ZINC SUPPLEMENTATION IN YOUNG CHILDREN WITH ACUTE DIARRHEA IN INDIA

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Abstract *Background.* In developing countries the duration and severity of diarrheal illnesses are greatest among infants and young children with malnutrition and impaired immune status, both factors that may be associated with zinc deficiency. In children with severe zinc deficiency, diarrhea is common and responds quickly to zinc supplementation.

Methods. To evaluate the effects of daily supplementation with 20 mg of elemental zinc on the duration and severity of acute diarrhea, we conducted a double-blind, randomized, controlled trial involving 937 children, 6 to 35 months of age, in New Delhi, India. All the children also received oral rehydration therapy and vitamin supplements.

Results. Among the children who received zinc supplementation, there was a 23 percent reduction (95 percent confidence interval, 12 percent to 32 percent) in the risk of continued diarrhea. Estimates of the likelihood of recovery according to the day of zinc supplementation revealed a reduction of 7 percent (95 percent confidence interval, −9 percent to +22 percent) in the risk of continued diarrhea during days 1 through 3 and a reduction of 38 percent (95 percent confidence interval, 27 percent to 48 percent) after day 3. When zinc supplementation was initiated within three days of the onset of diarrhea, there was a 39 percent reduction (95 percent confidence interval, 7 percent to 61 percent) in the proportion of episodes lasting more than seven days. In the zinc-supplementation group there was a decrease of 39 percent (95 percent confidence interval, 6 percent to 70 percent) in the mean number of watery stools per day (P = 0.02) and a decrease of 21 percent (95 percent confidence interval, 10 percent to 31 percent) in the number of days with watery diarrhea. The reductions in the duration and severity of diarrhea were greater in children with stunted growth than in those with normal growth.

Conclusions. For infants and young children with acute diarrhea, zinc supplementation results in clinically important reductions in the duration and severity of diarrhea. (N Engl J Med 1995;333:839-44.)

AMONG children in developing countries, diarrheal illnesses, especially those of prolonged duration, are important causes of growth retardation and death.[1-5] Episodes of diarrhea, which usually resolve within a few days in a healthy child, persist longer in children with malnutrition,[6,7] impaired cellular immunity,[7-9] or recurrent diarrhea.[10]

We hypothesized that zinc deficiency is a link between these risk factors and the duration of diarrhea. Diarrhea is consistently found in children with severe zinc deficiency,[11,12] as well as in animals with zinc depletion[13]; it responds quickly to zinc supplementation.[14] Zinc deficiency can result in growth retardation, especially stunting,[15] and impairment of immune function.[16,17] Finally, diarrhea leads to excess zinc losses and could contribute to zinc deficiency,[18-21] especially if the zinc content of the diet is limited.[22,23] To evaluate this hypothesis, we investigated the effect of zinc supplementation on the duration and severity of diarrhea in a community-based, double-blind, controlled trial.

METHODS

The trial was conducted in the Kalkaji neighborhood of New Delhi, India, an urban population of low socioeconomic status, between

September 1992 and November 1994. The incidence of diarrhea in this population is nine episodes per year for children up to 11 months of age and five episodes per year for children 12 to 36 months of age.[24]

Screening and Selection of Patients

All patients with diarrhea who presented to the dispensary at Kalkaji, where a special diarrhea clinic was operated by research physicians, were screened for enrollment in the trial. Children 6 to 35 months of age who were reported to have passed at least four unformed stools in the previous 24 hours, who had had diarrhea for less than seven days, and who were permanent residents of Kalkaji were selected for inclusion. Children who presented to the clinic a second time, those who were judged by the physician to have malnutrition requiring hospitalization, and those whose parents did not provide consent were excluded. The parents of children without dehydration or with mild dehydration were advised to give them 50 ml of oral rehydration solution per kilogram of body weight at home and were given packets of oral rehydration salts. Parents were advised to continue feeding the child his or her usual diet. Children who presented with dehydration of more than 7 percent as assessed clinically were enrolled, supplementation was started, and the children were referred to the All India Institute of Medical Sciences for rehydration.

The study was approved by the human research review committees at the All India Institute of Medical Sciences, Johns Hopkins School of Hygiene and Public Health, and the World Health Organization (WHO). The consent form was read to a parent, and written informed consent was obtained for each child's enrollment.

