Efficacy of zinc in young infants with acute watery diarrhea[1–3]

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ABSTRACT

Background: Recent studies reported that zinc significantly reduced the duration and volume of acute watery diarrhea in children aged ≥4 mo, but there were no data specifically on infants aged <6 mo.

Objective: This study investigated the effect of zinc on the duration of illness and the stool quantity in acute watery diarrhea of infants aged 1–6 mo by comparing a 20 mg Zn/d dose with a 5 mg Zn/d dose.

Design: Infants hospitalized with at least some dehydration (by World Health Organization classification) were enrolled in a double-blind, randomized, placebo-controlled trial. Infants were randomly assigned to receive 20 mg Zn (acetate)/d, 5 mg Zn/d, or placebo for the duration of illness.

Results: Two hundred seventy-five infants were enrolled between 20 September 1998 and 18 December 2000. Neither diarrhea duration nor mean stool volume differed between groups. There were no significant differences in fluid intake, the need for unscheduled intravenous fluid, weight gain, or vomiting rates between the groups.

Conclusions: Zinc supplementation did not affect diarrhea duration or stool volume in young infants. Young infants tolerated both zinc doses. A beneficial effect on subsequent illness cannot be ruled out. *Am J Clin Nutr* 2005;82:605–10.

KEY WORDS Acute watery diarrhea, dehydration, zinc, infants, children, Bangladesh, International Center for Diarrhoeal Diseases Research, Bangladesh, ICDDR,B, hospitalized children

INTRODUCTION

Diarrhea is associated with 18% mortality among children aged <5 y, accounting for 1.9 million deaths (1), primarily in developing countries. Despite gains in the use of oral rehydration therapy, diarrhea-associated mortality has not declined in the past few years in Bangladesh (2). The continued high mortality underscores a need for further improvements in case management and primary prevention.

Studies have shown that zinc is involved in epithelial barrier integrity, tissue repair, and immune function (3–6). However, diarrhea can be associated with increases in fecal zinc loss (7). Thus, in cases of diarrhea, the very condition under which the body needs optimum zinc balance, the disease itself accelerates zinc loss.

Studies have reported significant reductions in both the duration of acute watery diarrhea (AWD) and the stool volume during AWD with the administration of zinc in the acute phase of the illness (7–10). A pooled analysis showed a mean 15% (95% CI: 5%, 24%) lower likelihood that diarrhea will continue on a given day in zinc-supplemented children (8). Subgroup analysis by age showed significant effects for children aged <12 mo with AWD, but most of these studies were conducted on children aged ≤4 mo. To date, there have been no reported studies specifically in infants aged <6 mo. Concerns have been raised about tolerance to zinc among hospitalized children, because it was reported that zinc-supplemented children have an increased tendency for vomiting (10).

Our study sought to determine the effect of zinc on the severity of AWD in infants aged 1–6 mo by using the standard 20 mg Zn/d dose used in other trials (9, 11) and also to ascertain whether the effects were similar with a 5 mg Zn/d dose.

SUBJECTS AND METHODS

Subjects

This study was a double-blind, randomized, placebo-controlled clinical trial. All infants were admitted to the hospital of the Centre for Health and Population Research of the International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR,B) in Dhaka, Bangladesh. Patients were enrolled if they were male infants aged 1–6 mo at the time of diarrheal illness, if the onset of illness was ≤ 72 h before admission, and if they had ≥ 3 watery stools in the preceding 24 h. Male patients were selected to facilitate the separation of urine and stool. Patients were enrolled if they had at least some dehydration, as defined by the method of the World Health Organization (12), or had ≥100 mL of watery stool within a 4-hobservation period, which made the infant eligible for hospitalization according to ICDDR,B criteria.

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A study health worker screened for eligible patients at the intake triage desk, and they were then brought to a senior team member for consent and random assignment. A trained staff member read the consent form in its entirety to the parent, and answered any questions from a standardized set of key points for each section of the consent form. If the parent indicated that he or she understood the terms and agreed to let the child participate, then the parent was asked to sign the consent form or, if illiterate, to provide a thumb impression.

