aminotransferase* (IU/l)	237 ± 163	102 ± 71	0.01
Total bilirubin (mg/dl)*	10.5 ± 5.9	9.3 ± 4.1	0.53
Direct bilirubin (mg/dl)*	8.5 ± 5	6.2 ± 2.6	0.25

* Mean ± standard deviation

For patients with serial Cu measurements, the clinical characteristics are those taken at the time of the last serum Cu measurement

Table 3

Multiple linear regression analysis model with Serum Alanine Aminotransferase as the primary outcome

N = 28	Coefficient	Standard error	P value
Intercept	− 404	114	0.001
Gestational age	10.6	3.4	< 0.01
Total bilirubin	13.8	3.3	< 0.01
Serum Cu	1.8	0.5	0.004