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ORIGINAL ARTICLE

Zinc supplementation in the management of shigellosis in malnourished children in Bangladesh

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Objective: To assess the impact of zinc supplementation on clinical recovery, weight gain and subsequent growth and morbidity in moderately malnourished children with shigellosis.
Design: A randomized, double-blind, controlled trial.
Setting: Dhaka hospital of ICDDR,B: Centre for Health and Population Research, Dhaka, Bangladesh.
Subjects: Fifty-six moderately malnourished children, aged 12–59 months with culture-proven shigellosis.
Methods: Subjects were randomly allocated to receive zinc (20 mg/day elemental) in multivitamin syrup (intervention) or multivitamin syrup without zinc (control) in two equally divided doses daily for 2 weeks. All children received pivmecillinam in a dose of 15 mg/kg every 6 h for 5 days. After supplementation, children were followed in their respective homes every 2 weeks for 6 months.
Results: Children receiving zinc recovered from acute illness significantly faster than the control children ($P<0.05$). The medians time (days) to recovery and disappearances of blood and mucous were significantly 50% shorter in the zinc-supplemented group compared to the control group. The mean body weight of zinc supplemented children increased significantly from 8.8 kg on admission to 9.2 kg ($P<0.01$) at recovery, which was not observed in the control children (from 9.3 to 9.6 kg; $P=0.12$). During the 6-month follow-up period, zinc-supplemented children had significantly fewer mean episodes of diarrhoea compared to the control children (2.2 vs 3.3; $P=0.03$).
Conclusion: Zinc supplementation significantly shortens the duration of acute shigellosis, promotes better weight gain during recovery and reduces diarrhoeal morbidity during the subsequent 6 months.
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Keywords: zinc supplementation; children; shigellosis; clinical recovery

Introduction

Shigellosis is a major cause of childhood morbidity and mortality globally (Kotloff *et al.*, 1999). Malnourished children experience higher case fatality rates compared to better nourished children during shigellosis. Shigellosis, an invasive disease of human colonic mucosa, typically manifests as bloody-mucoid stools and/or febrile diarrhoea (Speelman *et al.*, 1984). It places a heavy nutritional burden on children, including loss of important micronutrients such as vitamin A through urine (Mitra *et al.*, 1998). Increased loss of zinc in diarrhoeal stool precipitates zinc deficiency in children (Castillo-Duran *et al.*, 1988). During shigellosis, anorexia and vomiting reduces net food intake, high fever leads to increased catabolism and intestinal inflammation causes enteric protein loss, all of which contribute to deterioration in the nutritional status of children during and following shigellosis (Bennish *et al.*, 1993), and the disease adversely affects growth (Black *et al.*, 1984; Henry *et al.*, 1987). Severely malnourished children with shigellosis and those with hypoglycaemia, hypothermia, altered consciousness and/or bronchopneumonia are at higher risk of death (Broek *et al.*, 2005). Although antimicrobial therapy is a key element in the management (Khan *et al.*, 1997), provision of adequate nutritional support during the acute illness and nutrition supplementation during convalescence reduces nutritional deterioration, including its negative impact on growth (Kabir *et al.*, 1993; Hossain *et al.*, 1998).

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A pooled analysis of studies of zinc supplementation to children in different countries proved that zinc supplementation reduces the severity and the duration of the acute and persistent diarrhoea (The Zinc Investigators' Collaborative Group, 2000). Zinc supplementation has been shown to enhance linear growth in malnourished children with acute and persistent diarrhoea (Roy *et al*., 1999, 2007) and improved intestinal permeability in children with shigellosis (Alam *et al*., 1994). Zinc supplementation had a significant preventive effect on the incidence of persistent diarrhoea and dysentery in certain groups of preschool children (Sazawal *et al*., 1996). However, role of zinc in the treatment of acute shigellosis in children has not been previously reported.

This study aimed to examine if zinc supplementation, in addition to the standard rehydration, antimicrobial, dietary and other supportive therapy, reduces the clinical severity and hastens recovery from and lessens negative impact of shigellosis on growth and morbidity.

Materials and methods

Study design
A randomized, double-blind, controlled clinical trial with subsequent follow-up for 6 months.

