# Plasma zinc as a predictor of diarrheal and respiratory morbidity in children in an urban slum setting[1–3]

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**ABSTRACT** The association between low initial plasma zinc concentration and risk of morbidity over the subsequent 3 mo was examined in a cohort of 116 children aged 12–59 mo recovering from acute diarrhea. Children with low initial plasma zinc (≤ 8.4 μmol/L) had more episodes of diarrhea [risk ratio (RR): 1.47; 95% CI: 1.03, 2.49) and severe diarrhea, defined as passage of ≥ 5 liquid stools in a 24-h period, (RR: 1.70; 95% CI: 1.06, 2.72) than did children with normal plasma zinc (> 8.4 μmol/L). The mean prevalence rate of diarrhea associated with fever was 4 times higher in the zinc-deficient group ($P = 0.01$). Overall, the difference in the number of episodes of acute lower respiratory tract infections (ALRIs) between the two groups was not statistically significant (RR: 1.76; 95% CI: 0.88–3.53) but the mean prevalence rate of ALRIs was 3.5 times higher in children with low plasma zinc ($P = 0.05$). The increased risk of diarrhea and ALRIs episodes in zinc-deficient children was larger in boys than in girls. These results show that children with low plasma zinc concentrations are at risk for increased diarrheal and respiratory morbidity. *Am J Clin Nutr* 1998(suppl); 68:414S–7S.

**KEY WORDS** Plasma zinc, zinc deficiency, diarrhea, acute respiratory infections, morbidity, children, India

## INTRODUCTION

Mild to moderate zinc deficiency is common in several developing countries, including India, because the commonly consumed staple foods have low zinc contents and are rich in phytates, which inhibit the absorption and utilization of zinc. Zinc deficiency results in impaired immunity, which may increase the risk of infections, including diarrhea (1–4). In experimental studies, zinc deficiency has been shown to have direct effects on the gastrointestinal tract, including villus atrophy, decreased brush border disaccharidase activity, and impaired intestinal transport (5, 6). Diarrhea is a cardinal feature of the clinical syndrome of acrodermatitis enteropathica, a congenital disorder in which the clinical manifestations can be reversed by supplementation with zinc (7). It is thus plausible that zinc deficiency may increase the incidence of acute diarrhea and, once it is acquired, worsen the episode outcome.

We report herein on the association between initial low plasma zinc concentrations and increased risk of diarrheal and respiratory morbidity over 3 mo in a cohort of children aged 12–59 mo. A preliminary report of this study was published previously (8) and this article presents further analysis of the data.

## SUBJECTS AND METHODS

### Study site

The study was conducted in the urban slum of Kalkaji, New Delhi. One hundred twenty-five 12–59-mo-old children attending an outpatient facility with nondysenteric acute diarrhea were enrolled in the study. The patients were treated with an oral rehydration salts solution and visited at their homes every other day until they recovered from diarrhea.

Ten days after recovery from the diarrheal illness, the children were revisited; 120 children who were asymptomatic at that time were enrolled after informed consent was obtained from the family. Socioeconomic characteristics, weight [to the nearest 100 g (Salter Weigh-Tronix, West Midlands, United Kingdom)], and length or height (to the nearest 0.1 or 0.2 cm) were obtained at baseline. The mothers of enrolled children were instructed not to feed the child after 0800 the next day until a blood sample was obtained. The next morning at ≈1100, just before the next meal was given to the child, a venous blood sample was obtained, without use of a tourniquet, for plasma zinc estimation in zinc-free tubes containing heparin. The specimens were stored at −20 °C and shipped to the University of Colorado for zinc estimation by atomic absorption spectrophotometry (9). The specimens were analyzed only after the completion of morbidity surveillance. The study was approved by the All India Institute of Medical Sciences Ethics Committee.

### Follow-up and morbidity ascertainment

The blood specimen for zinc estimation was obtained 10 d after the subjects' recovery from the initial diarrheal episode; this day marked the beginning of the 90-d observation period, during which morbidity was ascertained through household visits conducted every third day. At each visit mothers of the subjects were queried by trained field investigators about coughing, the number of formed and liquid stools passed in a 24-h period, and fever for each day since the previous visit. If cough was

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**TABLE 1**
Baseline characteristics of children with low and normal plasma zinc concentrations

| Characteristic | Plasma zinc concentration | |
|---|---|---|
| | ≤ 8.4 μmol/L (*n* = 37) | > 8.4 μmol/L (*n* = 79) |
| Mean age (mo) | 31.9 ± 12.8 | 30.7 ± 14.4 |
| Boys [*n* (%)] | 23 (62.2) | 39 (49.4) |
| Girls [*n* (%)] | 14 (37.8) | 40 (50.6) |
| Breast-fed [*n* (%)] | 11 (29.7) | 31 (39.2) |
| Weight-for-height ≤ 80% of NCHS[1] median [*n* (%)] | 6 (16.2) | 12 (15.2) |
| Literate mothers [*n* (%)] | 3 (8.1) | 12 (15.2) |
| Mean yearly family income (rupees) | 10 659 ± 4002 | 10 922 ± 3496 |

[1] National Center for Health Statistics reference population (16).

present at visitation, respiratory rate was counted twice for 1 min each. Diarrhea and acute lower respiratory tract infections (ALRIs) were treated according to standard World Health Organization case management guidelines (10, 11).

