Randomised controlled trial of zinc supplementation in malnourished Bangladeshi children with acute diarrhoea

S K Roy, A M Tomkins, S M Akramuzzaman, R H Behrens, R Haider, D Mahalanabis, G Fuchs

Abstract
Objective—To evaluate the impact of zinc supplementation on the clinical course, stool weight, duration of diarrhoea, changes in serum zinc, and body weight gain of children with acute diarrhoea.

Design—Randomised double blind controlled trial. Children were assigned to receive zinc (20 mg elemental zinc per day) containing multivitamins or control group (zinc-free multivitamins) daily in three divided doses for two weeks.

Setting—A diarrhoeal disease hospital in Dhaka, Bangladesh.

Patients—111 children, 3 to 24 months old, below 76% median weight for age of the National Center for Health Statistics standard with acute diarrhoea. Children with severe infection and/or oedema were excluded.

Main outcome measures—Total diarrhoeal stool output, duration of diarrhoea, rate of weight gain, and changes in serum zinc levels after supplementation.

Results—Stool output was 28% less and duration 14% shorter in the zinc supplemented group than placebo (p < 0.06). There were reductions in median total diarrhoeal stool output among zinc supplemented subjects who were shorter (less than 95% height for age), 239 v 326 g/kg (p < 0.04), and who had a lower initial serum zinc (< 14 mmol/l), 279 v 329 g/kg (p < 0.05); a shortening of mean time to recovery occurred (4.7 v 6.2 days, p < 0.04) in those with lower serum zinc. There was an increase in mean serum zinc in the zinc supplemented group (+2.4 v −0.3 mmol/l, p < 0.001) during two weeks of supplementation, and better mean weight gain (120 v 30 g, p < 0.03) at the time of discharge from hospital.

Conclusions—Zinc supplementation is a simple, acceptable, and affordable strategy which should be considered in the management of acute diarrhoea and in prevention of growth faltering in children specially those who are malnourished.

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Keywords: zinc supplementation; acute diarrhoea; weight gain; malnutrition

Diarrhoea remains a major cause of morbidity and mortality in less developed countries. A significant proportion of children who suffer from diarrhoea are malnourished, and malnutrition is associated with increased severity and duration of diarrhoea in such children. These malnourished children are often reported to have associated micronutrient deficiencies. An association between zinc deficiency and impaired growth has also been observed. Furthermore, zinc is important in the control of growth of the intestinal mucosa. Zinc supplementation improves the transport of water and electrolytes across the intestinal mucosa in experimental zinc deficiency. Severe zinc deficiency syndrome in humans, known as acrodermatitis enteropathica, responds to treatment with oral zinc supplements.

Methods

Design
A double blind, randomised, controlled clinical trial was conducted in children with diarrhoea of less than three days’ duration who were admitted to the International Centre for Diarrhoeal Disease Research, Bangladesh. Children aged between 3 and 24 months were eligible for the study provided they had no systemic infection or oedema, and their weight was below the 76th centile of weight for age according to the
The sample size was determined on the assumption that zinc supplementation would decrease intestinal fluid losses at least by 20% and reduce the duration by 20%. The estimation of sample size was performed according to Kirkwood, taking the probability at the 5% level and assuming a power of 80%.

A block randomisation procedure was performed using a random table to assign eligible patients equally to a treatment group receiving zinc acetate with multivitamin syrup or the control group receiving only multivitamin syrup of the same basic ingredients (prepared by Square Pharmaceutical Co, Bangladesh). The syrups were identical in appearance and flavour and were packaged in identical containers. Randomised patient numbers were labelled on the bottles to maintain the double blind design. Fifty seven children received zinc and 54 received the multivitamin syrup.