Study participants
The study was conducted between January 1999 and October 2002 on 56 moderately malnourished (weight/age 61–75% of NCHS median) children of both sexes, aged 12–59 months, attending the Dhaka hospital ICDDR,B. Patients with dysentery, for example, bloody-mucoid diarrhoea or febrile diarrhoea less than 5 days' duration were initially screened for the study. Stool samples were examined microscropically and cultured for enteric bacterial pathogens. Children with culture-confirmed *Shigella* spp. were finally enrolled. Children with severe malnutrition, history of receiving zinc supplementation or measles in the last 6 months and those with complications such as haemolytic uraemic syndrome or other systemic illness, including pneumonia, meningitis and septicaemia were excluded. Those who lived beyond 2-h of travel time from the Dhaka hospital were also excluded to ensure effective follow-up. The mean duration of diarrhoea in all patients receiving pivmecillinam was 3.3 ± 1.8 days (Kabir *et al*., 1984). The sample size was estimated to detect an assumed 25% reduction by zinc in the time to clinical recovery between the intervention and the placebo group at 5% significance level with 80% power, using the formula of sample size calculation for comparison of two means (Kirkwood, 1988). The actual sample size was 23 in each group.

Ethical Review Committee of the ICDDR,B approved the study and signed informed consent was obtained from the parent/guardian of each child before her/his enrolment.

Study groups
Enrolled children were randomized to two groups: (i) the intervention group that received 20 mg zinc daily for 2 weeks (10 mg of elemental zinc in 5 ml) added to multivitamin syrup and (ii) the control group receiving multivitamin syrup which did not contain zinc. Each 5 ml of multivitamin syrup contained vitamin A (3000 IU), vitamin D (600 IU), thiamin (1.2 mg), riboflavin (2.0 mg), nicotinamide (6.0 mg) and calcium pantothanate (6 mg). All children in the two groups received the standard antimicrobial therapy for shigellosis of this hospital, pivmecillinam in a dose of 15 mg/kg every 6 h for 5 days.

Procedure
A permuted block randomization with a block length of six was performed using a random table to assign equal number of children to the zinc-supplemented and control group, that is, 28 children in each group. Double blinding was ensured by using identical coloured bottles of syrups, labelled with sequential numbers, which had been allocated to either intervention or control according to the randomization. Acme Laboratories (Dhaka, Bangladesh) prepared the study and the control syrups, which were indistinguishable in taste, consistency and appearance. The children received syrup in twice-daily doses. During hospitalization, all the study children received a standard hospital diet of 100–125 kcal and 3–5 g protein per kg body weight daily. At the time of discharge, mothers of respective children were provided with the remaining portion of the assigned syrup and were instructed to administer that to their children at home to complete the total 14 days of supplementation. On the 14th day, a designated health assistant visited the household, checked the bottle and asked about any problems encountered during feeding of the syrup. After discharge from hospital, the health assistants visited all children every 2 weeks for 6 months. Data were collected on body weight, height and morbidity.

Clinical evaluation
Presence of blood and mucous in stool, straining at defecation and body weights were recorded daily. Clinical cure by study day 7 was the primary outcome measured for comparison between the groups. Children were considered to have been clinically cured if they had three or fewer formed stools in a day, were a febrile, did not have visible blood or mucous in stools and did not have abdominal pain or tenderness.

Assessment of growth and growth velocity
Weight and height were measured using standard techniques of WHO (1983), before randomization and at discharge. After completion of 14-day supplementation, children's body weight and height/length were measured every

2 weeks in their respective homes. Weights were measured to the nearest 10 g using an electronic digital scale (Seca, model 770, Seca, Hamburg, Germany; Sweden) standardized with 5 and 10 kg standard weights each morning and evening. Recumbent length was measured for children younger than 2 years using a slide board infantometer (Harpenden, St Albans, England) during hospitalization and a locally made length board with precision of 1 mm at follow-up in homes. For older children, standing height was measured using a stadiometer. Health assistants were trained in anthropometric measurements until attainment of minimum intra- and interindividual variation (<1% coefficient of variation).