Base-Line Assessment

A base-line assessment, including a detailed physical examination, was performed by a research physician at the time of enrollment. Weight was measured using an electronic scale with a sensitivity of ±10 g (SECA, Columbia, Md.) by two independent observers; length (for children less than 24 months old) or height (for children 24 to 35 months old) was measured using Shorr length boards (Shorr Productions, Olney, Md.) to within 0.1 cm. For dehydrated children the examination was repeated after hydration. Stunting was defined as a z score of less than −2 for length or height for age (indicating a value

more than 2 SD below the median for the reference population) and wasting as a z score of less than −2 for weight for length; the reference population used was that of the National Center for Health Statistics.[25]

A venous blood sample for the estimation of zinc levels was collected with Monovette trace element–free heparinized syringes (Sarstedt, Newton, N.C.); plasma was separated within 15 minutes after collection of samples. Zinc was analyzed with standard methods.[26]

Randomization and Blinding

Randomization schedules with permuted blocks of 10[27,28] were used for children with four combinations of characteristics: those with z scores of −2 or greater for weight for length who were partially or exclusively breast-fed (stratum A1); those with z scores below −2 who were breast-fed (A2); those with z scores of −2 or greater who were not breast-fed (A3); and those with z scores below −2 who were not breast-fed (A4). Within each stratum, enrolled children were assigned sequential serial numbers indicating whether they would receive the zinc preparation or placebo. The solutions were identical in appearance and taste. The code, which was kept by WHO personnel, was not available to the investigators until the end of the study.

Intervention

The liquid preparations given to the children in the two groups were made by Sandoz India (Bombay). Each daily 10-ml dose contained vitamins A (1600 units), B_1 (1.2 mg), B_2 (1.0 mg), B_6 (1.0 mg), D_3 (200 IU), and E (6 mg) and niacinamide (20 mg). The zinc preparation contained zinc gluconate (20 mg of elemental zinc). Bottles were given to each child's mother and kept at home. A separate team of field assistants dispensed the assigned preparation to the child in the home every day except Sundays and holidays, when they left a measured dose in a separate vial for the mother to give to the child. Compliance was checked by other workers who visited to assess the child's condition and by study supervisors. The supplement was actually fed by the field worker on 78 percent of the days in the zinc-supplementation group and 79 percent of the days in the control group; on 2 to 3 percent of the days in each group no supplement was consumed.

Follow-up Visits

Each enrolled child was visited at home by a trained field worker every fifth day, and information for each of the previous five days, including the number and consistency of stools, was recorded. Children who were unavailable were visited again the next day. Mothers were asked to contact the study physicians at the clinic if they felt that their children were sick between the visits. Packets of oral rehydration salts were provided by the field worker, and the mother was advised about treating the child's diarrhea. Parents were given a card to show to any non-study health worker stating that the child should not be given any additional vitamin or mineral preparation. Children who had dysentery or who had diarrhea for 10 days or more after enrollment were given antibiotics. None of the medications used contained zinc.

Study Groups

Of 960 children screened, 13 were excluded because their parents did not give consent; no child was excluded because of malnutrition. A total of 947 children were assigned to treatment groups: 462 to the zinc-supplementation group and 485 to the control group; 576 were from stratum A1, 177 from A2, 143 from A3, and 51 from A4. Ten children were excluded from analysis (six in the zinc group and four in the control group); in five cases consent was withdrawn at the first home visit, in two the diarrhea stopped before supplementation could be begun, and in three there was missing information for two or more consecutive visits. For six children, three in each group, the total duration of diarrhea was unknown; they were included in the survival analysis but not in analyses of the total duration of diarrhea. In

eight cases, consent to collect a blood sample for the estimation of the zinc concentration was refused.

Definitions of Primary Outcomes

A day of diarrhea was defined as the passage of four or more unformed stools in 24 hours, and an episode of diarrhea was considered terminated on the last day of diarrhea that was followed by a 72-hour diarrhea-free period. A day with watery stools was defined as the passage of three or more watery stools in 24 hours.