CLINICAL PROCEDURES
On admission, dehydration was assessed using World Health Organisation (WHO) guidelines and corrected within four hours with World Health Organisation (WHO) guidelines. On admission, dehydration was assessed using World Health Organisation (WHO) guidelines for children and corrected within four hours with WHO/Unicef oral rehydration solution. Body weight after rehydration was measured with a scale having a precision of 20 g on the day of admission and daily thereafter between 9 and 10 am. Supine length was measured on a locally constructed length board to a precision of 1 mm. Nutritional status was expressed as a per cent of the median NCHS standard. Shorter children were defined as less than 95% height for age. Serum zinc concentrations were considered low if they were below 14 µmol/l (as found in malnourished and zinc deficient children).

To examine a possible relation between serum zinc and diarrhoeal or nutritional outcome, children with hypozincemia on admission were considered to be zinc deficient. However, as the number of children with very low serum zinc concentrations (below 10 µmol/l), was very small, the 66th centile value for serum zinc was taken as the lower cut off point for comparing the effect of zinc supplementation among the more zinc deficient children.

A normal hospital diet (milk/cereal/liquid diet, appendix 1) was given ad libitum and breast feeding was encouraged. Zinc was given as 20 mg elemental zinc per day in three divided doses for a period of two weeks in a multivitamin syrup. This was prepared by Square Pharmaceuticals Co, Bangladesh, and contained in each 5 ml: elemental zinc 6.5 mg, vitamin A 3000 IU, vitamin D 600 IU, vitamin B-1 1.2 mg, riboflavin 2.0 mg, vitamin B-6 0.6 mg, nicotinamide 6.0 mg, and calcium d-pantothenate 6.0 mg. One millilitre of zinc syrup contained 1.3 mg of elemental zinc. The control group received the same multivitamin syrup but without zinc. The syrup was given by the mothers under direct continuous supervision of nurses during the hospital inpatient stay and by community health workers on alternate day home follow up visits.

LABORATORY INVESTIGATIONS
Blood samples were obtained on admission, after correction of dehydration, and at the end of the supplementation and were stored at −20°C. Urine was collected separately from stool using adhesive single use paediatric urine collection bags. Stool was collected in a preweighed container beneath a cholera urine cot and measured every eight hours on a sensitive scale to the nearest 1 g. Clinical recovery from diarrhoea was defined as the passage of a soft formed stool. Serum zinc was measured using an atomic absorption spectrophotometer (Pye Unicam, SP9) and serum retinol by high performance liquid chromatography (HPLC, Waters 510, USA). Enteric pathogens were isolated according to the WHO manual for laboratory investigations of acute enteric infections. Informed written consent was obtained from the parents of all patients before inclusion of the children in the study. The study was approved by the ethics review committee of the International Centre for Diarrhoeal Disease Research.

STATISTICAL ANALYSIS
Data were recorded on questionnaires and transferred to coding sheets. Data cleaning, validation, and analyses were performed using SPSS/PC+, EPISTAT, and NCHS anthropometric software. Student’s unpaired and paired t tests were used on normally distributed data and the non-parametric Mann-Whitney test and Wilcoxon’s matched paired tests were used for stool weight and body weights of malnourished and zinc deficient children respectively, where the distribution was skewed. Statistical significance was considered to be at the 5% probability level.

Results
Fifty four subjects were allocated to control group not receiving zinc but only multivitamin syrup, and 57 subjects received zinc supplementation with multivitamins. The oral zinc supplementation was palatable and well accepted by the children and without any apparent complications.

Diarrhoeal pathogens were isolated in 67% of the children. Single infections were noted with rotavirus (25%), enteropathogenic Escherichia coli (7%), enterotoxigenic E coli (6%), Vibrio cholerae (2%), and shigella or campylobacter spp (7%). Fifteen per cent of the children had a mixed infection. The distribution of pathogens was similar between the zinc supplemented and placebo groups.

BASELINE COMPARISON
On admission, the mean values of the general characteristics of the children of the control group were comparable with those of the zinc supplemented group, for example: age, 11 ± 11 months; per cent of median weight for age, 67...
could be obtained, to ensure that they had received two weeks of supplementation.

