Efficacy of zinc supplementation in reducing the incidence and prevalence of acute diarrhea—a community-based, double-blind, controlled trial\textsuperscript{1–3}

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ABSTRACT A community-based, double-blind, randomized trial was conducted in a population of low socioeconomic status in urban India to determine whether daily zinc supplementation reduces the incidence and prevalence of acute diarrhea, especially in those with zinc deficiency. Children 6–35 mo of age were randomly assigned to zinc (n = 286) and control (n = 293) groups and received a supplement daily for 6 mo. Zinc gluconate (10 mg elemental Zn) was given, with both zinc and control groups also receiving multivitamins. The primary outcome measures determined by home visits every fifth day and physician examinations were the number of acute diarrheal episodes (incidence) and total diarrheal days (prevalence). Zinc supplementation had no effect in children 6–11 mo old. In children aged > 11 mo there was significantly less diarrhea in the zinc group. In boys > 11 mo old, supplementation resulted in a 26% (95% CI: 13%, 38%) lower diarrheal incidence and a 35% (95% CI: 20%, 50%) lower prevalence. In zinc-supplemented girls > 11 mo of age, the incidence was 17% (95% CI: 2%, 30%) lower and the prevalence was 19% (95% CI: 4%, 47%) lower. Overall, zinc supplementation resulted in a 17% (95% CI: 1%, 30%) lower diarrheal incidence in children with plasma zinc concentrations < 9.18 \mu mol/L at enrollment and a 33% (95% CI: 6%, 52%) lower incidence in children with concentrations < 50 \mu mol/L. In conclusion, zinc supplementation had a significant effect on acute diarrheal morbidity in children > 11 mo old and in children with low plasma zinc concentrations.

KEY WORDS Infantile diarrhea, diarrhea prevention, humans, India, malnutrition, randomized trial, zinc

INTRODUCTION

Inadequate dietary intake and diarrhea, associated with negative zinc balance, may contribute to zinc deficiency (1–6), which can result in growth retardation (7) and impairment of immune function (8–13). Each of these factors related to zinc nutriture, ie, recent diarrhea, malnutrition, and impaired cellular immunity, have been found to be risk factors for diarrhea in developing countries (14–19). Furthermore, in clinical conditions with severe zinc deficiency (20, 21) or in animal experiments with zinc depletion (22), diarrhea is consistently found and responds quickly to zinc supplementation.

We hypothesized that zinc deficiency, possibly mediated by impaired immunity, is an important determinant of diarrheal morbidity. Because the evaluation of zinc status is complicated by the lack of a specific and sensitive laboratory indicator (23), response to zinc supplementation remains the most valid approach to investigation. Therefore, we evaluated in a community-based, double-blind randomized trial the effect of daily zinc supplementation on diarrheal morbidity.

SUBJECTS AND METHODS

The details of the study population, baseline assessments, randomization, and subject selection were reported previously (24, 25). Briefly, a subgroup of children enrolled in a trial of the therapeutic effect of zinc supplementation (24) were randomly selected at the time of initial enrollment to enter a 6-mo follow-up trial after recovery from the enrollment diarrheal episode. The study was approved by the human research review committees at the All India Institute of Medical Sciences, the Johns Hopkins School of Hygiene and Public Health, and the World Health Organization (WHO).

Eligibility

Children aged 6–35 mo with reported passage of at least four unformed stools in the previous 24 h, a diarrheal duration of < 7 d, and permanent residence in the study area were enrolled in a trial evaluating the therapeutic effect of zinc supplementation on acute diarrhea (24). A child could only be enrolled once. Of 947 children enrolled in that trial, 609 were designated at the beginning of the study to participate in the follow-up trial to determine whether continued zinc supplementation reduced the occurrence of diarrhea. At the first home visit after recovery from the enrollment diarrheal episode (pass-
sage of at least three stools for 3 consecutive days) these children were enrolled in the 6-mo follow-up trial. Before enrollment, a parent of the child was given a full explanation of the study and written informed consent was obtained. The supplementation trial was conducted between September 1992 and November 1994.