Study outcomes

Study outcome variables during hospitalization were time to resolution of diarrhoea, time (days) to disappearance of blood from stool, time (days) to disappearance of mucous from stool, time (days) to resolution of straining, weight and height. Data on morbidity (diarrhoea, fever and acute respiratory tract infection (ARI)) and weight and height were recorded biweekly in the follow-up visits.

Statistical analysis

Data analyses were performed using SPSS/PC + (Windows 10.0) and WHO Anthro, 2005. Consistency check was done using logical programs. Student's and paired *t*-tests were used when data were normally distributed and nonparametric Mann–Whitney *U* test and Wilcoxon test were used when the distribution was skewed. Proportions were compared using χ^2 test. In case of non-normal distribution, data was logarithmically transformed before using *t*-test. Repeated-measure analysis of variance was used to assess the changes in weight and length during the follow-up period. Time to proportions of clinical recovery between the groups was compared using Kaplan–Meier survival analysis. Statistical significance was set at 5% probability level. The analysis of the baseline characteristics, patients during hospitalization and morbidity during follow-up were done on intent to treat basis, with all patients assigned to the study, Kaplan–Meier survival analysis on clinical outcomes and weight and height gain during follow-up were analysed as per protocol with patients who completed the study.

Results

Enrolment of the study participants

During the enrolment period, 98 eligible children with history of bloody-mucoid diarrhoea were initially screened; their stool specimens were examined microscopically and cultured for isolation of enteric bacterial pathogens. Forty-two children were excluded for not meeting the inclusion criteria or for meeting the exclusion criteria. Twenty-eight children were randomized to zinc and 28 were for control group. All children enrolled in the study were infected with *Shigella flexneri*. Two study children from zinc group and four from control group were withdrawn from the study due to different reasons (three for nonresponse to treatment, two for parents seeking early discharge and continuation of treatment in homes and one left from hospital against medical advice (LAMA) and not followed up). Finally, 50 children (26 in zinc group and 24 in control group) completed the clinical study and 30 children (14 in zinc and 16 in control group) completed the follow-up (Figure 1).

Baseline characteristics

Baseline characteristics of the children, such as age, weight/age (NCHS median), height/age (NCHS median), weight/height (NCHS median), duration of diarrhoea, fever and vomiting before enrolment were comparable between the children in the zinc-supplemented and control group (Table 1a). When data were restricted for children who completed the study, there was no significant difference in baseline characteristics between children of the two groups (Table 1b). When data were limited for children who did not complete the study, there was no significant difference in baseline characteristics between children of the two groups (Table 1c).

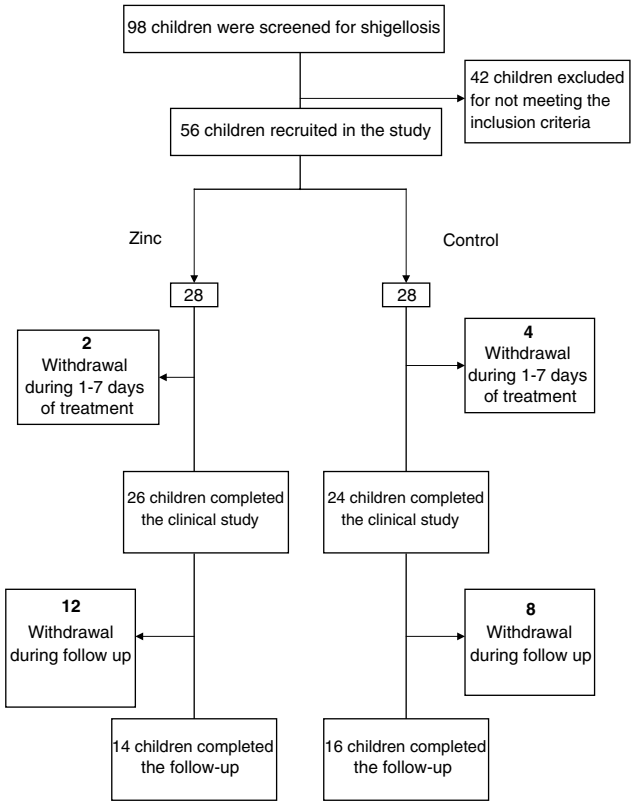

Figure 1 Trial profile.