### CLINICAL OUTCOME

The median total diarrhoeal stool output (g/kg/day) was 28% less in the zinc supplemented group as a whole (p = 0.06). Zinc supplementation reduced median stool weight (279 v 326 g/kg, p < 0.04) in those children who had lower serum zinc (< 14 µmol/l) on admission. There was reduction in median total diarrhoeal stool output with zinc supplementation in the shorter children (< 95% height/age) compared to similar children receiving placebo (279 v 326 g/kg, p < 0.049) (table 1).

The duration of diarrhoea was shorter by 14% (NS) with zinc supplementation among the entire group of children. Children with a lower serum zinc (< 14 µmol/l) achieved a 22% reduction (p < 0.04) in the duration of diarrhoea with zinc supplementation (table 2). No such difference in duration among the stunted or wasted children was found.

Weight gain at discharge and improved serum zinc levels were noted in the zinc supplemented children. The zinc supplemented group had gained in mean body weight (6.31 v 6.19 kg, p = 0.03) but there was no gain in the placebo group (6.46 v 6.43 kg, p = 0.68). The control group showed a slight reduction in mean serum zinc (12.6 v 12.3 µmol/l, p = 0.63), whereas the supplemented group had a significant increase in mean serum zinc (11.2 v 13.6 µmol/l, p < 0.001) (table 3).

### NUTRITIONAL OUTCOME

The body weight of the zinc supplemented stunted and wasted children increased by the time of recovery from diarrhoea. The change was significant in the supplemented stunted group (median 6.20 kg from 6.08 kg, p < 0.006) compared to their counterparts in the control group (6.27 kg from 6.28 kg, p = 0.7). Similarly in children who had lower serum zinc, the supplemented group had a significant increase in body weight (6.20 kg from 6.04 kg, p = 0.05) compared to the control group (6.23 kg from 6.31 kg, p = 0.49) (table 4).

### Discussion

Our study examined several aspects of zinc supplementation. Zinc deficiency in children with diarrhoea occurs as a result of increased zinc loss in the stool together with reduced dietary intake. Many studies have stressed that serum zinc is not a good indicator of overall zinc status. Yet we have been able to show clearly that our group of children with moderate to severe malnutrition showed a rise in serum zinc after supplementation, even when they were suffering from acute diarrhoeal illness. On the other hand, the group given multivitamins alone did not show any improvement in serum zinc concentrations. This result suggests that serum zinc does have value as an indicator of zinc nutrition. However, we do not know whether this rise in serum zinc is maintained. The significant rise in serum zinc following zinc supplementation suggested an improvement in zinc status. We feel that the

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**Table 1** Impact of zinc supplementation on stool output in children during acute diarrhoea

<table>
<thead>
<tr>
<th>Total stool weight/kg body weight, median (range)</th>
<th>Placebo</th>
<th>Zinc supplemented</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children</td>
<td>329 (32–1446) n=37</td>
<td>238 (35–2416) n=37</td>
<td>0.06</td>
</tr>
<tr>
<td>Height/age &lt;95%</td>
<td>326 (31–1460) n=33</td>
<td>239 (33–2496) n=37</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Serum zinc (&lt;14 µmol/l)</td>
<td>326 (99–1446) n=25</td>
<td>279 (43–2416) n=30</td>
<td>&lt;0.049</td>
</tr>
</tbody>
</table>

*Mann-Whitney U test.

**Table 2** Impact of zinc supplementation on duration of diarrhoea in days. Values are mean (95% confidence interval)

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Zinc</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children</td>
<td>5.80 (4.93 to 5.84) n=37</td>
<td>5.0 (4.16 to 5.84) n=37</td>
</tr>
<tr>
<td>Serum zinc &lt;14 µmol/l</td>
<td>6.0 (4.96 to 5.84) n=25</td>
<td>5.0 (4.16 to 5.84) n=30</td>
</tr>
<tr>
<td>Wasted &lt;80% weight/height</td>
<td>5.8 (4.53 to 5.07) n=18</td>
<td>4.9 (3.72 to 5.07) n=11</td>
</tr>
<tr>
<td>Stunted &lt;95% height/age</td>
<td>6.0 (5.0 to 6.94) n=33</td>
<td>5.0 (4.16 to 5.84) n=37</td>
</tr>
</tbody>
</table>

*Student’s t test.