**Intervention and follow-up**

The supplement was a liquid preparation (Sandoz India Ltd, Mumbai, India). Each 5 mL contained vitamin A (240 μg retinol equivalents), thiamine (0.6 mg), riboflavin (0.5 mg), vitamin B-6 (0.5 mg), cholecalciferol (2.5 μg), vitamin E (3 mg α-tocopherol equivalents), and niacin (10 mg niacin equivalents). The zinc preparation contained zinc gluconate (10 mg elemental Zn). A fixed dose of 5 mL per child was given daily for 6 mo to all enrolled children; during diarrheal illness this was increased to 10 mL to provide for excess zinc losses. Field assistants different from the morbidity surveillance fieldworkers visited the family every day and fed the preparation to the child. On Sundays or holidays, or if the child was not available, measured daily doses of the assigned liquid were left in separate vials for the mother to feed. A detailed log of days when the field assistant or mother did or did not feed the supplement was maintained. Of 90,421 d of follow-up, children were actually fed the supplement by a fieldworker on 78% of days in the zinc group and 79% in the control group. Additionally, a relative fed the supplement for 19% of days in the zinc group and 18% in the control group. There were no differences in the consumption of the supplements in the subgroups analyzed.

Each child was visited at home every fifth day for the 180-d intervention period (25). If the child was not available on the scheduled day, the house was revisited the following day. At these visits, information on the number of diarrheal stools, consistency of stools, fever, vomiting, and feeding history for each of the previous 5 d was recorded.

Anthropometric assessments (weight and length or height) were performed at baseline and at monthly intervals. Plasma zinc concentration was determined at baseline (during the enrollment diarrheal episode) and after 120 d of supplementation (24–26).

**Study outcomes**

The primary outcome measures for the present analysis were incidence and prevalence of acute diarrhea. A diarrheal day was defined as a 24-h period with passage of at least four uniformed stools and a diarrheal episode was considered terminated on the last day of diarrhea followed by a 72-h diarrhea-free period. The days of observation were determined by subtracting the days that the child was absent from the total days in the study. The number of new episodes of diarrhea occurring during the 180-d follow-up was used as the numerator for incidence. The number of diarrheal days was used for prevalence.

**Power estimation**

Before the study, the necessary sample size was determined based on detecting a 15% decrease in diarrheal incidence in all children and a 20% decrease in zinc-deficient children. Allowing a 20% increase in the sample to account for extra-Poisson variation and 20% more for possible attrition, we decided to enroll 300 children per group. Posttrial estimates, using the actual number of observed child-intervals, the incidence in the control group, and a factor for inflation of the SE, indicated that we had a power of 90% to detect a 16% reduction in incidence and power of 80% to detect a reduction of ≥ 14%. Within the subgroup of children 6–11 mo old, we had a power of 80% to detect a reduction ≥ 20%.

**Analysis**

Of 609 children (298 in the zinc group and 311 in the control group), 30 children (12 in the zinc and 18 in the control group) with actual follow-up of < 30 d were excluded from the analysis. The excluded children did not differ from other children in baseline characteristics. An analysis of 579 children (286 zinc and 293 control group) is presented. If all 579 children included in the analysis had completed follow-up (180 d), there would have been 104,220 d of observation. There were 90,421 d of actual follow-up, indicating that loss of data due to withdrawal, temporary nonavailability, or permanent attrition was only 13% (13% for the zinc and 14% for the control group). Analysis was performed by using SPSSPC+ (version 6.0; SPSS Inc, Chicago), EPI-INFO (version 6.0; US Centers for Disease Control and Prevention, Atlanta) and SAS (version 6.08; SAS Institute Inc, Cary, NC) software.

Children < −2.0 SDs (Z scores) from the height-for-age median compared with the National Center for Health Statistics (NCHS) US reference population (27, 28) were considered stunted and children < −2.0 Z scores from the weight-for-height median were considered wasted. EPI-INFO was used for these calculations.

Criteria for subgroups based on age (6–11 or 12–35 mo) and plasma zinc concentrations (< 9.18 μmol/L or ≥ 9.18 μmol/L) were selected before analysis; the 9.18-μmol/L (< 60 μg/dL) cutoff for deficiency was selected according to the literature (29) and the standard of the laboratory where the zinc assay was performed. A plasma zinc concentration < 7.65 μmol/L (< 50 μg/dL) was selected as a concentration indicating more severe deficiency.