Infants were excluded if they had clinical signs of zinc deficiency (eg, acro-oral dermatitis or alopecia), kwashiorkor, weight-for-age <60% based on the National Center for Health Statistics (NCHS) reference data (13), grossly bloody stool, or other comorbidity that required them to be managed in a different ward. Patients who had *Vibrio cholerae* in their stool or who were suspected of having cholera because of persisting dehydration were also ineligible, because they required antimicrobial management, and their responses to zinc might not be comparable to those of infants who were not also receiving antibiotics. All stools were tested before randomization.

Parents provided informed consent for all study participants. The Ethical Review Committee of ICDDR, B, approved the study in accordance with the standards of the Helsinki Declaration of 1975, as revised in 1983.

Methods

Patients' dehydration was corrected before enrollment as follows. Those with moderate ("some") dehydration received 100 mL/kg of a standard oral rehydration solution (ORS) over 4 h, with an interim evaluation at 2 h, and adjustments made to the ORS volume as needed. This procedure was repeated every 2 h for a maximum of 12 h. Patients who remained dehydrated after 12 h were treated as presumed cholera patients, were given intravenous fluid, and were managed according to hospital protocol, but were not enrolled in the study.

Those with severe dehydration were initially rehydrated intravenously. An intravenous solution (Dhaka solution) containing 133 mmol sodium/mL, 13 mmol potassium/L, and 48 mmol sodium bicarbonate/L was administered at a rate of 40 mL $\cdot$ $kg^{-1} \cdot h^{-1}$ over a period 2 h, until severe dehydration was corrected and the infant could tolerate oral rehydration. If severe dehydration persisted after the first 2 h, the intravenous infusion was repeated for a maximum of 3 attempts, after which those who remained severely dehydrated were treated as cholera patients, were transferred to the general ward, and were not enrolled in the study.

Nude body weight was obtained after rehydration and then daily until discharge from hospital. Length measurements were made with the use of a wooden sliding board. The mean of 2 consecutive measurements was recorded for all indexes. Measurements were compared with those from the reference population of the NCHS (13).

The zinc preparation was supplied by ACME Laboratories, Ltd (Dhamrai, Dhaka, Bangladesh), with the following specifications: 20 mg zinc acetate/5 mL (group A), 5 mg zinc acetate/5 mL (group B), and 5 mL placebo (group C). The zinc acetate was dissolved in a base substance normally used for vitamins but was vitamin free. The zinc and placebo were otherwise identical in appearance in color, taste, and smell.

After rehydration, patients were randomly assigned to 1 of the 2 treatment groups or to the placebo group. Group assignments were allocated by block randomization in permuted blocks of variable length between 3 and 12. The syrup was administered within 2 h of randomization and, thereafter, every morning at 0900. All study team members and patients were blind to group assignment.

The total fluid intake (including ORS and water) and the stool and urine output were recorded every 3 h. Stool weight was measured by using metabolic beds. Urine was collected in polyethylene urine bags adhesively attached to the patient's groin. Vomitus weight was measured along with previously weighed towels, and mass was converted to volume (1 g/mL). Food intake was also recorded every 8 h. All mothers stayed with their children throughout hospitalization, and breastfeeding was encouraged ad libitum, but measurement of breast milk intake was not attempted. If dehydration recurred during the maintenance phase, the calculated deficit was replaced with ORS over a period of 6 h, and an interim evaluation was performed at 3 h. If signs of dehydration persisted after 6 h of ORT, then intravenous fluids were administered at a rate of 40 mL/kg/h over a period of 2 h, as described above. Intravenous fluids were administered any time signs of severe dehydration developed.

The following outcomes were evaluated: first, duration of diarrhea, calculated from the time of admission until the end of diarrhea, defined as the formation of 3 soft stools or the absence of stool for ≥12 h, and, second, the total volume of the diarrhea stools during hospitalization. Secondary outcomes included the need for unscheduled intravenous fluids (defined as the need for intravenous fluids after preenrollment rehydration), frequency of diarrheal stools, total fluid intake, frequency and volume of vomiting, serum zinc concentration, and weight gain.