### Definitions

Diarrhea was defined as the passage of ≥ 3 liquid or watery stools in a 24-h period with reporting from the mother of a recent change in the character of the stools. Liquid stools had both fecal matter and separate water whereas watery stools had predominantly water with little or no fecal matter. Watery diarrhea was defined as the passage of ≥ 3 watery stools in a 24-h period. Severe diarrhea was defined as the passage of 5 liquid or watery stools on any day of the episode. Presence of a cough and 2 respiratory rate measurements of ≥ 40 breaths/min constituted a day with ALRI. Recovery from an episode of ALRI was defined as the absence of the combination of cough and fast breathing for 2 consecutive weeks. The respiratory rate obtained on a visit was extrapolated for the intervening days between subsequent visits.

Prebreakfast plasma zinc concentrations varying from ≤ 9.2 to ≤ 10.7 μmol/L, and postprandial concentrations ≈10% lower than the prebreakfast ones, are proposed cutoffs to indicate zinc deficiency (12–14). The lowest value of this range for postprandial samples, 8.4 μmol/L, being the most conservative, was selected for this study as the cutoff for low plasma zinc concentration.

### Analysis

The total number of episodes of diarrhea and ALRI during the days at risk were compared between the zinc-deficient and zinc-sufficient children. The total days at risk were calculated by subtracting the days of nonavailability from the maximum possible follow-up of 90 d per child. The prevalence of an illness for each child was calculated as the days with the illness per 100 d at risk; the mean prevalences of the 2 study groups were compared. The statistical comparisons were made by analysis of variance (ANOVA) and chi-square tests (15). Analysis was done using EPI INFO version 6 (Centers for Disease Control and Prevention, Atlanta, and World Health Organization, Geneva) and SPSS PC+ (SPSS Inc, Chicago) for Windows (Microsoft, Redmond, Wa) software.

## RESULTS

Nearly one-third (32%) of the children had low plasma zinc concentrations (≤ 8.4 μmol/L). The baseline characteristics of children with low and normal plasma zinc concentrations are shown in **Table 1** (16).

**TABLE 2**
Episodes of diarrhea and acute lower respiratory tract infections over the 90-d observation period by plasma zinc group

| | Plasma zinc concentration | | Relative risk |
|---|---|---|---|
| | ≤ 8.4 μmol/L | > 8.4 μmol/L | (95% CI) |
| | *n* | | |
| All children | 37 | 79 | |
| Diarrhea | 50 | 77 | 1.47 (1.03, 2.09) |
| Diarrhea for ≥ 7 d | 9 | 8 | 2.54 (0.98, 6.59) |
| Diarrhea with ≥ 5 stools/d | 30 | 40 | 1.70 (1.06, 2.72) |
| Acute lower respiratory tract infection | 14 | 18 | 1.76 (0.88, 3.53) |
| Boys | 23 | 39 | |
| Diarrhea | 35 | 38 | 1.67 (1.06, 2.64) |
| Diarrhea for ≥ 7 d | 5 | 3 | 3.02 (0.72, 12.64) |
| Diarrhea with ≥ 5 stools/d | 21 | 20 | 1.90 (1.04, 3.50) |
| Acute lower respiratory tract infection | 11 | 5 | 3.99 (1.39, 11.47) |
| Girls | 14 | 40 | |
| Diarrhea | 15 | 39 | 1.14 (0.63, 2.07) |
| Diarrhea for ≥ 7 d | 4 | 5 | 2.38 (0.64, 8.85) |
| Diarrhea with ≥5 stools/d | 9 | 20 | 1.34 (0.61, 2.93) |
| Acute lower respiratory tract infection | 14 | 18 | 0.69 (0.20, 2.41) |

Children with low initial plasma zinc had a 47% higher risk of a diarrheal episode during the 90-d observation period than those with normal plasma zinc (95% CI: 3–109%). The risk of severe diarrhea, defined as passage of ≥ 5 liquid or watery stools on any day of the episode, was 70% greater in the zinc-deficient group (95% CI: 6–172%). At baseline, there were more boys in the zinc-deficient group (62.2%) than in the normal plasma zinc group (49.4%). Therefore, we also analyzed the morbidity data in subgroups by sex. The increased diarrheal morbidity in the zinc-deficient group was predominantly observed in boys; whereas similar trends were observed in girls, none of the differences were statistically significant (**Table 2**).

The mean prevalence of diarrhea was 32.5% higher, and that of watery diarrhea 37.5% higher, in zinc-deficient than in zinc-sufficient children, but in neither case was the difference significant. The mean prevalence of diarrhea associated with fever was 75% higher in zinc-deficient children than in zinc-sufficient children ($P = 0.01$; **Table 3**).