**Statistical methods**

The primary interest in these analyses was the preventive effect of zinc supplementation on the incidence and prevalence of diarrhea in all children and in important subgroups. To assess the effect of zinc supplementation on incidence, a Poisson regression model was used (30, 31). The model with dependent variable y as the number of episodes, independent variable x as the group (zinc or control), and days (log transformed) of follow-up as an offset term provided the rate ratio for incidence between zinc and control groups. For the multivariate analyses, covariates were added as additional independent variables. To assess the effect of zinc supplementation on prevalence, a marginal-logistic-regression model was used (32). The model with dependent variable y (events/trials) as days of diarrhea/days of follow-up and independent variable x as the group (zinc or control) provided the odds ratio for prevalence between zinc and control groups. For multivariate analyses, covariates were added as additional independent variables.

Because multiple episodes or days of diarrhea contributed by a single child were used in analyses of morbidity rates and
because morbidity is correlated within children (ie, some children have high rates and some have low rates), traditional analyses of rate ratios result in spuriously narrow CIs, hence overestimating significance (33). Information from the regression analyses were used to correct for this error. The residual deviance and its degrees of freedom from each regression were used to calculate an adjustment factor phi (Φ = residual deviance/degrees of freedom). The SE from the regression was inflated by a factor of Φ and then adjusted SEs were used to calculate 95% CIs for rate ratios and odds ratios.

Interactions between the effect of zinc supplementation and age, sex, stunting, and zinc concentrations were assessed first in separate models, each fitting treatment group, one covariate, and a term for its interaction with treatment group. There were no significant interactions with age and sex, which were independent significant in a model fitting all interaction terms simultaneously. In the final analyses two Poisson or logistic regression models were constructed to provide an estimate for the rate ratio or odds ratio for each subgroup of interest. In the first analysis, β2 provided the estimate of the effect of zinc supplementation in boys > 11 mo of age, whereas β3 provided the estimate of this effect in girls > 11 mo of age. In the second analysis, β2 was the estimate of effect of supplementation in boys > 11 mo with initial plasma zinc < 9.18 μmol/L, β3 was the effect in boys > 11 mo with zinc ≥ 9.18 μmol/L, β4 was the effect in girls > 11 mo with zinc < 9.18 μmol/L, and β5 was the effect in girls > 11 mo with zinc ≥ 9.18 μmol/L.

Statistically significant results were those with P values < 0.05 and results considered of borderline significance had P values < 0.10.

RESULTS

The comparability of baseline characteristics and effects of supplementation on plasma zinc concentrations were reported previously for the children selected for the 6-mo follow-up trial (25). The two groups did not differ significantly in age, sex, anthropometric status, socioeconomic status, or family characteristics. The baseline plasma zinc concentrations were (± SD) 9.88 ± 3.39 μmol/L in the zinc group and 9.94 ± 3.20 μmol/L in the control group. After 120 d in the trial, the zinc concentrations in the zinc and control groups were 13.4 ± 5.48 and 9.76 ± 2.10 μmol/L, respectively (P < 0.001). This difference was consistent in children < 11 or ≥ 11 mo old and in boys and girls. Both boys and girls > 11 mo old in the zinc group had significantly higher plasma zinc concentrations than those in the control group after 120 d of supplementation (Table 1).

Zinc-supplemented children had an 8% lower incidence and a 6% lower prevalence of diarrhea (Table 2). These differences were not significant after correction for correlation within children. In the children with plasma zinc concentrations < 9.18 μmol/L at enrollment, the zinc-supplemented group had a 17% (95% CI: 1%, 30%) lower incidence of diarrhea whereas in children with plasma zinc < 7.65 μmol/L the zinc-supplemented group had a 33% (95% CI: 6%, 52%) lower incidence (Table 2).

There was a significant interaction between treatment group and age. There was no effect of zinc supplementation on either incidence or prevalence of diarrhea in children 6–11 mo of age; both incidence and prevalence were slightly higher in zinc-supplemented children in this age group, but the differences were not significant (Table 2). In the older children there was in addition a significant interaction of treatment group with sex and a moderate interaction (of borderline significance) with plasma zinc concentration.

For children > 11 mo of age, two models each for incidence and prevalence were fit, one estimating a separate effect by sex and the second estimating an effect by sex and initial plasma zinc concentration. Zinc supplementation resulted in a significantly lower incidence in both boys and girls > 11 mo of age (Table 3). In zinc-supplemented children the incidence of diarrhea was 26% (95% CI: 13%, 38%) lower in boys and 17% (95% CI: 2%, 30%) lower in girls. In boys, both zinc-deficient children and those with values above the cutoff used for zinc deficiency had lower diarrheal incidence with zinc supplementation. In girls, only those defined as zinc deficient had a significant effect of zinc supplementation.