The sample size calculation was based on a 365-d chart review, which gave a mean duration of AWD of 131 ± 47.8 h and a stool volume of 121 ± 65.9 mg $\cdot$ $kg^{-1} \cdot d^{-1}$. All calculations were based on a 5% chance of a Type I error and a 10% chance of a Type II error. We wanted to be able to detect a 20% reduction in duration of diarrhea and 30% reduction in stool volume, which required a sample size of 70 infants per group for both diarrhea duration and stool volume. To allow for 20% attrition, we required 84 children per group, or a total sample size of 252.

Two 1-mL blood specimens were collected, one at the time of randomization and one just before discharge, for measurement of baseline and postsupplementation serum zinc concentrations, respectively. Serum samples (150 μL) in polypropylene tubes were diluted 1 in 12 with nitric acid and 30% (wt:vol) polyoxyethylene 23 lauryl ether (Brij-35; Sigma Diagnostics, St. Louis, MO), a nonionic detergent. Zinc concentration was determined by flame atomic absorption spectrophotometry (AAS), as described previously (14).

The duration of illness was also compared between groups. A Cox proportional hazards model (15) was used to compare the relative hazard of the duration outcome in each zinc group with that in the placebo group and to explore associations between baseline covariates and outcome. Results are reported as relative hazard (RH) and 95% CIs. We also used the Cox hazard model to detect interactions between covariates and outcome for the 2 treatment groups. Because age and nutritional status, as ascertained by anthropometry and serum zinc concentrations, were biologically plausible explanatory variables, we developed a final model by using age, baseline and end-of-illness anthropometric indicators (ie, weight and weight-for-age), and serum zinc concentrations as continuous variables. Tied scores for duration

TABLE 1
Demographic and clinical characteristics of enrolled patients[1]

Demographic characteristics[2]	Placebo group ($n = 93$)	Zn05 group ($n = 91$)	Zn20 group ($n = 91$)
Age (mo)	4.2 (4.0, 4.4)[3]	3.9 (3.7, 4.2)	3.9 (3.6, 4.1)
Household income/mo (US$/mo)	59.84 (54.59, 65.09)	64.56 (57.13, 71.99)	58.02 (51.90, 64.14)
Rent (US$/mo)	14.95 (12.52, 17.38)	18.28 (13.58, 22.99)	14.32 (11.91, 16.74)
Father's education (y)	5.0 (4.1, 5.8)	4.7 (3.8, 5.7)	5.5 (4.6, 6.4)
Mother's education (y)	3.3 (2.6, 4.0)	3.4 (2.7, 4.2)	3.3 (2.6, 4.1)
Duration of illness before hospitalization (h)	46.2 (43.1, 49.3)	45.3 (42.0, 48.5)	44.6 (41.6, 47.7)
Amount of ORS if given (packets)	2 (2, 2)	2 (1, 2)	2 (1, 2)
Blood in stool (%)	0	0	0
Fever (%)	11.8	16.5	11.0
ORS given at home (%)	93.6	90.1	96.7
No change in feeding (%)	46.2	49.4	50.6
Exclusive breastfeeding (%)	10.8	3.3	6.6
Prehydration weight (g)[4]	5890[a] (5704, 6075)	5469[b] (5265, 5673)	5584[a,b] (5392, 5775)
Posthydration weight (g)[4]	5985[a] (5798, 6171)	5573[b] (5372, 5774)	5680[a,b] (5491, 5870)
Discharge weight (g)[4]	5920[a] (5746, 6095)	5510[b] (5312, 5709)	5584[a,b] (5406, 5761)
Admission weight-for-age (%)[4]	87.3 (85.1, 89.6)	83.9 (81.7, 86.1)	86.2 (83.9, 88.6)
Discharge weight-for-age (%)[4]	84.7 (82.4, 87.0)	80.7 (78.5, 83.0)	83.3 (81.1, 85.6)
Serum zinc baseline (μg/dL)[4]	69.9 (66.9, 72.8)	67.0 (64.3, 69.7)	68.6 (65.8, 71.4)
Serum zinc after supplement (μg/dL)[4]	69.1 (65.6, 72.5)	84.1 (79.9, 88.4)	101.0 (93.6, 107.3)