Table 1a Comparison of baseline characteristics of children who were recruited in the study[a]

Characteristic	*Zinc (n = 28)**	*Control (n = 28)**
Age (months)	22.2 (18.2, 26.2)	27.9 (23.0, 32.9)
WAZ	−2.2 (−2.7, −1.8)	−2.5 (−2.8, −2.1)
HAZ	−1.8 (−2.4, −1.3)	−2.2 (−2.5, −1.9)
WHZ	−1.8 (−2.2, −1.4)	−1.8 (−2.3, −1.4)
Serum zinc (mg/l)	0.59 (0.42, 0.65)	0.57± (0.51, 0.62)
Duration of diarrhoea (days)	6.6 (5.4, 8.0)	5.6 (4.7, 6.5)
Duration of fever (days)	5.4 (3.9, 6.9)	4.3 (3.4, 5.2)
Duration of vomiting (days)	1.3 (0.4, 2.3)	2.1 (1.2, 3.0)

Abbreviations: HAZ, height for age Z score; WAZ, weight for age Z score; WHZ, weight for height Z score.
Figures indicate mean (95% CI). *Student's *t*-test. There were no significant differences between the two groups.
[a]All children (intention to treat analysis).

Table 1b Children who completed the follow-up study (per protocol)

Characteristic	*Zinc (n = 14)**	*Control (n = 16)**
Age (months)	24.4 (18.9, 29.8)	32.5 (25.7, 39.3)
Weight for age *Z* score (WAZ)	−2.6 (−3.1,−2.1)	−2.4 (−2.7 −2.1)
Height for age *Z* score (HAZ)	−2.1 (−2.9, −1.4)	−2.3 (−2.8, −1.8)
Weight for height *Z* score (WHZ)	−2.1 (−2.6, −1.6)	−1.7 (−2.1, −1.3)
Duration of diarrhoea (days)	6.9 (5.6, 8.1)	6.0 (4.6, 7.4)
Duration of fever (days)	5.7 (2.8, 8.7)	4.2 (2.8, 5.6)
Duration of vomiting (days)	1.1 (0.01, 2.4)	2.1 (0.8, 3.3)

Figures indicate mean (95% CI). *Student's *t*-test. There were no significant differences between the two groups.

Table 1c Children who did not complete the follow up study (dropout)

Characteristic	*Zinc (n = 14)**	*Control (n = 12)**
Age (months)	20.0 (13.6, 26.3)	22.5 (15.5, 29.6)
Weight for age *Z* score (WAZ)	−1.9 (−2.6, −1.2)	−2.5 (−3.4, −1.6)
Height for age *Z* score (HAZ)	−1.6 (−2.4, −0.7)	−2.1 (−2.5, −1.7)
Weight for height *Z* score (WHZ)	−1.5 (−2.2, −0.8)	−2.0 (−3.1, −0.08)
Duration of diarrhoea (days)	6.4 (4.05, 8.8)	5.08 (4.2, 5.9)
Duration of fever (days)	5.00 (3.6, 6.4)	4.42 (3.0, 5.6)
Duration of vomiting (days)	1.5 (0.01, 3.1)	2.17 (1.2, 4.02)

Figures indicate mean (95% CI). *Student's *t*-test. There were no significant differences between the two groups.

Clinical outcome

The zinc-supplemented children and the controls were fed the study syrup without any side effects. Diarrhoea resolved within 7 days in 25 (89%) and 21 (75%) children in the zinc-supplemented and control group, respectively ($P = 0.01$) (Table 2). Two (7%) children in the zinc group and four (14%) in the placebo group ($P = 0.35$) withdrew from the study during hospitalization (Table 2) and the reasons for withdrawal included: nonresponse to treatment ($n = 3$), parents seeking early discharge and continuation of treatment in homes ($n = 2$) and LAMA and not followed up ($n = 1$).