**Table 3** Impact of zinc supplementation on weight gain and serum zinc levels during management of acute diarrhoea. Values are mean (95% confidence interval)

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>On admission</th>
<th>At discharge</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n=34)</td>
<td>6.43 (6.1 to 6.7)</td>
<td>6.46 (6.2 to 6.8)</td>
<td>0.68</td>
</tr>
<tr>
<td>Zinc (n=57)</td>
<td>6.19 (5.9 to 6.5)</td>
<td>6.31 (6.0 to 6.6)</td>
<td>0.03</td>
</tr>
<tr>
<td>Serum zinc (µmol/l)</td>
<td>11.2 (10.4 to 12.0)</td>
<td>11.2 (12.5 to 14.7)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Paired t test.

**Table 4** Impact of zinc supplementation on weight gain in stunted, wasted, and zinc deficient children. Values are median (range)

<table>
<thead>
<tr>
<th>On admission (weight in kg)</th>
<th>At discharge (weight in kg)</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height for age &lt;95% of median NCHS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 42)</td>
<td>6.28 (4.52–8.80)</td>
<td>6.27 (4.50–8.22)</td>
</tr>
<tr>
<td>Zinc (n = 50)</td>
<td>6.08 (4.30–8.52)</td>
<td>6.20 (4.20–8.80)</td>
</tr>
<tr>
<td>Weight for height &lt;80% of median NCHS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 26)</td>
<td>6.65 (4.68–9.16)</td>
<td>6.77 (5.02–9.86)</td>
</tr>
<tr>
<td>Zinc (n = 22)</td>
<td>5.90 (4.85–8.80)</td>
<td>6.0 (5.10–8.80)</td>
</tr>
<tr>
<td>Serum zinc &lt;14 µmol/l</td>
<td>6.31 (4.52–9.16)</td>
<td>6.23 (4.50–9.86)</td>
</tr>
<tr>
<td>Placebo (n = 40)</td>
<td>6.04 (4.22–8.80)</td>
<td>6.20 (4.20–8.80)</td>
</tr>
</tbody>
</table>

*Wilcoxon’s matched pair test.
Zinc supplementation in acute diarrhoea

20 mg of daily zinc supplementation reduced diarrhoeal stool in shorter children
Zinc reduced diarrhoeal stool in those with low serum zinc
Zinc reduced duration of diarrhoea in children with low serum zinc
Zinc increased body weight of children during acute diarrhoeal episode
Malnourished children are likely to benefit more from zinc supplementation

quantity of zinc given, which was double the recommended nutrient intake, was adequate to allow a physiological effect despite the known reduction in absorption during diarrhoea. The results of zinc supplementation might have been even greater if the control group had not received multivitamin syrup. However, the children were malnourished, and hence multivitamin supplementation during a diarrhoeal episode was appropriate. The WHO has suggested that children below 1 year of age are given 100 000 IU vitamin A at six month intervals, with an additional dose during diarrhoea. Our patients were given 126 000 IU vitamin A during their diarrhoeal episode over a 2 week period in both study groups. During diarrhoea, the absorption of vitamin A is significantly reduced, while the requirement in malnourished children is increased; hence the dose given to our children should have been adequate. The cost of the zinc and multivitamin syrup was £0.33 (US $0.50) per child for a two week course. In Bangladesh and most developing countries, a large majority of children suffer from malnutrition and they contract about three to four episodes of diarrhoea in a year. The study was conducted in a malnourished group of children who are likely to be depleted of endogenous zinc from earlier diarrhoeal episodes and low dietary intake.

