Predictors of plasma zinc concentrations in children with acute diarrhea

Tor A Strand, Ramesh K Adhikari, Ram K Chandyo, Pushpa R Sharma, and Halvor Sommerfelt

ABSTRACT
Background: Plasma and serum zinc concentrations are the most widely used markers of zinc status in individual persons and populations.

Objective: The objective was to identify factors that influence plasma zinc concentrations during acute childhood diarrhea.

Design: This was a cross-sectional study of 1757 cases of acute diarrhea in 6–35-mo-old Nepalese children. The association between plasma zinc concentration and several clinical, anthropometric, socioeconomic, and biochemical variables was estimated in simple and multiple linear regression analyses.

Results: We observed a reduction in the mean plasma zinc concentration of 0.59 (95% CI: 0.44, 0.74) μmol/L per degree (°C) increase in axillary temperature. Having dysentery and an elevated plasma C-reactive protein concentration was also independently associated with lower plasma zinc. Children with clinical features of dehydration had higher plasma zinc concentrations than did those who were not dehydrated. Furthermore, a decrease in plasma albumin of 1 g/L was associated with a decrease in plasma zinc of 0.25 (95% CI: 0.21, 0.29) μmol/L. The plasma albumin concentration confounded the associations between some clinical variables and plasma zinc, but the association between axillary temperature and dehydration on one hand and plasma zinc on the other was not substantially influenced by the albumin concentration. Moreover, the plasma zinc concentration increased with an increase in observed hemolysis.

Conclusions: Dehydration, clinical and biochemical indicators of inflammation and hemolysis, and, when possible, plasma albumin concentrations should be taken into account when the plasma zinc concentration and various other variables in a cross-sectional study of Nepalese children with acute diarrhea in childhood. Am J Clin Nutr 2004;79:451–6.

KEY WORDS Plasma zinc, acute diarrhea, inflammation, body temperature, C-reactive protein, plasma albumin, children, Nepal, dehydration

INTRODUCTION
The importance of adequate zinc nutriture in human health is well documented and zinc deficiency is a large public health problem, especially among children in developing countries (1–4). Less than 0.2% of the total body zinc content circulates in the plasma (5), and several factors other than the diet and total body zinc affect the plasma zinc concentration (2, 6, 7). Evidence from human and animal studies shows that plasma zinc decreases during infection (6, 8–10) and that the magnitude of change is related to the severity of infection (11). A study in Ghana showed that children with elevated C-reactive protein (CRP) concentrations had lower plasma zinc concentrations than did those with low CRP concentrations (12). However, the association between plasma zinc concentrations on one hand and markers of the severity of infection on the other has been weak in other studies of children in developing countries (13–15). In studies undertaken in Pakistan, only severe infections such as pneumonia and sepsis were found to be associated with low plasma zinc concentrations (16). Moreover, a review by Brown (6) suggests that common acute infections encountered in community settings may not have a major effect on the plasma zinc concentration. However, this review also states that “fever might be a useful marker of whether or not a particular infection is affecting the plasma zinc concentration although this hypothesis must be subjected to empirical testing.”

Zinc is transported in the plasma bound to albumin and other proteins (2), and it is therefore conceivable that alterations in the plasma protein concentration—such as during hemoconcentration and increased turnover—may influence the plasma zinc concentration. Plasma and serum zinc are the most widely used markers of zinc status and specimens are often collected during ongoing illness. Identification of factors and conditions that influence the plasma zinc concentration is therefore important. The objective of this study was to examine the association between the plasma zinc concentration and various other variables in a cross-sectional study of Nepalese children with acute diarrhea.

SUBJECTS AND METHODS
Subjects
The study participants were residents of Bhaktapur, which is located 12 km east of Kathmandu, the capital of Nepal. Bhaktapur is situated ≈1350 m above sea level; there is a wet and hot season from July until the end of September, a dry and cool season from October to March, and a dry and hot season from...
March until June. There are ≈80 000 inhabitants in Bhaktapur, and most practice Hinduism, Buddhism, or a combination of these 2 religions. The staple foods are rice, lentils, potatoes, and green leafy vegetables. A substantial proportion of the population eats meat occasionally. Most of the inhabitants have their own piece of land, where they grow vegetables and rice according to the season. In addition, some are employed in the tourist industry and some work in carpet or brick factories.