The zinc-supplemented boys > 11 mo of age had a 35% (95% CI: 20%, 50%) lower prevalence of diarrhea. In girls, the difference was smaller (19%) and not significant (Table 3). In both boys and girls the supplementation effect on prevalence was greater in zinc-deficient children, but the difference in effect comparing zinc-deficient and nondeficient children was greater in girls (35% compared with 7%) than in boys (39% compared with 34%).

DISCUSSION

With daily supplementation of 10 mg elemental Zn (which was doubled during diarrhea), we observed a clinically and statistically significant reduction of 17–26% in incidence and

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**TABLE 1**

Plasma zinc concentrations after 120 d of supplementation in boys and girls > 11 mo of age by intervention group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Zinc group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc concentrations after 120 d</td>
<td>12.43 ± 5.04 [69]^T</td>
<td>9.95 ± 2.64 [73]^T</td>
</tr>
<tr>
<td>Mean of difference in plasma zinc concentration between baseline and 120 d per child</td>
<td>2.68 ± 5.04 [69]^T</td>
<td>0.20 ± 2.69 [73]^T</td>
</tr>
<tr>
<td>Girls</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc concentrations after 120 d</td>
<td>13.53 ± 6.10 [76]^T</td>
<td>9.59 ± 3.49 [73]^T</td>
</tr>
<tr>
<td>Mean of difference in plasma zinc concentration between baseline and 120 d per child</td>
<td>4.02 ± 6.31 [76]^T</td>
<td>0.23 ± 2.74 [73]^T</td>
</tr>
</tbody>
</table>

^T x ± SD; n children with plasma zinc concentrations at both baseline and after 120 d in brackets.
^T^T Significantly different from control group: ^T P < 0.001, ^T^T P < 0.0001.
TABLE 2
Effect of zinc supplementation on incidence and prevalence of diarrhea in children during 180 d of follow-up

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Zinc group</th>
<th>Control group</th>
<th>(\Phi^a)</th>
<th>Rate ratio or odds ratio(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of children</td>
<td>286</td>
<td>293</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of diarrhea(^c)</td>
<td>934 (7.6)</td>
<td>1033 (8.3)</td>
<td>1.23</td>
<td>0.92 (0.82, 1.02)</td>
</tr>
<tr>
<td>Prevalence of diarrhea(^d)</td>
<td>3854 (31.4)</td>
<td>4150 (33.3)</td>
<td>3.54</td>
<td>0.94 (0.80, 1.10)</td>
</tr>
<tr>
<td>Follow-up (d)</td>
<td>44 866</td>
<td>45 555</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children with plasma zinc &lt; 9.18 (\mu)mol/L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of children</td>
<td>110</td>
<td>115</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of diarrhea</td>
<td>333 (6.9)</td>
<td>429 (8.5)</td>
<td>1.86</td>
<td>0.83 (0.70, 0.99)</td>
</tr>
<tr>
<td>Prevalence of diarrhea</td>
<td>1311 (27.1)</td>
<td>1725 (34.1)</td>
<td>3.50</td>
<td>0.83 (0.63, 1.09)</td>
</tr>
<tr>
<td>Follow-up (d)</td>
<td>17 656</td>
<td>18 467</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children with plasma zinc &lt; 7.65 (\mu)mol/L.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of children</td>
<td>29</td>
<td>34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of diarrhea</td>
<td>83 (6.5)</td>
<td>145 (9.2)</td>
<td>1.20</td>
<td>0.67 (0.48, 0.94)</td>
</tr>
<tr>
<td>Prevalence of diarrhea</td>
<td>330 (25.6)</td>
<td>635 (40.2)</td>
<td>3.89</td>
<td>0.60 (0.34, 1.04)</td>
</tr>
<tr>
<td>Follow-up (d)</td>
<td>4661</td>
<td>5764</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children 6–11 mo old</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of children</td>
<td>109</td>
<td>112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence of diarrhea</td>
<td>432 (9.6)</td>
<td>419 (9.1)</td>
<td>1.07</td>
<td>1.05 (0.91, 1.21)</td>
</tr>
<tr>
<td>Prevalence of diarrhea</td>
<td>1933 (42.3)</td>
<td>1816 (39.6)</td>
<td>3.32</td>
<td>1.09 (0.87, 1.37)</td>
</tr>
<tr>
<td>Follow-up (d)</td>
<td>16 521</td>
<td>16 760</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Estimated correction factor for the SE of \(\beta\) (residual deviance/degrees of freedom).