As the number of children with any specific enteric pathogen was small, we analysed the results from all the children without stratifying them according to the type of pathogenic organism, assuming that with random allocation patients with particular pathogens would be equally distributed in the two groups. In doing this, we recognise that those with a limited response to zinc might dilute the effect of zinc on those with mucosal damage. None of the children received 40 mg of elemental zinc daily during acute diarrhoea responded with a shorter duration of diarrhoea than a control group. The response was linked to a significantly earlier recovery in the children who had lower rectal zinc concentration and were shorter children.16 31 Clearance of intestinal parasites such as Strongyloides ratti in zinc deficient rats occurred significantly earlier and more efficiently after zinc supplementation.32 Zinc supplementation has also been found to reduce the duration of infection with rhinovirus in elderly patients.8 33 It may be assumed that zinc inhibits viral activity but it is difficult to say whether zinc supplementation reduces diarrhoea because of an effect on viruses such as rotavirus, which was present in about one third of our subjects. It is known that diarrhoea may cause growth faltering in children34 by a variety of mechanisms including decreased dietary intake, malabsorption, and loss of endogenous nutrients, and altered metabolism. The rate of catch up weight gain in our children was greater in those receiving zinc than

Key messages

- Zinc deficiency in children has been associated with dwarfism and it is well recognised that a chronic zinc deficient state may be reflected in slow growth or height faltering in children. Shorter children may thus be zinc deficient and this may explain why this group had a better response to zinc supplementation.

- Zinc increased body weight of children during acute diarrhoeal episode. Zinc supplementation helps to improve cell mediated immunity and it increases salivary secretory IgA in malnourished children.30 31 Clearance of intestinal parasites such as Strongyloides ratti in zinc deficient rats occurred significantly earlier and more efficiently after zinc supplementation.32 Zinc supplementation has also been found to reduce the duration of infection with rhinovirus in elderly patients.8 33 It may be assumed that zinc inhibits viral activity but it is difficult to say whether zinc supplementation reduces diarrhoea because of an effect on viruses such as rotavirus, which was present in about one third of our subjects. It is known that diarrhoea may cause growth faltering in children34 by a variety of mechanisms including decreased dietary intake, malabsorption, and loss of endogenous nutrients, and altered metabolism. The rate of catch up weight gain in our children was greater in those receiving zinc than.
in the control group. This might be a result of better absorption of nutrients and increased protein synthesis after zinc supplementation. Zinc supplementation of malnourished Jamaican children during nutritional rehabilitation was associated with increased lean tissue synthesis. Zinc supplementation also promoted an increased weight gain velocity of 8.8 g/kg/day among children recovering from severe malnutrition in Bangladesh. The effect of zinc on diarrhoeal pathogens could reduce loss of endogenous zinc, electrolytes, and water.

In conclusion, our study shows overall weight gain in children with zinc supplementation, an increase in serum zinc in the face of acute diarrhoea, and significant benefit in reduction of the duration of diarrhoea and diarrhoea stool output among the children who had linear growth faltering and who had lower serum zinc values.

We propose that zinc supplementation is a simple, acceptable, and affordable strategy which should be considered in the management of acute diarrhoea and prevention of growth faltering in children, specially those who are malnourished.

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Appendix 1
Composition of diet for acute diarrhoea patients (per litre).
Low lactose milk based diet

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk powder</td>
<td>35 g</td>
</tr>
<tr>
<td>Rice powder</td>
<td>30 g</td>
</tr>
<tr>
<td>Sugar</td>
<td>25 g</td>
</tr>
<tr>
<td>Oil</td>
<td>20 ml</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1 g</td>
</tr>
<tr>
<td>Water to 1 litre</td>
<td></td>
</tr>
<tr>
<td>Energy</td>
<td>2800 KJ (670 kcal)</td>
</tr>
<tr>
<td>Protein</td>
<td>17 g</td>
</tr>
<tr>
<td>Elemental zinc</td>
<td>1.4 mg</td>
</tr>
</tbody>
</table>

10 Roy SK, Drasar BS, Tomkins AM. The impact of zinc deficiency on intestinal response to cholera toxin [abstract]. Proc Natl Acad Sci 1986;85:39A.