The study had ethical clearance from the Nepal Health Research Council in Kathmandu and the Human Research Ethical Committee of the Medical Faculty at the University of Bergen. The implementation of all aspects of the project was in agreement with the international ethical guidelines for research involving human subjects as stated in the latest version of the Helsinki Declaration. Informed and, when possible, written consent was obtained from at least one of the parents.

Between June 1998 and September 2000, we enrolled 1792 cases of acute diarrhea in 6–35-mo-old children (17). The children were recruited through weekly surveillance and spontaneous visits to a field clinic. Diarrhea was defined as having ≥3 watery or loose stools in the preceding 24 h and a recent change in stool character. The study physicians determined eligibility; children with parental consent and diarrhea lasting for <96 h were included. Children with coexisting illness were included unless the condition required hospitalization.

Data collection

History on symptoms of diarrhea and respiratory tract and other infections in the preceding 24 h was obtained in the clinic, and a complete physical examination was performed by one of the study physicians. The children were weighed nude with the use of a UNICEF electronic scale (SECA, Hamburg, Germany) with an accuracy of 100 g. Length was measured with the use of a locally made length board while the children were in the supine position. Each measurement was made at least twice with 2 fieldworkers present.

Between 0900 and 1300 on the day of enrollment, one of the study physicians drew a nonfasting venous blood specimen (=5 mL) into zinc-free heparin-containing polypropylene tubes (Monovette; Sarstedt, Nümbrecht, Germany) from the child’s cubital vein while he or she was in the supine position. Time since the last meal or breastfeeding was recorded. Immediately after specimen collection, the blood was centrifuged (760 × g, 10 min, room temperature) and the plasma was transferred to zinc-free polypropylene vials (Eppendorf, Hinz, Germany), which were stored at −20 °C until analyzed.

Laboratory analyses

After the specimens were thawed, the degree of hemolysis was visually categorized as mild, moderate, and extensive by trained laboratory technicians. The plasma specimens were analyzed for zinc by using inductively coupled plasma atomic emission spectrometry from Thermo Jarrell-Ash (Franklin, MA) at the Laboratory for Clinical Biochemistry, Haukeland Hospital, Bergen, Norway, Seronorm (Sero AS, Billingstad, Norway) was used as the reference standard. All samples were analyzed twice, and the mean concentration was calculated. The zinc analysis was repeated in the specimens in which the concentration of the 2 measurements deviated >5% from the mean. The CV between the runs was <7.5%. One hundred of the samples were concomitantly analyzed in an external laboratory at the University of Colorado Health Sciences Center; agreement between the laboratories was good, with an intraclass correlation coefficient of 0.95. CRP and albumin were estimated by using immunoturbidimetric assays (Tina-Quant, Roche, Germany) on a Hitachi 917 (Tokyo). We used different assays for estimating CRP concentrations ≤10 mg/L (micro-CRP) and of CRP concentrations >10 mg/L (18). Some of the samples contained too little material to obtain reliable concentration estimates for zinc, albumin, or CRP. We accordingly estimated plasma zinc, albumin, and CRP concentrations in 1757 (98%), 1649 (92%), and 1686 (94%) of the collected specimens, respectively.

Data analysis

The CRP concentration was categorized as <8, 8–15, and >15 g/L. A cutoff of 8 g/L was used previously (12); we included a second cutoff of 15 g/L to create 2 similarly sized categories of elevated CRP concentrations. Stunting and wasting was defined as being less than −2 z scores compared with the reference population for length-for-age and weight-for-length, respectively, as described previously (17).