**TABLE 3**
Prevalence of diarrheal and respiratory morbidity over the 90-d observation period by plasma zinc group[1]

| Prevalence | Plasma zinc concentration | | *P* |
|---|---|---|---|
| | ≤ 8.4 μmol/L (*n* = 37) | > 8.4 μmol/L (*n* = 79) | |
| Diarrhea | 4.3 ± 4.9 | 2.9 ± 4.3 | 0.12 |
| Watery diarrhea | 0.8 ± 2.2 | 0.5 ± 1.5 | 0.13[2] |
| Diarrhea with fever | 0.8 ± 1.7 | 0.2 ± 0.8 | 0.01[2] |
| Cough | 28.8 ± 22.4 | 22.2 ± 16.9 | 0.20[2] |
| Acute lower respiratory tract infection | 1.4 ± 3.6 | 0.4 ± 0.9 | 0.05[2] |

[1] $\bar{x}$ ± SD.
[2] Nonparametric test used.

**TABLE 4**
Incidence of diarrhea over the 90-d observation period in children categorized by plasma zinc concentration

| | Plasma zinc[1] | | | |
|---|---|---|---|---|
| | < 33 percentile ($n$ = 37) | 33-66 percentile ($n$ = 42) | > 66 percentile ($n$ = 37) | Chi-square for linear trend: $P$ |
| | | $n$ | | |
| Diarrhea | 50 | 42 | 35 | 0.05 |
| Diarrhea for ≥ 7 d | 9 | 3 | 5 | 0.18 |
| Diarrhea with ≥ 5 stools/d | 30 | 24 | 16 | 0.02 |
| Days at risk | 3080 | 3701 | 3265 | — |

[1] 33rd percentile corresponds to plasma zinc concentration of 8.4 μmol/L and 66th percentile to 9.9 μmol/L.

To look for a trend of diarrheal morbidity with decreasing plasma zinc concentrations, we classified the enrolled children into 3 groups based on initial plasma zinc concentration: < 33rd percentile, between 33rd and 66th percentile, and > 66th percentile (**Table 4**). There was a significant increasing linear trend for diarrheal and severe diarrheal morbidity with decreasing plasma zinc concentration ($P$ = 0.05 and $P$ = 0.02, using chi-square for trend).

Overall, the risk of ALRI episodes was not significantly higher in the low plasma zinc group. However, males with low plasma zinc had a 4-fold higher risk of suffering from ALRIs (Table 2). Also, among all children, the prevalence of ALRIs was about 3-fold higher in zinc-deficient children, presumably as a result of longer duration of ALRI episodes ($P$ = 0.05; Table 3).

## DISCUSSION

The findings of this study suggest that children with low plasma zinc concentrations are at increased risk of diarrheal and possibly respiratory morbidity. This effect of zinc deficiency in increasing morbidity is particularly large among males and for severe diarrheal episodes. A greater effect of zinc supplementation on growth in boys has been previously reported (17, 18). In recent Indian studies, the effect of zinc supplementation was also greater on prevention of severe or prolonged diarrheal episodes than for short-lasting or milder episodes (14, 19, 20).

Excess morbidity as a result of zinc deficiency is biologically plausible. In experimental models, zinc deficiency is associated with impaired antibody response, decreased cutaneous delayed hypersensitivity response, lower thymic hormone activity, and impaired cytotoxic T cell and natural killer cell activity (1–4). Zinc deficiency also has direct effects on the gastrointestinal tract, which may tend to increase severity of gastrointestinal tract infections once they are acquired. These direct effects include mild villus atrophy, decreased brush border disaccharidase activity, and enhanced secretory response to cholera toxin (5, 6, 12). The findings of this study are consistent with the results of recent zinc supplementation trials that showed substantial reductions in the incidence of acute and persistent diarrhea when zinc intakes were increased through daily supplementation (13, 14, 19–22).

Although plasma zinc is the most widely used of the laboratory assays for the assessment of zinc status, concern has been raised about its usefulness, because concentrations vary with the time of consumption of the last meal and the presence of infection or hypoalbuminemia (23–25). Also, homeostatic mechanisms tend to maintain plasma zinc in the normal range even in the presence of severe dietary zinc restriction (26). Currently, the way to evaluate plasma zinc as a measure of zinc status is to validate it against isotope studies. Nevertheless, in this study, low plasma zinc predicted the anticipated increase in diarrheal morbidity and showed a clear dose-response relation. These results suggest that, despite its limitations, plasma zinc can serve as a useful measure of zinc status in epidemiologic studies.

This study has some important limitations. The excess morbidity observed in association with zinc deficiency may have been due to coexisting deficiencies of other micronutrients, which were not measured. Also, the prevalence of zinc deficiency in this study may have been higher than that in the general population of children in this setting because the study subjects had recently recovered from acute diarrhea, which is characterized by excess intestinal zinc losses.

In conclusion, our study shows that low plasma zinc places children at an increased risk of morbidity from diarrhea and ALRIs. Globally, efforts to improve micronutrient status have focused on iodine, iron, and vitamin A. Our findings, and results of recent supplementation trials, suggest that greater priority should also be given to the correction of zinc deficiency in susceptible populations.

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