Statistical Analysis

Statistical analysis was performed using SPSSPC+ (version 6.0), Epi-Info (version 6.0), and SAS (version 6.08) software. Relative risks and 95 percent confidence intervals were estimated by the Taylor series method.[29] The total duration of episodes was modeled with Cox survival regressions with a time-dependent covariate (PHREG in SAS 6.07 on a VMS mainframe had to be used to enable the use of the "exact option" for the handling of ties).[30,31] The time-dependent covariate was assigned a value of 0 (for children in the control group) or 1 (for those in the zinc-supplementation group) on the day that supplementation began. In addition, a second model that allowed the effect of supplementation to change between days 1 through 3 and day 4 or later after the beginning of supplementation[32] was fitted with two time-dependent variables. Finally, logistic-regression models used the duration of diarrhea (>7 days vs. ≤7 days) from the time of enrollment as the dependent variable; the treatment group, potential covariates, and interaction terms were the independent variables.[27]

RESULTS

The base-line characteristics of the children in the two groups were similar (Table 1). Identical proportions (0.4 percent) were exclusively breast-fed. One child in each group was initially hospitalized for the treatment of dehydration. Among socioeconomic indicators, the average annual incomes (about $532) and the rate of ownership of a number of household items (data not shown) were similar in the two groups.

Duration of Episodes of Diarrhea

Of 931 episodes of diarrhea, 44.4 percent resolved within three days after enrollment, and 83.5 percent resolved by day 7. Supplementation with zinc was associated with a 23 percent reduction in the risk of continued diarrhea on a given day (model 1 in Table 2). For the subgroups of children who had stunted growth or low plasma zinc concentrations at enrollment, the reductions in risk were 25 percent and 27 percent, respectively (model 1). In a model including stunting, age group, and plasma zinc concentration as covariates (model 2 in Table 2), zinc supplementation was associated with a 21 percent reduction in the risk of continued diarrhea.

Kaplan–Meier curves (data not shown), although they did not take into account the duration of diarrhea before enrollment, indicated that the reduction in the duration of diarrhea became evident on the fourth day after the beginning of supplementation. Therefore, we used a model that included the effect of supplementation for days 1 through 3 and for day 4 and subsequent days; the resulting fit was better than with model 1.[32] The estimated relative risk of continued diarrhea in

Table 1. Base-Line Demographic Characteristics and Features of the Episodes of Diarrhea, According to Study Group.*

CHARACTERISTIC	ZINC SUPPLEMENTATION (N = 456)	CONTROL (N = 481)
Age at enrollment (% of children)		
6–11 mo	40.6	40.7
12–23 mo	39.9	39.5
24–35 mo	19.5	19.8
Male sex (%)	50.9	52.4
Any breast-feeding at enrollment (%)	76.3	75.5
Duration of diarrhea before enrollment (days)	3.4±1.8	3.4±1.9
No. of unformed stools in previous 24 hr	7.8±3.6	7.7±3.6
No. of watery stools in previous 24 hr	3.7±4.9	4.0±4.7
z Score for weight for age <−2.0 (%)	21.3	21.4
z Score for height for age <−2.0 (%)	54.2	53.8
Stunted growth (%)	40.4	40.5
Wasting (%)	7.5	8.1
Stunted growth and wasting (%)	13.8	13.3
Episode of diarrhea in past 2 mo (%)	67.1	65.5
Fever during index episode (%)	37.7	36.4
Vomiting in 24 hr before enrollment (%)	17.3	20.2
Intake of a drug during episode (%)	22.1	24.9
Plasma zinc <60 μg/dl at enrollment (%)†	36.5	37.7

*Plus–minus values are means ±SD.

†Zinc levels were estimated for 452 children in the zinc-supplementation group and 477 in the control group. To convert values for zinc to micromoles per liter, multiply by 0.1530.

the zinc-supplementation group as compared with the control group was 0.93 (95 percent confidence interval, 0.78 to 1.09) during days 1, 2, and 3 of supplementation and 0.62 (95 percent confidence interval, 0.52 to 0.73) after day 3.

Persistence and Severity of Diarrhea

In the zinc-supplementation group there was a significant reduction (39 percent) in episodes of diarrhea lasting more than seven days after enrollment when supplementation was started within three days of the onset of diarrhea (Table 3). To evaluate the effect of supplementation in a multivariate analysis, we used a logistic-regression model in which the dependent variable was the duration of diarrhea (≤ 7 vs. >7 days from the onset of treatment); sex, the use of a drug during the episode (yes or no), breast-feeding (yes or no), years of schooling of the child's mother, study group (zinc supplementation or control), z score for height for age (<-2.0 or ≥ -2.0), and the plasma zinc concentration (<60.0 or ≥ 60.0 μg per deciliter [<9.2 or ≥ 9.2 mmol per liter]) were entered as independent variables. In this model, the odds ratio for diarrhea lasting more than seven days was 0.79 with zinc supplementation (95 percent confidence interval, 0.64 to 0.96). The odds ratio was 0.74 (95 percent confidence interval, 0.57 to 0.95) when the model was restricted to the children enrolled by day 3 of the episode of diarrhea.