The plasma zinc concentration was symmetrically distributed, and the association between plasma zinc and the variables of interest were assessed in simple and multiple linear regressions analyses. The variables that were included in the initial assessments were loose, watery stools and total stool frequency 24 h before enrollment, general condition, presence of respiratory symptoms, pneumonia, maternal age, maternal and paternal occupation and education level, household size, number of siblings, and those variables in the models described in Table 1. On the basis of these analyses, we selected variables for the multiple regression models as described elsewhere (19). In short, after we assessed the associations between plasma zinc concentration and the variables of interest by using simple linear regression, all variables were assessed in a fuller model. The sequence of which the variables were added to this model was based on their significance level in the initial assessments. Explanatory variables that had a considerable effect on the association between other explanatory variables and the plasma zinc concentration and those that were independently associated with the outcome were retained in the final multiple regression models. Differences between categories were expressed as the mean differences with the corresponding 95% CIs. All independent variables, except albumin and axillary temperature, were categorical. For the variables with multiple categories, eg, CRP and age categories, we used the category with the lowest values as the reference and adjusted the P values of these differences for multiple comparisons with the use of Holm’s method (20). We also assessed many relevant interactions. One hundred ninety-nine children were included in the study more than once; adjustment for repeated entry of the same child was undertaken by using generalized estimation equations (21) with an exchangeable covariance structure. The correlation between CRP and measured temperature was undertaken by using Spearman rank-order correlation. The statistical analyses was conducted with the use of STATA, version 8 (StataCorp, College Station, TX). A P value < 0.05 was considered to represent statistical significance. A single-measure
The intraclass correlation coefficient was calculated by using SPSS version 9 (SPSS Inc., Chicago). The graph describing the relation between plasma zinc and axillary temperature in the 3 CRP categories was constructed by using locally weighted scatter plot smoothing by the STATA "ksm" command with the option "lowess" and a bandwidth of 0.8. The geographic distribution of the children’s homes was visualized by using a global positioning system–based computerized plot.
RESULTS

Children from all of the 17 administrative areas in the Bhaktapur municipality were well represented in the study. The mean (± SD) plasma zinc concentration was 8.4 ± 2.3 μmol/L, and the mean age of the children in which we assessed plasma zinc was 15.5 ± 7.8 mo; 40% of the children were infants and 82% were breastfed, 3% exclusively so. The mean period of time from the last intake of breast milk was 63 ± 76 min and from the last intake of other food was 176 ± 74 min. There were 775 children whose caretaker gave us information on the duration from the last breast-milk feeding or last meal was 78 ± 79 min.

Stunting was observed in 514 (29%) and wasting in 393 (22%) of the enrollments. Seventy-one percent of the families whose children participated in the study owned their own piece of land. The mean (± SD) number of family members was 7.1 ± 3.8, and 34% of the families lived in only one room. The mean maternal age was 25 ± 4.5 yr; the median numbers of maternal and paternal years of schooling were 1 [interquartile range (IQR): 1–5] and 8 (IQR: 4–10) yr, respectively. Of the mothers, 54% were employed in agriculture, 17% were employed as daily wage earners, and 17% were unemployed. Twenty-three percent of the fathers had their main income from agriculture, 41% were daily wage earners, 21% were government employees, and <2% were unemployed.

Almost half of the children had fever during the episode, as reported by the caretaker, 40% had respiratory symptoms, 0.7% had pneumonia diagnosed by one of the study physicians, 3% had a reduced general condition, and 9.8% had dysentery. There were 206 (11.7%) children with some dehydration; none had severe dehydration. The mean (± SD) total number of stools 24 h before enrollment was 9.0 ± 4.1; the median number of loose and watery stools were 6 (IQR: 0–9) and 0 (IQR: 0–6), respectively. The mean number of days with diarrhea before inclusion and blood sampling was 2.2 ± 1.1 d.

Eighty-eight percent of the specimens had no hemolysis, whereas 5.7% had moderate or extensive hemolysis. Exclusion of the samples with hemolysis, instead of adjustment for it in the models, did not change the strength of the observed associations.