[1] Zn05, zinc given at a dose of 5 mg/d; Zn20, zinc given at a dose of 20 mg/d; ORS, oral rehydration solution. Means in a row with different superscript letters are significantly different, $P < 0.05$ (Bonferroni-adjusted Student's t test).
[2] Percentages are the proportion of children in each group with the finding.
[3] $\bar{x}$; 95% CI in parentheses (all such values).
[4] 2-Factor repeated-measures ANOVA with interaction.

were treated by using the exact partial (conditional logistic) calculation, because time was measured in discrete 8-h shifts. Proportional hazards assumptions were tested by using tests of specification (reestimation, analysis, and plotting of Schoenfeld residuals) and goodness-of-fit (15). Comparisons between groups were performed by using one-way analysis of variance (ANOVA) for all continuous variables and the Bonferroni adjustment for corrected P values. Two-factor repeated-measures ANOVA was used to assess the time × treatment interaction to ascertain whether the pattern of change over time was the same in the 2 groups. All data analyses were performed by using the STATA/SE statistical analysis package (version 8.2; Stata Corporation, College Station, TX).

RESULTS

We recruited 275 infants from a group of 280 eligible infants between 20 September 1998 and 18 December 2000. Ninety-one were randomly assigned to the 5 mg Zn/d group (Zn05), 91 to the 20 mg Zn/d group (Zn20), and 93 to the placebo group.

There were no significant differences between groups in demographic characteristics (**Table 1**). The mean duration of illness before hospitalization was 44.6 h (95% CIs: 43.9, 45.3 h) from the onset of illness, and there were no significant differences between groups. There were no significant differences between groups in the other aspects of the history of the current illness (Table 1).

Fifteen infants were withdrawn from the study by their parents, and 260 infants successfully completed the protocol. The completion rates in the placebo, Zn05, and Zn20 groups were 95.7%, 93.4%, and 94.5% respectively (**Figure 1**). These rates represent an overall completion rate of 94.5%, and there were no significant differences in attrition between groups by Pearson's chi-square test ($P = 0.967$). There were no deaths or serious adverse events.

Whereas there were no significant differences between the Zn20 and placebo groups in baseline anthropometric measurements, the Zn05 group weighed significantly less than did the other groups, which resulted in significant differences in baseline prehydration and posthydration weight (Table 1). All groups lost weight by discharge, but the percentage decrease in weight at discharge in the placebo, Zn05, and Zn20 groups (1.1%, 1.1%, and 1.7%, respectively) did not differ significantly ($P = 0.468$). The admission and discharge weight-for-age did not differ significantly between groups. Although serum zinc concentrations at discharge were higher in both zinc groups than at baseline, which indicated good compliance, the differences were not significant. There were 15 children in the Zn05 group and 12 in the Zn20 group who vomited, compared with 7 in the placebo group. There were no differences in the number of children who vomited, in vomiting frequency, or in the volume vomited among children who did vomit (**Table 2**).

There were no significant differences between groups in outcome variables (Table 2). The RH of the Cox proportional hazards model showed no significant reduction in the duration of diarrhea in either the Zn05 or the Zn20 group. Adjustment for age, admission and discharge weights, weight-for-age, and serum zinc concentrations as continuous variables had no effect (**Table 3**). There was no interaction between covariates in the proportional hazards model.

DISCUSSION

We did not find a significant difference in disease severity or duration between either the Zn05 or Zn20 group and the placebo group. It is of particular interest that there was no measurable

