# A randomized controlled clinical trial of zinc, vitamin A or both in undernourished children with persistent diarrhea in Bangladesh

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In a double-blind randomized controlled clinical trial, moderately malnourished Bangladeshi children (61–75% of the median weight/age) were studied for the effect of zinc and/or vitamin A supplementation on the clinical outcome of persistent diarrhea. Children 6 mo to 2 y of age with diarrhea for more than 14 d were randomly allocated into 4 groups of 24 receiving a multivitamin syrup and (i) zinc (20 mg elemental), (ii) vitamin A, (iii) both zinc and vitamin A, or (iv) neither, in 2 doses daily for 7 d. Clinical data on recovery and on stool output, consistency and frequency were recorded for 7 d, and weight change from day 1 to day 7 was assessed. The baseline characteristics of the four study groups were comparable. The mean daily stool outputs from days 2 to 7 of therapy were significantly less in the zinc and zinc plus vitamin A groups, but not in the vitamin A group, in comparison with the control group. In children receiving zinc, the cumulative stool weight in the 7 d was 39% less than in the control group ($p < 0.001$) and 32% less than in the vitamin A group ($p = 0.006$). The cumulative stool weight in the zinc plus vitamin A group was 24% less than in the control group ($p < 0.001$), but the 14% lower output than in the vitamin A group was not statistically different. The change in body weight over the 7 d study period was significantly different between the group receiving zinc and the control group (+111 g vs −90 g, $p = 0.045$). The rate of clinical recovery of children within 7 d was significantly greater in the zinc group (88%) compared with the control group (46%, $p = 0.002$) or vitamin A group (50%, $p = 0.005$), but not statistically different from the zinc plus vitamin A group (67%, $p = 0.086$).

*Conclusion*: The results indicate that zinc, but not vitamin A, supplementation in persistent diarrhea reduces stool output, prevents weight loss and promotes earlier recovery.

**Key words:** *Malnutrition, persistent diarrhea, randomized controlled trials, vitamin A, zinc*

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Malnutrition, which often includes deficiencies of micronutrients such as zinc and vitamin A, may contribute to increased severity and duration of diarrhea (1, 2), as well as diarrhea-associated mortality (3). Children are at risk of zinc deficiency because of inadequate dietary intake combined with excess fecal losses during diarrhea. Up to 300 µg of zinc per kilogram of body weight per day can be lost in children with persistent diarrhea (4, 5). Vitamin A deficiency may also occur in underprivileged children because of dietary inadequacies and excess losses (6, 7).

Previous trials of zinc supplementation as an adjunct to therapy in acute and persistent diarrhea have found reductions in illness duration and stool output (8, 9). The substantial reductions in diarrheal mortality with vitamin A supplementation found in community-based trials (10, 11) and the therapeutic effect of vitamin A on measles complications and case fatality (12, 13) suggest that vitamin A may have therapeutic benefits in diarrhea. Two previous trials of vitamin A supplementation in acute diarrhea in Bangladesh found no benefit (14, 15), but no trial has been done in persistent diarrhea, which is more strongly associated with malnutrition than is acute diarrhea (16). It is important to determine whether the effects of zinc and any possible effects of vitamin A are overlapping, possibly working through the same mechanism, or whether there are synergistic benefits of providing both zinc and vitamin A. Therefore, this four-cell randomized controlled trial was performed to determine the individual and combined benefits of zinc and vitamin A on the outcome of persistent diarrhea.

## Patients and methods

A double-blind randomized controlled clinical trial was conducted at the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) from April 1996 to March 1997. Children irrespective of sex were eligible for the study if they presented with persistent diarrhea, defined as diarrhea for more than 14 d duration and having no systemic infection or clinical signs of vitamin A deficiency and having not received vitamin A supplementation within 3 mo. Children with bloody mucoid diarrhea or kwashiorkor, or who no longer received breast milk, were excluded. Subjects given prior antimicrobial therapy were not excluded. One-hundred moderately malnourished children, [61–75% of the median National Center for Health Statistics (NCHS) median weight for age] between 6 mo and 3 y of age were randomly allocated to four groups (17). Sample size was calculated on the assumption that the zinc-supplemented group would have 20% fewer who failed to recover by day 7 than the control group [a previous trial in this hospital found that 27% fewer zinc-supplemented children failed to recover (18)], using an alpha error of 5% and power of 82%. Informed written consent was obtained from the child's parents before enrolment into the study. The study was approved by the Ethical Review Committee of the ICDDR,B.