\(^b\) Rate ratio for incidence estimated by using Poisson regression and odds ratio for prevalence estimated by using marginal logistic regression, with corrected 95% CIs in parentheses.

\(^c\) Number of episodes of diarrhea (incidence in episodes per child-year).

\(^d\) Number of days of diarrhea (prevalence in days per child-year).

19–35% in prevalence of diarrhea in children > 11 mo of age. We did not find an effect on diarrheal morbidity in infants 6–11 mo old. The somewhat greater effect on prevalence than incidence suggests that there was also an effect on the duration of episodes in this 6-mo trial, as we reported previously from the enrollment episode therapeutic trial (24).

This study should be interpreted as an effect of zinc supplementation in the presence of a multivitamin supplement because this was given to both groups. A previous study in a similar group of Indian children found no benefit of high-dose vitamin A supplementation on diarrheal incidence (34). Other studies of vitamin A supplementation have had widely varying results ranging from modest reductions to increases in diarrheal incidence or prevalence (35–39). It is possible that adequate vitamin A nutrition is necessary for the effect of zinc to be seen or vice versa because many biological interactions between vitamin A and zinc have been shown (40, 41). Although it would be of scientific value to determine the relative effects of single micronutrients or combinations, it is pragmatic to give multivitamin-mineral supplements to populations that may be deficient in multiple micronutrients. Additional knowledge of the role of various specific deficiencies could, however, guide interventions to improve dietary quality.

A previous community-based, controlled trial of zinc supplementation did not detect a difference in clinic visits for diarrhea; no active morbidity surveillance was performed in that study (42). A trial of zinc supplementation (10 mg/d) in growth-retarded Vietnamese children showed a 71% lower

TABLE 3
Effect of zinc supplementation in children > 11 mo of age within subgroups by sex and baseline plasma zinc status

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Supplementation group</th>
<th>Incidence</th>
<th>Prevalence</th>
<th>(\Phi^a)</th>
<th>Rate ratio(^b)</th>
<th>(\Phi^e)</th>
<th>Odds ratio(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Zinc</td>
<td>Control</td>
<td>(\Phi^a)</td>
<td>Rate ratio(^b)</td>
<td>(\Phi^e)</td>
<td>Odds ratio(^c)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>91 (14 639)(^d)</td>
<td>93 (14 777)</td>
<td>1.21</td>
<td>0.74 (0.62, 0.87)</td>
<td>3.46</td>
<td>0.65 (0.50, 0.80)</td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>86 (13 906)</td>
<td>88 (14 018)</td>
<td>1.21</td>
<td>0.83 (0.70, 0.98)</td>
<td>3.46</td>
<td>0.81 (0.53, 1.04)</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>Zinc concentration &lt; 9.18 (\mu)mol/L.</td>
<td>29 (4727)</td>
<td>30 (4821)</td>
<td>1.21</td>
<td>0.70 (0.53, 0.93)</td>
<td>3.46</td>
<td>0.61 (0.40, 0.95)</td>
</tr>
<tr>
<td></td>
<td>Zinc concentration ≥ 9.18 (\mu)mol/L.</td>
<td>62 (9912)</td>
<td>63 (9956)</td>
<td>1.21</td>
<td>0.76 (0.69, 0.92)</td>
<td>3.46</td>
<td>0.66 (0.49, 0.90)</td>
</tr>
<tr>
<td>Females</td>
<td>Zinc concentration &lt; 9.18 (\mu)mol/L.</td>
<td>32 (5907)</td>
<td>41 (6841)</td>
<td>1.21</td>
<td>0.73 (0.57, 0.94)</td>
<td>3.46</td>
<td>0.65 (0.44, 0.96)</td>
</tr>
<tr>
<td></td>
<td>Zinc concentration ≥ 9.18 (\mu)mol/L.</td>
<td>49 (7999)</td>
<td>47 (7177)</td>
<td>1.21</td>
<td>0.90 (0.73, 1.10)</td>
<td>3.46</td>
<td>0.93 (0.70, 1.26)</td>
</tr>
</tbody>
</table>

\(^a\) Estimated correction factor for the standard error of \(\beta\) (residual deviance/degrees of freedom).