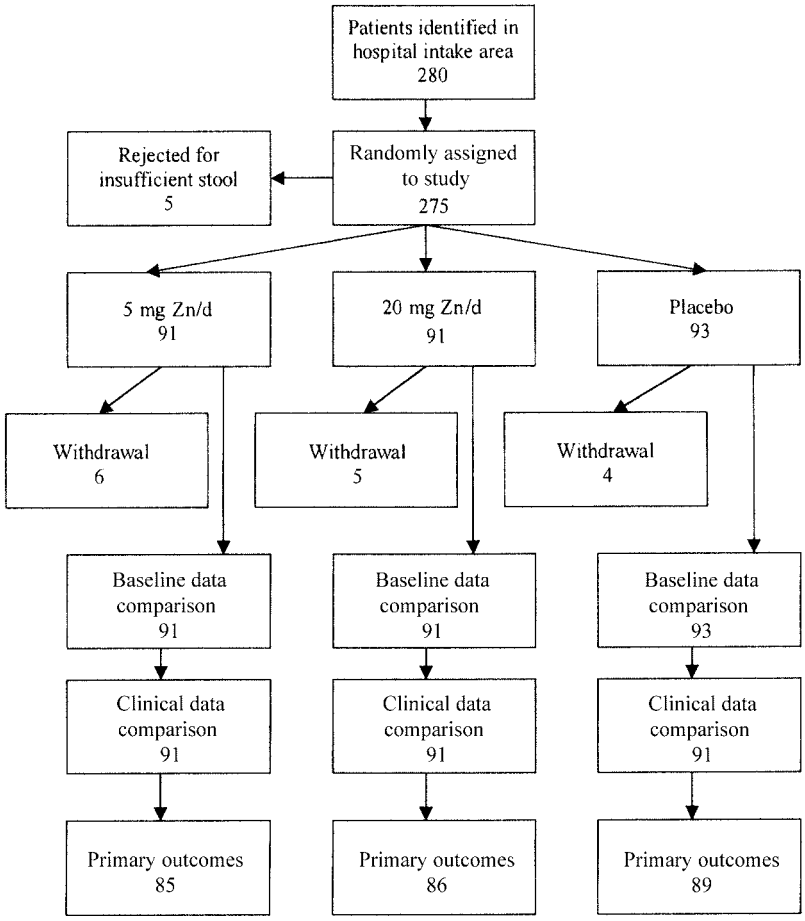

FIGURE 1. The trial profile.

effect on illness duration. Although the Zn05 group had a significantly lower mean admission weight than did the placebo group, none of the children in the Zn05 group were severely malnourished. Given the lack of difference between groups in illness characteristics at admission and in the history of current illness, this difference in mean admission weight is not likely to have been clinically significant or to have affected outcome. This lack of effect is supported by the weight-for-age, which did not differ significantly between groups. Adjustment for these factors in the Cox model had no effect. This absence of effect on either duration of illness or stool volume is at variance with the findings in most studies of zinc treatment and acute diarrhea in older children (8, 10, 16–18).

There may be several reasons for this absence of effect. First, very young infants may have adequate zinc bioavailability, acquired in utero, that may persist for the first few months of life. Evidence suggests that there is preferential zinc shunting across the placenta (19), and infants, excluding those of low birth weight, may be able to obtain adequate total body zinc from maternal sources, even when maternal stores are suboptimal

TABLE 2
Primary and secondary clinical outcome variables for study subjects during hospitalization for acute watery diarrhea[1]

Outcomes	Placebo group (n = 89)	Zn05 group (n = 85)	Zn20 group (n = 86)
Total IVF (mL)	300 (100, 500)	300 (200, 400)	240 (213, 504)
Total ORS (mL)	500 (500, 572)	500 (500, 527)	500 (500, 500)
Total water (mL)	35 (20, 59)	50 (15, 100)	35 (13, 87)
Vomiting frequency[2]	1 (1, 3)	1 (1, 1)	1 (1, 2)
Vomiting volume (mL)	37 (7.7, 63.9)	26 (11.8, 36.8)	18.5 (5.4, 34.9)
Stool output (mL)	202 (180, 246)	229 (180, 256)	240 (200, 266)
Stool frequency (no./d)	5 (4, 6)	5 (5, 6)	5 (5, 6)
Duration of diarrhea (d)	5 (4, 6)	5 (4, 6)	5 (4, 6)

[1] All values are $\bar{x}$; 95% CI in parentheses. Zn05, zinc given at a dose of 5 mg/d; Zn20, zinc given at a dose of 20 mg/d; IVF, intravenous fluids; ORS, oral rehydration solution. There were no significant differences between groups according to Bonferroni-adjusted Student's t test.

[2] The number of times a child vomited, if he vomited.