Dehydration was assessed on admission according to World Health Organization (WHO) guidelines and corrected within 4 h using either intravenous poly-electrolyte solution or WHO/UNICEF oral rehydration salt solution. Stool was collected with urine separated from stool, every 8 h for 7 d. Body length and weight were measured on the day of admission and weights were measured daily thereafter. Anthropometric indicators were calculated by comparison with the National Center for Health Statistics reference population (17). The usual milk-free ICDDR,B hospital diet, which is based on rice, glucose and egg white, for persistent diarrhea was given to the patients during the 7 d of study at 100 kcal $kg^{-1}$ per day. Subjects were encouraged to continue breastfeeding.

One group received 20 mg of elemental zinc as zinc acetate in 2 doses of 5 ml daily for a period of 1 wk in a multivitamin syrup. The multivitamin syrup contained vitamin D (640 IU), vitamin C (50 mg), vitamin $B_1$ (1.6 mg), vitamin $B_2$ (1.37 mg), vitamin $B_6$ (1.0 mg), nicotinamide (10.0 mg), D-pantothenol (6.0 mg), glycerine (1.2 ml), propylene glycol (0.75 ml), sorbitol 70% (2 ml), methyl paraben (3.5 mg), polysorbate 80 (50 mg), lemon oil (0.0125 ml) and caramel brown color (1.0 mg), in 75 ml total volume. The second group received vitamin A plus multivitamin syrup; the vitamin A dose was 100000 IU for children below 1 y old and 200000 IU for children above 1 y old. The vitamin A was added to the syrup and thus was given in doses of 13333 IU $d^{-1}$ for infants and 26666 IU $d^{-1}$ for older children. The third group received zinc plus vitamin A in multivitamin syrup. The fourth group received only multivitamin syrup. The syrup was administered by the mothers, who were assisted by health workers.

After correction of dehydration on admission, blood was collected and sera were stored at −20°C. Serum retinol concentration was measured by high-performance liquid chromatography (Waters 510, USA). Serum zinc concentration was measured by Atomic Absorption Spectrophotometry (Shimadzu Corporation, Japan).

Clinical recovery from persistent diarrhea was defined as the time of the last diarrheal stool followed by passage of soft or formed stool for more than 48 h of observation in the hospital.

### *Statistical analysis*

Data entry, cleaning and analysis were carried out using SPSS/PC+ and Statpack Gold software. For statistical comparison, *t*-test and one-way ANOVA were used on normally distributed data and non-parametric Mann–Whitney test and Wilcoxon's signed rank test were used when the distribution was skewed. To analyse for the effect of zinc supplementation on continuation of diarrhea beyond 1 wk, log-rank test statistics with Kaplan–Meier survival analysis were done. Statistical significance was considered at the 5% probability level.

## Results

Supplementation was accepted by the children without any side effects. One subject in each group dropped out during the study because of religious festivals or family problems, leaving 24 per group. No children died during the study. The children in the four groups were comparable on admission with respect to mean age, anthropometric indices, baseline serum vitamin A and zinc concentrations, family income and duration of diarrhea before enrolment (Table 1).

The mean daily stool output (data not shown) was less in the zinc and zinc plus vitamin A groups from day 2 to day 7 compared with the control group ($p = 0.028$ to $< 0.0001$). There was a significantly lower stool output on each day from day 3 to day 7 in the zinc group ($p = 0.030$ to 0.001) or the zinc plus vitamin A group ($p = 0.024$ to 0.001) in comparison with the vitamin A group. No significant differences were found in comparisons of the vitamin A group to the control group or in the zinc to the zinc and vitamin A group. There were significantly lower stool outputs on each day from day 2 to day 7 in the combined zinc groups compared with the non-zinc groups ($p = 0.034$ to $< 0.001$).

The mean cumulative stool weights in the zinc group and in the zinc plus vitamin A group were significantly lower than the control group by day 2 and day 3, respectively (Table 2). The mean cumulative stool weight in 7 d was 39% lower in children receiving zinc compared with the controls ($p < 0.001$) and 32% lower