\(^b\) Rate ratio for incidence estimated by using Poisson regression with corrected 95% CI in parentheses.

\(^c\) Odds ratio for prevalence estimated by using marginal logistic regression with corrected 95% CIs in parentheses.

\(^d\) Number of children in the subgroup; days of follow-up in the subgroup in parentheses.
incidence of diarrhea (43) and a trial in Mexico found a 36% lower incidence of diarrheal episodes in zinc-supplemented (20 mg/d) children (44). We reported previously a significant reduction in the incidence of persistent diarrhea in children > 11 mo old and in the incidence of dysentery in boys (25). Two small hospital-based trials evaluating the therapeutic effect of zinc supplementation on acute (45) and persistent (46) diarrheal episodes found significantly reduced duration and stool frequency in acute diarrhea in children with low rectal mucosal zinc concentrations and reduced duration and severity in persistent diarrhea, which were not significant. For the enrollment episode in our large trial, we found a significant effect of zinc supplementation on the duration and severity of acute diarrhea (24).

The sizable effect of zinc supplementation and the high proportion (37%) of children with plasma zinc concentrations < 9.18 μmol/L are suggestive of a high prevalence of zinc deficiency in this setting. There have been reports of negative zinc balance and depressed plasma or tissue zinc concentrations during diarrhea in children (1–5, 47, 48). Although we cannot exclude the possibility that the low plasma zinc concentrations at enrollment were a consequence of the diarrheal episode, this seems unlikely because the mean zinc concentrations were similar between the baseline and 120-d samples in the control group and the number of children with a zinc concentration < 9.18 μmol/L actually increased. A decline in plasma zinc concentration between 6 and 9 mo of age was reported previously (49). Although plasma zinc concentration may not be an optimal measure of zinc status in an individual, when used at a population level it does appear to indicate a subgroup of children that will benefit more from zinc supplementation.

Our observation that zinc supplementation had no effect among infants 6–11 mo old needs further investigation. It is possible that extensive breast-feeding in these infants may result in less zinc deficiency in this age group, although the similar plasma zinc concentrations in younger compared with older children at baseline argue against this explanation. Furthermore, in our analysis of the therapeutic effect of zinc in the enrollment episode, there was equal benefit in younger and older children (24).

The reason for a possibly greater effect of zinc supplementation in boys than girls is unknown; however, this finding is consistent with previous observations of a greater effect of zinc supplementation on growth in boys (7, 50). Estimates of zinc requirements for infant growth are higher for boys than for girls, which has been given as a possible explanation for the observed differences in zinc effect by sex (51). Our finding of no appreciable difference in the effect of supplementation between boys and girls with low plasma zinc suggests that all zinc-deficient children will benefit regardless of sex. The different effect by sex was in children with baseline plasma zinc concentrations above the value for zinc deficiency used in this analysis. In this subgroup, zinc-supplemented boys had significantly lower rates of diarrhea than did control subjects, but girls did not.

The mechanisms for the protective effect of zinc supplementation may be a restoration of immune function in zinc-deficient children. Zinc deficiency has been shown to be associated with atrophy of lymphoid tissues, reduced lymphocyte count, and proportion of CD4 helper T cells (21, 52, 53), decreased lymphocyte proliferation (54), impaired delayed cutaneous hypersensitivity (55–57), reduced cytotoxic activity of lymphocytes (10, 58), and reduced natural killer cell activity (59). All of these respond dramatically to repletion of zinc (60). Indeed, zinc-supplemented children, in comparison with control children, had significantly better responsiveness to skin tests and a significantly increased proportion of helper T cells, indicating improved cellular immune status (61).

In conclusion, zinc supplementation had a significant effect on diarrheal morbidity in children > 11 mo old. These findings, if confirmed in other settings, would have important implications for efforts to improve the health and survival of children in developing countries.

We thank Michael Hambidge and Jaime Westcott for help with plasma zinc estimations. Scott Zegar and Larry Moulton for statistical suggestions. Dhirminder Kashyap and Usha Dhirgna for data management, all the field staff of the project for their effort and cooperation and the staff of “Asha” and “Dipalaya” for allowing us to use their clinic space. We are especially appreciative of the pains that Ashok Aggarwal of Sandoz took to make the supplement, maintain its quality, and ensure delivery on time throughout the study.

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341