At discharge from the hospital, the serum zinc concentration of zinc-supplemented children was significantly higher than that of the control children (0.73 vs 0.68 mg/l, $P = 0.01$). The median time to clinical recovery (2 vs 4 days, $P = 0.03$), disappearance of blood (2 vs 4 days, $P = 0.04$) and mucous from stool (2 vs 4 days, $P = 0.04$) were all 50% shorter in the zinc-supplemented children (Table 2). Kaplan–Meier survival analysis showed that proportion of children clinically recovering from shigellosis by days 2 (52 vs 13%, $P = 0.02$, Breslow test, $P = 0.07$, log rank test) and 4 (84 vs 64%, $P = 0.02$, Breslow test, $P = 0.07$, log rank test) were significantly higher in the zinc-supplemented children (Figure 2).

Weight gain was significantly higher in the zinc group compared to the control group ($P < 0.01$) (Table 2).

Follow-up outcomes

Fourteen patients in the zinc-supplemented group and 12 patients in the control group were dropped out during follow-up. Children gained weight and height during the 6-month follow-up; however, they did not differ significantly from the treatment group. The mean velocity of weight gain was 7.9 and 6.5 g/kg per week for children in the zinc and control group, respectively ($P = 0.6$), and the linear growth was also similar in both the groups (0.65 and 0.58 cm per month, respectively; $P = 0.8$).

Zinc-supplemented children experienced significantly fewer mean episodes of diarrhoea than those in the control group (2.2 vs 3.3, $P = 0.03$) during the follow-up period; however, the number of febrile episodes and respiratory infections were not different between the groups (Table 3).

Discussion

We investigated the effect of zinc supplementation, as an adjunctive to standard fluid and antimicrobial therapy for shigellosis, in malnourished children. The findings indicate that zinc supplementation resulted in faster recovery from diarrhoea; the 50% shorter median duration of illness as observed in this study is greater than that has been observed in our earlier studies in young children with acute watery diarrhoea, (Roy *et al*., 1997) and persistent diarrhoea (Roy *et al*., 1998; The Zinc Investigators' Collaborative Group, 2000) and cholera (Roy *et al*., 2006a).

This benefit might have resulted from improved immunity due to zinc supplementation (Raqib *et al*., 2004). We earlier reported beneficial effects of zinc supplementation in modulating systemic humoral and cellular immune

Table 2 Comparison of clinical features of zinc-supplemented and nonsupplemented children during hospitalization[a]

	Zinc (n = *28*)	*Control* (n = *28*)	P *value*
Withdrawal between 1–7 days hospitalization, *n* (%)	2 (7)	4 (14)	0.35
Recovery by day 7, *n* (%)	25 (89)	21 (75)	0.01[b]
Delayed recovery beyond day 7, *n* (%)	1 (4)	3 (11)	0.06[b]
	n = 26	*n* = 24	
Serum zinc concentration (mg/l) at discharge, mean (95% CI)	0.73 (0.69,0.76)	0.68 (0.66,0.71)	0.01[c]
Time (days) to recovery, median (range)	2 (1–8)	4 (1–8)	0.03[d]
Time (days) to disappearance of blood from stool, median (range)	2 (1–4)	4 (2–5)	0.04[d]
Time (days) to disappearance of mucous from stool, median (range)	2 (1–7)	4 (1–7)	0.04[d]
Time (days) to resolution of straining, median (range)	2 (1–6)	2 (1–5)	0.5[d]
Body weight (kg)			
On admission, mean ± s.d.	8.75 ± 1.2[e]	9.38 ± 1.4[e]	
At discharge, mean ± s.d.	9.20 ± 0.4	9.60 ± 1.8	
P value**	0.000	0.12	

Abbreviation: CI, confidence interval.
[a]Data are expressed in days unless otherwise indicated.
[b]χ^2 test.
[c]Student *t*-test.
[d]Mann–Whitney *U* test
[e]paired *t*-test.