*Table 1.* Characteristics on admission of children with persistent diarrhea

| Characteristics | Placebo ($n = 24$) | Zinc ($n = 24$) | Vitamin A ($n = 24$) | Zinc + vitamin A ($n = 24$) |
|---|---|---|---|---|
| Wt/age % of median NCHS | 69.1 ± 4.6 | 68.8 ± 5.1 | 67.1 ± 5.3 | 68.1 ± 5.0 |
| Ht/age % of median NCHS | 93.0 ± 2.9 | 93.0 ± 2.8 | 92.2 ± 3.4 | 91.9 ± 3.0 |
| Wt/ht % of median NCHS | 82.1 ± 6.9 | 81.0 ± 6.7 | 81.1 ± 6.1 | 82.6 ± 5.2 |
| Age (mo) | 10.0 ± 2.6 | 12.0 ± 5.4 | 11.0 ± 4.1 | 10.6 ± 3.8 |
| Body weight (kg) | 6.3 ± 0.7 | 6.6 ± 1.1 | 6.2 ± 1.0 | 6.4 ± 0.9 |
| Height weight (cm) | 67.8 ± 4.0 | 69.4 ± 5.5 | 67.4 ± 4.9 | 67.5 ± 4.2 |
| MUAC (mm) | 121 ± 6.4 | 125 ± 16 | 119 ± 7 | 120 ± 7.4 |
| Duration of diarrhea (d) | 21 ± 6 | 25 ± 18 | 23 ± 12 | 22 ± 11 |
| Serum vitamin A (μmol $l^{-1}$) | 0.51 ± 0.27 | 0.51 ± 0.24 | 0.52 ± 0.25 | 0.50 ± 0.23 |
| Serum zinc (μmol $l^{-1}$) | 15.0 ± 0.06 | 15.0 ± 0.05 | 14.9 ± 0.06 | 14.9 ± 0.06 |
| Family income (Taka $mo^{-1}$) | 4859 ± 3702 | 4666 ± 3969 | 4405 ± 2841 | 4204 ± 2493 |

Data are means ± SD.
No significant difference among any study groups.

*Table 2.* Comparison of cumulative stool output (mg $kg^{-1}$ body weight) of children with persistent diarrhea during hospitalization

| Day | Control ($n = 24$) | Zinc ($n = 24$) | Vitamin A ($n = 24$) | Zinc + vitamin A ($n = 24$) |
|---|---|---|---|---|
| 1 | 137 ± 41 | 127 ± 35 (ns) | 128 ± 29 (ns) | 129 ± 53 (ns) |
| 2 | 280 ± 76 | 232 ± 50 (0.028) | 253 ± 59 (ns) | 239 ± 72 (ns) |
| 3 | 400 ± 89 | 334 ± 66 (0.034) | 385 ± 78 (ns) | 333 ± 90 (0.032) |
| 4 | 522 ± 113 | 401 ± 98 (0.007) | 488 ± 102 (ns) | 424 ± 101 (0.010) |
| 5 | 640 ± 121 | 460 ± 125 (0.002) | 586 ± 126 (ns) | 505 ± 120 (0.003) |
| 6 | 744 ± 124 | 524 ± 171 (0.003) | 681 ± 153 (ns) | 585 ± 131 (0.001) |
| 7 | 866 ± 127 | 528 ± 140 (<0.001) | 767 ± 154 (ns) | 656 ± 145 (<0.001) |

Data are means ± SD ($p$-value, compared with control group).
ns: non-significant ($p > 0.05$).

*Table 3.* Weight change from day 1 to day 7 of hospitalization for persistent diarrhea by study group

| Characteristics | Control ($n = 24$) | Zinc ($n = 24$) | Vitamin A ($n = 24$) | Zinc + vitamin A ($n = 24$) |
|---|---|---|---|---|
| Day 1 weight (kg) | 6.34 ± 0.67 | 6.72 ± 1.16 | 6.24 ± 1.02 | 6.54 ± 0.77 |
| Day 7 weight (kg) | 6.25 ± 0.64 | 6.83 ± 1.37 | 6.31 ± 1.00 | 6.61 ± 0.89 |
| Change in weight (kg) | −0.09 | 0.11 | 0.07 | 0.06 |
| $p$-Value for change in weight compared with control group | – | 0.045 | 0.207 | 0.074 |

Data are means ± SD.

*Table 4.* Outcome of persistent diarrhea within 1 wk of supplementation in hospitalized children

| Outcome | Control | Zinc | Vitamin A | Zinc + vitamin A |
|---|---|---|---|---|
| Recovered | 11 | 21 | 12 | 16 |
| Failed to recover | 13 | 3 | 12 | 8 |
| Total | 24 | 24 | 24 | 24 |

$\chi^2 = 10.9$ ($p = 0.012$).

than in children receiving vitamin A alone ($p = 0.006$). Children who received zinc plus vitamin A had a 24% lower cumulative stool weight by day 7 ($p < 0.001$) than the control group, and a 14% lower stool weight than the vitamin A group, which was not statistically significant. The cumulative stool weights in the vitamin A and control groups were not statistically different. The combined zinc groups had a mean cumulative stool weight of 623 ± 152 mg $kg^{-1}$ body weight, which was 24% lower than that of the combined non-zinc groups (817 ± 147 mg $kg^{-1}$ body weight, $p < 0.001$).