Clinical variables associated with plasma zinc concentration

Increasing axillary temperature was associated with a lower plasma zinc concentration. In the simple model, a 1–°C increase in body temperature corresponded to a 0.55-μmol/L lower mean plasma zinc concentration. Adjustment for the other clinical variables only slightly altered this estimate (Table 1, model 1). Having dysentery was also strongly and independently associated with a lower zinc concentration. Dehydration and vomiting in the 24 h before enrollment was independently associated with a higher plasma zinc concentration. Children with a 72–96-h pre-enrollment duration of diarrhea had a lower mean zinc concentration than did children with a duration of <72 h (Table 1).

Other variables associated with plasma zinc concentration

The mean plasma zinc concentration was lower in children aged >2 y (Table 1). Wasting was not significantly associated with plasma zinc, whereas stunted children had a plasma zinc concentration that was 0.24 (95% CI: 0.01, 0.48) μmol/L lower than that of other children in the crude analysis. However, neither stunting nor wasting was associated with plasma zinc concentrations in the multiple regression models. Similarly, breastfeeding status and the time since the last meal were not strongly or independently associated with plasma zinc concentration, although these variables were associated with plasma zinc in the crude analysis. Adjustment for plasma albumin concentration reduced the effect of having diarrhea during the wet season (Table 1). Girls had a slightly lower mean plasma zinc concentration than did boys. The socioeconomic variables were only weakly associated with plasma zinc.

Biochemical variables associated with plasma zinc concentration

The plasma zinc concentration increased with increasing degree of hemolysis, whereas elevated CRP concentrations were associated with lower plasma zinc concentrations (Table 1). CRP and axillary temperature were correlated (ρ = 0.26, P < 0.0005). The observed change in mean zinc concentration per 1–μmol/L increase in body temperature was 0.59 μmol/L after adjustment for the clinical variables (Table 1, model 1). This estimate was reduced to 0.42 (95% CI: 0.26, 0.58) when further adjusted for CRP. The association of each of these 2 variables with the plasma zinc concentration is shown in Figure 1. The observed zinc concentration was lower as the axillary temperature increased within each of the 3 CRP concentration categories.

The mean plasma albumin concentration was 40.9 ± 2.7 g/L, and 277 children (16.8%) had <39 g/L (22). An increase in plasma albumin of 1 g/L corresponded to a zinc concentration that was 0.25 (95% CI: 0.21, 0.29) μmol/L higher. Except for axillary temperature and dehydration, the strength of the associations between the other clinical variables and plasma zinc was substantially reduced when plasma albumin concentration was added to the model (Table 1, model 2). Thus, in this model, the apparent effects of these variables on the plasma zinc concentration were less than when plasma albumin was not included.

DISCUSSION

The aim of this study was to describe variables that could influence the plasma zinc concentration in children with acute diarrhea. We found that the zinc concentration was lower in those with dysentery, fever, and elevated CRP concentrations. Caution is required, however, when attempting to draw conclusions regarding the direction of the causal pathway from cross-sectional studies such as ours. There is a possibility that children with low plasma zinc concentrations before their illness developed a more severe disease followed by higher body temperature and elevated CRP concentrations. Zinc deficiency causes stunting and underweight (3). In our study, being stunted, wasted, or underweight was not associated with having dysentery, fever, or an elevated CRP concentration (data not shown). Malnutrition, with its frequently accompanied zinc deficiency (3), did not seem to lead to conditions that cause elevated CRP concentrations or an increased temperature. This indicates that an increased severity of inflammation resulted in reduced plasma zinc concentrations rather than the reverse. Furthermore, studies in humans and animals (6, 8–10, 12) support our assumption that inflammation reduces the plasma zinc concentration. A reduction in plasma or
serum zinc concentration has been found to be proportional to the level of induced parasitemia and the dose of administered bacterial endotoxin (9, 10). Fever is partly and directly mediated through an interleukin 1 response, which is followed by an increase in the hepatic synthesis of the metal binding protein metallothionein that again increases zinc uptake from plasma (23). Thus, the strong negative association between fever or CRP and plasma zinc may have been mediated by an interleukin 1–metallothionein-induced zinc uptake in the liver (24). Furthermore, plasma CRP concentration and axillary temperature were strongly associated with plasma zinc concentration even when both variables were included in the linear regression model. The temperature may vary during the course of an infection, and CRP may be elevated even if the temperature is low at the time of measurement. Some infections that are caused by viruses do not induce high CRP concentrations but may cause high fever. Thus, these 2 variables may complement each other in describing the associations between the inflammatory process and the plasma zinc concentration.