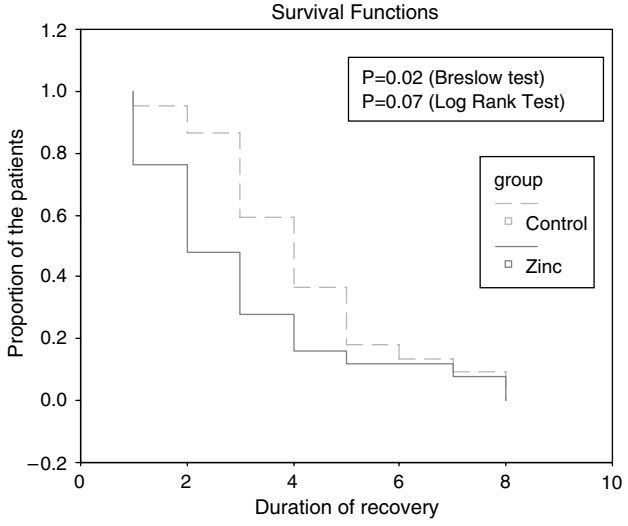

Figure 2 Proportion of children with probability of having diarrhoea by day of interventions (Kaplan–Meier survival graphic of the overall survival in the zinc-supplemented group ($n = 26$) and in the control group ($n = 24$). The median survival was 2 days in zinc-supplemented group and 4 days in control group (Breslow test, $P = 0.02$, log rank test, $P = 0.07$)).

Table 3 Effect of zinc supplementation on morbidity among moderately malnourished children during the 6-month follow-up

	Zinc	*Control*	P *value*
All diarrhoea incidence	2.24 (1.6–3.1)	3.3 (2.7–4.1)	0.03
Duration of diarrhoea episodes (days)	7.1 (3.2–12.6)	9.8 (6.0–15.9)	0.1
ARI incidence	3.6 (2.5–5.3)	3.3 (2.4–4.6)	0.9
Fever incidence	2.2 (1.6–3.2)	2.3 (1.7–3.2)	0.2

Abbreviation: ARI, acute respiratory tract infection.
Data presented as geometric means (95% CI). Data were log transformed before using Student's *t*-test of significance.

responses as zinc therapy was associated with enhanced antigen-specific antibody responses, bactericidal antibody titers against *Shigella*, increased proportions of B cells and plasma cells and higher lymphocyte proliferation responses in the peripheral circulation during the early convalescent phase of shigellosis (Rahman *et al*., 2005).

In earlier studies, supplementation of zinc to deficient rats was associated with rapid regeneration of intestinal mucosa and significant increase in their mass and reduced cholera toxin-induced secretion of water and electrolytes in the ileum (Roy and Tomkins, 1989; Roy *et al*., 2006b). Earlier morphological studies have demonstrated improvement in the structure of the intestinal mucosa in zinc-deficient animals in response to zinc (Koo and Turk, 1977; Roy and Tomkins, 1989). Zinc supplementation also improves mucosal integrity as evidenced by improved mucosal permeability, particularly in malnourished children (Roy *et al*., 1992). The results of the present study suggest that similar changes might have had taken place in malnourished children.

In this study, the weight gain of the zinc-supplemented children during recovery from acute illness was significantly higher. An earlier Bangladeshi study had also observed significant increase in the mean body weight of children with acute diarrhoea who received zinc (Roy *et al*., 1997). Another Jamaican study observed increase in the lean tissue mass of children during their nutritional rehabilitation (Golden and Golden, 1981). Zinc supplementation has also been reported to promote velocity of weight gain in children

recovering from severe malnutrition (Simer *et al*., 1988). However, in our study the beneficial effect of zinc on growth, as observed during hospitalization, was not sustained during the follow-up period. This could result from one or more of the following: relatively shorter duration of zinc supplementation to overcome the strong catabolic effects of shigellosis, which was not adequate for sustained growth thereafter, but reported earlier (Roy *et al*., 1999) and significant enteric protein and micronutrient loss in stool during acute-stage diarrhoea (Castillo-Duran *et al*., 1988; Bennish *et al*., 1993).

In this study, zinc supplementation reduced the number of diarrhoeal episodes during the 6-month follow-up, which corroborates the findings of our earlier studies on short-term zinc supplementation during acute diarrhoea (Roy *et al*., 1999) and persistent diarrhoea (The Zinc Investigators' Collaborative Group, 2000; Roy *et al*., 2007). This effect could have resulted from immunological and nutritional benefits of zinc supplementation, better mucosal repair and growth (Alam *et al*., 1994). Notably, we did not observe any reduction in the episodes of ARI during follow-up. Protective effects of zinc supplementation against respiratory illness, especially viral respiratory diseases, have been described (Brooks *et al*., 2005).