The net change in body weight during the 7 d study period was significantly greater in children receiving zinc compared with the control group (+111 g vs −90 g, $p = 0.045$) (Table 3). Both the vitamin A and zinc plus vitamin A groups had gains in weight, but these were not significantly different from the weight losses in control group children. The clinical recovery

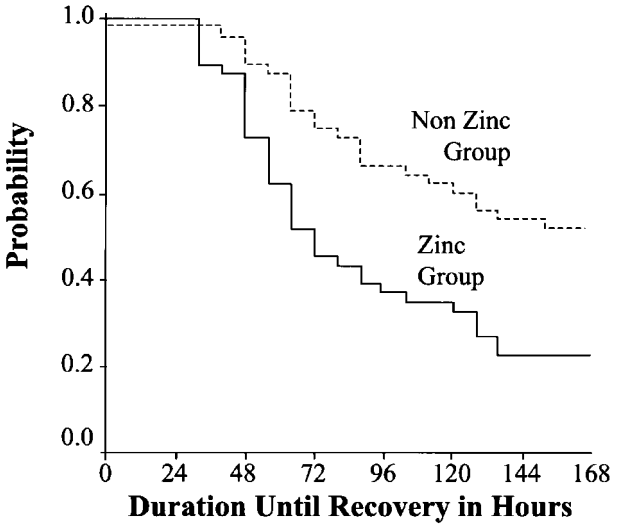


*Fig. 1.* Probability of diarrhea persisting (Kaplan–Meier estimates) by treatment group and hours since admission.

rate of children within 7 d of observation was significantly greater in the zinc group compared with the control group (88% vs 46%, $p = 0.002$) or vitamin A group (50%, $p = 0.005$), but not that of the zinc plus vitamin A group (67%, $p = 0.086$) (Table 4). The recovery rate in the zinc plus vitamin A group was not significantly greater than that in the control or vitamin A groups ($p = 0.146$ and $p = 0.242$, respectively). Overall, the recovery rate in the combined zinc groups (77%) was significantly higher ($p = 0.004$) than that in the non-zinc groups (48%). In a survival analysis, the zinc groups had a significantly ($p = 0.001$) lower probability of continuing diarrhea during the week of study (Fig. 1).

## Discussion

This study found significantly decreased stool output, greater weight gain and faster recovery in zinc-supplemented children compared with control or vitamin A-supplemented children. Vitamin A had no significant effect on any outcome and there were no synergistic or additive effects of vitamin A given with zinc on the outcome of persistent diarrhea in this group of malnourished patients. In fact, there was a trend for the zinc plus vitamin A group to have less effect on both the outcome of diarrhea and weight gain than the zinc-alone group, but this study did not have the power to determine whether there was an antagonistic effect of vitamin A. These findings are similar to those from a zinc and vitamin A factorial trial in acute diarrhea in Bangladesh (15). In that study zinc-supplemented children with acute diarrhea had 13% reduced duration, but low-dose daily vitamin A had no effect. Likewise, a previous trial of high-dose vitamin A in Bangladesh had no therapeutic efficacy in acute watery diarrhea (14).

This trial shows large effects of zinc supplementation in persistent diarrhea. Some previous studies have also suggested benefits. A small trial in India found that zinc-supplemented children with persistent diarrhea had shorter diarrheal durations and lower stool frequencies than children in the placebo group, but these differences were not statistically significant (9). Studies in Bangladesh and Peru found a 15–18% reduced probability of continuing diarrhea with zinc supplementation compared with the control groups, but these differences were not statistically significant (18, 19). A study in Pakistan found no effect of zinc supplementation (20). As in a previous trial of zinc supplementation in persistent diarrhea in the same hospital, faster recovery and greater weight gain were seen with zinc supplementation (18), but this trial, by performing more complete studies of stool output, was also able to document important changes in diarrheal stool losses. It should be noted that this study excluded children with severe systemic infections who might have been included in other studies, such as in Pakistan (20), so one can only generalize to this population.

Previous studies have demonstrated that zinc supplementation in zinc-deficient rats significantly increased the intestinal mucosal mass and transport of water and sodium and reduced secretion of water and electrolytes (21). Morphological studies have shown that zinc supplementation improved the structure of the intestinal mucosal in zinc-deficient animals as well as in patients with acrodermatitis enteropathica (22, 23). Improved mucosal integrity with zinc supplementation as evidenced by reduced permeability, particularly in malnourished children, has been observed (24, 25). Another mechanism that could explain enhanced recovery from persistent diarrhea is the restoration of immune function, especially cell-mediated immunity and production of secretory immunoglobulin A (26).

Malnutrition is widely present in developing countries: approximately 65% of all children under 5 y in Bangladesh (27) and nearly all children with persistent diarrhea globally are malnourished (14). These results strongly suggest that zinc supplementation should be used as part of the standard case management of childhood persistent diarrhea. In fact, multiple micronutrient supplements, which include 2 RDA of zinc, are now recommended by the WHO for therapy of this illness (28).

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