There was a 39 percent reduction (95 percent confidence interval, 6 percent to 70 percent) in the mean number of watery stools per day in the zinc-supple-

mentation group (P = 0.02) and a 21 percent reduction (95 percent confidence interval, 10 percent to 31 percent) in the number of days with watery stools. In the zinc-supplementation group the mean (±SD) number of watery stools per day was 3.1±9.9, as compared with 5.1±14.9 in the control group. Fewer children in the zinc-supplementation group were taken to a physician at least once during follow-up because their parents were concerned about their health, but this difference was not significant (P = 0.12).

Analyses of Subgroups

The reduction in the likelihood of diarrhea lasting more than seven days was 65 percent in the subgroup of children with stunted growth who had had diarrhea for less than four days before enrollment (Table 4). The effect of zinc on the number of days with watery stools was greater in children with stunted growth than in those with normal growth (relative risk in the zinc-supplementation group as compared with the controls, 0.59 [95 percent confidence interval, 0.48 to 0.73] and 0.95 [95 percent confidence interval, 0.79 to 1.15], respectively). In children with wasting (z score for weight for length <-2), there was an even greater reduction in the number of days of watery diarrhea (relative risk in the zinc-supplementation group, 0.48; 95 percent confidence interval, 0.39 to 0.68). There was no clear trend with respect to the effect within subgroups defined according to the plasma zinc levels.

Adverse Reactions

Four children, two in each group, reported vomiting immediately after consuming the supplement on one or two occasions.

DISCUSSION

This study, conducted in New Delhi, documents the effectiveness of zinc supplementation as an adjunct to oral rehydration therapy and early continued feeding among preschool children with acute diarrhea. Zinc supplementation was associated with a clinically im-

Table 2. Relative Risk of Continued Diarrhea in the Cox Regression Models, According to Study Group.*

MODEL No.	COVARIATES†	ALL CHILDREN (N = 937)	CHILDREN WITH STUNTING (N = 506)	CHILDREN WITH PLASMA ZINC <60 μg/dl (N = 315)
		relative risk (95% confidence interval)		
1	Study group	0.77 (0.68–0.88)	0.75 (0.63–0.90)	0.73 (0.59–0.91)
2	Study group, age group, plasma zinc level, stunting	0.79 (0.69–0.90)	—	—

*Relative risks are of continued diarrhea on a given day during the episode, calculated as the rate in the zinc-supplementation group as compared with that in the control group.

†Age group was categorized as <12, 12–23, or >23 months, plasma zinc levels as <60.0 or ≥60.0 μg per deciliter (<9.2 or ≥9.2 mmol per liter), and stunting as a z score for height for age <−2 or ≥−2.

portant and statistically significant overall reduction of 23 percent in the risk of continued diarrhea and a 39 percent reduction in the frequency of episodes persisting more than seven days after treatment began. It also resulted in a 21 percent reduction in the number of days with watery stools and a 39 percent reduction in the mean number of watery stools per day. Reductions in the frequency of prolonged diarrhea and the number of days with watery stools may decrease the risk of dehydration and the need for fluid and electrolyte replacement. We could not measure the effect of zinc supplementation on dehydration because oral-rehydration solution was provided from the day of enrollment. Reductions in the frequency of diarrhea may also improve growth.[33]

Because the study was conducted with the children at home rather than in the hospital, they were able to continue their usual diets. We made relatively few visits to assess morbidity, so that the natural history of the illness could be evaluated without intensive medical intervention, although this practice might have limited the precision of the information on daily outcomes.