TABLE 3
Cox proportional hazards model comparing mean duration of illness in the Zn05 and Zn20 intervention groups and the placebo group[1]

	Relative hazard (95% CI)	
	Zn05 group vs placebo group (n = 174)	Zn20 group vs placebo group (n = 175)
Duration of illness		
5d, unadjusted	0.88 (0.64, 1.13)	1.00 (0.80, 1.24)
5d, adjusted[2]	0.91 (0.72, 1.14)	1.00 (0.81, 1.26)

[1] Zn05, zinc given at a dose of 5 mg/d; Zn20, zinc given at a dose of 20 mg/d.
[2] Adjusted for age, admission and discharge weights, weight-for-age, and serum zinc concentrations as continuous variables.

(20). Such infants may not derive immediate benefit from supplemented zinc during a first bout of acute diarrhea.

Second, most young infants are unlikely to have had a previous zinc-depleting illness. One study reported that zinc appears to prevent subsequent or recurrent infections (21). This suggests that an acute illness, particularly a zinc-depleting enteropathy such as diarrhea, may reduce tissue zinc stores below a critical concentration. Perhaps, among young infants, there would be a clinical benefit from supplemental zinc only after an illness. Only 5.5% of the infants in this study were reported by their mothers to have had a prior illness requiring medical attention (data not shown), and only 3.6% had ever been hospitalized. It is possible, then, that most of these young infants were not physiologically zinc deficient.

Third, because nearly all of the infants in this study were breastfed, the additional zinc given to the 2 treatment groups may not have created a significant difference in zinc tissue bioavailability. Zinc is present in breast milk, especially during the first 4 mo of breastfeeding (22, 23), so that most of these breastfed infants had a similar physiologic exposure (24). Thus, they may have been less likely to have a therapeutic benefit from zinc than were the older children who receive little or no breast milk. Apart from zinc, there may be other benefits to breastfeeding that have a stronger effect on diarrhea than does zinc, which essentially would mask any therapeutic effect.

Fourth, the study possibly was underpowered, and this null effect is a type II error. Our sample size was based on an assumption of 5.5 d (95% CI: 5.3, 5.7 d) of diarrhea, which was not significantly different from the observed mean duration of 5.9 d (95% CI: 5.5, 6.3 d) ($P = 0.103$). Thus, from a duration-of-illness standpoint, the sample size should have been adequate. The effect size, however, may have been more modest among these young infants. If the effect were closer to the 10% reductions suggested by the Zn05 group, and if we still assumed a mean duration of illness of 5.5 d, we would require 972 infants per group for a similar placebo-controlled trial or, allowing for 20% attrition, a study population of 2333. Even in Dhaka, such a sample size for infants aged <6 mo would require a multisite study. The current study would then have had a power of only 40.7%. It is biologically plausible, given the discussion above, that the zinc dose effect is smaller in young infants, and that this study was underpowered.

Infants were able to tolerate the once-daily zinc supplements at either the 5 mg/d or 20 mg/d dose. Most (87.6%) of the infants in this study did not experience vomiting, but there were more vomiting infants among the Zn05 and Zn20 groups than among the placebo group (ie, 15, 12, and 7, respectively). This difference, however, was not significant and had no effect on clinical course or outcome. This therapeutic regimen resulted in no other complications, which suggests that both zinc doses are clinically safe and tolerated.

Current recommendations for zinc administration in children aged <5 y who have acute diarrhea are 20 mg/d for those aged 6–59 mo and 10 mg/d for those aged <6 mo (11). Although this study found no apparent benefit of zinc in the treatment of acute diarrhea in young infants, studies of children aged <5 y showed that zinc administered during acute diarrhea illness results in a reduction of subsequent diarrhea episodes (16, 25). This is also pointed out in the joint WHO/UNICEF statement on diarrhea management (11). Thus, young infants given zinc may also have a reduction in subsequent diarrhea incidence, as suggested by the higher serum zinc concentrations among supplemented infants. Future studies on this age group should include a follow-up observation period to ascertain whether there is such an effect.

This study should be conducted in other populations. Future studies in this age group should include a follow-up period to complete our understanding of the effects of zinc on young infants.

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