The concentration of plasma proteins may be reduced because of increased protein catabolism, reduced synthesis during infection, and intestinal protein loss during diarrhea. The plasma albumin concentration was strongly associated with the zinc concentration; adjustment for albumin changed some of the associations between plasma zinc and the other explanatory variables. The associations between dysentery, measured temperature, or CRP and plasma zinc concentrations, however, were still substantial and significant after adjustment for the plasma albumin concentration. It therefore seems likely that the associations of dysentery, body temperature, and CRP with plasma zinc were only to a limited extent mediated by the plasma albumin concentration. Dysentery is an invasive bacterial infection in the gut accompanied by fever and bloody stools and may affect the plasma zinc concentration through an increased protein and zinc loss in the stools and through the metabolic responses associated with inflammation and fever. The fact that the malnourished children in our study were not more prone to dysentery favors the interpretation that the dysentery-associated inflammation, enteric protein loss, or both reduced the plasma zinc concentration.

Although zinc is excreted in the stool and is lost with diarrhea (25, 26), we found no association between high stool frequency or the duration of illness and low zinc concentrations in the multiple regression models. Dehydration is more likely to occur during diarrhea with high stool frequency and could thereby be associated with an increased zinc loss followed by reduced plasma zinc concentrations. Dehydration and vomiting, however, were associated with increased zinc concentrations. This may be due to hemoconcentration, ie, an increased concentration of plasma proteins in which the bulk of the plasma zinc is bound. Although the association between plasma zinc and vomiting was reduced after adjustment for albumin, the observed association between the plasma zinc concentration and dehydration was not weakened.

Studies in healthy adults in industrialized countries have shown that the plasma zinc concentration varies with the time of day and the time since the last meal (27–29). In our study, we collected the blood samples at the same time of day and thus were not able to assess diurnal variation. Assessment of diurnal variation and the determination of postprandial changes in plasma zinc in children of developing countries require that blood samples be taken from healthy persons and a different study design than we used.

Older children had lower plasma zinc concentrations than did the younger children, even after adjustment for breastfeeding status and the other variables in the model. This could be explained by an increased intake of zinc inhibitors as the children’s diet became more similar to that of the grown-ups. However, age-specific reference values for plasma zinc in healthy children living in developing countries should be established by using a different design. The lower plasma zinc concentration in girls than in boys may seem to be at odds with results from studies in healthy children (2). However, the difference between boys and girls was <3%, and it should be emphasized that our children were ill.
We observed a significant association between stunting and low plasma zinc concentrations in the crude analysis, which disappeared in the multiple regression analyses. Stunted children are more likely to have a lower zinc intake and plasma zinc concentration (3, 30). The lack of an association between plasma zinc and stunting in our study, combined with our finding of a strong relation between inflammation and plasma zinc, suggests that the zinc concentration may not be a good marker of zinc nutrition when assessed during acute diarrhea.

The zinc concentration is higher in red blood cells than in plasma, and our data confirm that accounting for the level of hemolysis in blood specimens is important when plasma zinc is evaluated (2, 31, 32). Samples with moderate or extensive hemolysis should be reassessed; however, such a reassessment may not be necessary when the degree of hemolysis is mild, ie, only slight red discoloration after centrifugation and separation. In conclusion, the prevalence and severity of infections and dehydration and, when available, plasma albumin concentrations should be taken into account when interpreting plasma zinc values in individuals or in population groups.

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TAS was the principal investigator and led the planning and undertaking of the study, conducted the data analysis, and wrote the first draft of the manuscript. RKA was the Nepalese coordinator and contributed to the planning and the undertaking of the study. RKC and PRS were involved in the planning and undertaking of the study, conducted the data analysis, and writing of the manuscript. All authors critically reviewed the manuscript. None of the authors had any financial or personal interest in any company or organization that supported the research.

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