In a previous trial of supplementation with 20 mg of zinc daily in children with acute diarrhea, there were no differences in outcomes overall, but the children with low zinc concentrations in the rectal mucosa had shorter episodes of diarrhea and less frequent stools.[34] In a small study of children with persistent diarrhea, the administration of a 20-mg zinc supplement was associated with a 20 percent reduction in the duration of diarrhea and the frequency of stools, although these differences were not statistically significant.[35]

The finding of a sizable effect of supplementation and the observation that 37 percent of the children with diarrhea had plasma zinc levels below 60 μg per deciliter suggest a high prevalence of zinc deficiency in this population. Since the plasma zinc level was meas-

Table 4. Duration and Severity of Episodes of Diarrhea among Children with Stunted Growth and Children with Plasma Zinc Levels <60 μg/dl at Enrollment, According to Study Group.

OUTCOME MEASURE	ZINC SUPPLEMENTATION	CONTROL	RELATIVE RISK (95% CI)*
Children with stunted growth			
No. of children	247	259	—
Episodes lasting >7 days — % of children	13.4	17.5	0.77 (0.51–1.16)
Episodes occurring <4 days before enrollment, lasting >7 days — % of children†	5.8	16.7	0.35 (0.17–0.71)
Watery stools — % of children	20.6	31.5	0.59 (0.48–0.73)
Children with plasma zinc levels <60 μg/dl			
No. of children	153	162	—
Episodes lasting >7 days — % of children	13.1	17.9	0.73 (0.43–1.23)
Episodes occurring <4 days before enrollment, lasting >7 days — % of children‡	12.1	16.8	0.72 (0.36–1.44)
Watery stools — % of children	19.1	19.1	1.00 (0.75–1.33)

*Relative risks are the rate of the outcome in question in the zinc-supplementation group as compared with that in the control group. CI denotes confidence interval.

†Refers to 156 children in the zinc-supplementation group and to 156 children in the control group.

‡Refers to 99 children in the zinc-supplementation group and to 95 children in the control group.

ured during the episode of diarrhea, a transient effect of the episode on plasma zinc levels cannot be eliminated[36-42]; however, similar plasma zinc levels were found in the study children who did not receive the supplement 120 days after episodes of diarrhea (unpublished data). Studies from other developing countries suggest that dietary zinc deficiency can be highly prevalent among preschool-age children.[22,23] The diets of young children in our Indian study population share two characteristics with other zinc-deficient populations: a low intake of meat or dairy products, which contain zinc, and a high intake of phytates, which interfere with the bioavailability of zinc.

The comparison of the effects of supplementation among subgroups indicates that stunting, wasting, or both can be used to select the children most likely to benefit from treatment with zinc. The initial plasma zinc level did not differentiate those who would derive greater benefit from supplementation from those who would benefit less.

The possible mechanisms for the effect of zinc supplementation on diarrhea include improved absorption of water and electrolytes by the intestines,[43-49] regeneration of gut epithelium or the restoration of its function,[50-58] increased levels of enterocyte brush-border enzymes,[59-65] and enhanced immunologic mechanisms for the clearance of infection, including cellular immunity and higher levels of secretory antibodies.[66-68] It is also possible that improved appetite and dietary intake resulted in shorter episodes of diarrhea, as a result of the effect of staple foods on stool consistency.[69]

In conclusion, a dietary supplement of zinc, along

Table 3. Frequency of Diarrhea Lasting More Than Seven Days and Severity of Episodes of Diarrhea, According to Study Group.*

OUTCOME MEASURE	ZINC SUPPLEMENTATION (N = 456)	CONTROL (N = 481)	RELATIVE RISK (95% CI)†
Episodes lasting >7 days (% of children)			
All children	15.4	18.8	0.87 (0.65–1.16)
Children enrolled by day 4‡	10.2	16.8	0.61 (0.39–0.93)
Watery stools (% of children)	23.6	29.8	0.79 (0.69–0.90)§
Taken to a physician (% of children)	12.7	17.3	0.78 (0.57–1.07)
Multivariate analysis of episodes lasting >7 days			
All children			0.79 (0.64–0.96)
Children enrolled by day 4			0.74 (0.57–0.95)

*Plus–minus values are means ±SD.

†Relative risks are the rate of the outcome in question in the zinc-supplementation group as compared with that in the control group. CI denotes confidence interval.

‡Refers to 284 children in the zinc-supplementation group and to 285 children in the control group.

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with selected vitamins, resulted in clinically important reductions in the duration and severity of diarrhea among preschool-age children. Because these findings may have important implications for the reduction of morbidity and mortality due to diarrhea in children, they need to be confirmed in other developing countries.

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