In conclusion, zinc supplementation in children with acute shigellosis hastens recovery from acute illness and helps maintain better growth suggesting blunting of the malnutrition-inducing effects of shigellosis, combined with reduced diarrhoeal morbidity over a 6-month period. Zinc treatment is now recommended by the World Health Organization as part of the routine management of acute childhood watery diarrhoea, but not for shigellosis. Results of our study provide support to extend the recommendation for supplementation of zinc to children with shigellosis.

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The Trial Registration:

The trial was registered on 08/05/2006 according to the clinical trial protocol registration system and the registration number is 'NCT00321126'.

References

Alam AN, Sarker SA, Wahed MA, Khatun M, Rahman MM (1994). Enteric protein loss and intestinal permeability changes in children during acute shigellosis and after recovery: effect of zinc supplementation. *Gut* **35**, 1707–1711.

Bennish ML, Salam MA, Wahed MA (1993). Enteric protein loss during shigellosis. *Am J Gastroenterol* **88**, 53–57.

Black RE, Brown KH, Becker S (1984). Effects of diarrhoea associated with specific enteropathogens on the growth of children in rural Bangladesh. *Pediatrics* **73**, 799–804.

Broek JM, Roy SK, Khan WA, Ara G, Chakrobarty B, Islam S *et al*. (2005). Risk factors for mortality due to shigellosis: a case control study among severely-malnourished children in Bangladesh. *L Health Popul Nutr* **23**, 250–258.

Brooks WA, Santosham M, Naheed A, Goswami D, Wahed MA, Diener-west M (2005). Effect of weekly zinc supplements on incidence of pneumonia and diarrhoea in children younger than 2 years in an urban, low-income population in Bangladesh: randomized controlled trial. *Lancet* 17–23 **366**, 999–1004.

Castillo-Duran C, Vial P, Uauy R (1988). Trace mineral balance during acute diarrhoea in infants. *J Pediatr* **113**, 452–457.

Golden MHN, Golden BE (1981). Effect of zinc supplementation on the dietary intake, rate of weight gain and energy cost of tissue deposition in children recovering from severe malnutrition. *Am J Clin Nutr* **34**, 900–908.

Henry FJ, Alam N, Aziz KMS, Rahman MM (1987). Dysentery, not watery diarrhoea, is associated with stunting in Bangladeshi children. *Hum Nutr Clin Nutr* **41c**, 243–249.

Hossain S, Biswas R, Kabir I, Sarker S, Michael Dibley, Fuchs G *et al*. (1998). Single dose vitamin A treatment in acute shigellosis in Bangladeshi children: randomized double blind controlled trial. *BMJ* **316**, 422–426.

Kabir I, Malek Ma, Mazumder RN, Rahman MM, Mahalanabis D (1993). Rapid catch up growth of children fed a high-protein diet during convalescence from shigellosis. *Am J Clin Nutr* **57**, 441–445.

Kabir I, Rahaman MM, Ahmed SM, Akhter SQ, Butler T (1984). Comparative efficacies of pivmecillinam and ampicillin in acute shigellosis. *Antimicrob Agents Chemother* **25**, 643–645.

Khan WA, Seas C, Dhar U, Salam MA, Bennish ML (1997). Treatment of shigellosis: V. Comparison of azithromycin and ciprofloxacin. A double blind, randomized, controlled trial. *Ann Intern Med* **126**, 697–703.

Kirkwood BR (1988). *Essential of Medical Statistics*. Blackwell Scientific Publications, Oxford, London: Edinburgh, 45: 39A, pp 197.

Koo SI, Turk DE (1977). Effect of zinc eficiency on the ultrastuctures of the pancreatic aciner cell and intestinal epithelium I rat. *J Nutr* **107**, 896–908.

Kotloff KL, Winicoff JP, Ivanoff B, Clemens JD, Swerdlow DL, Sansonetti PJ *et al*. (1999). Global burden of *Shigella* infection: implications for vaccine development and implementation of control strategies. *Bull World Health Organ* **77**, 651–666.

Mitra A, Alvarez JO, Guay-Woodford LG, Fuchs GJ, Wahed MA, Stephenson CB (1998). Urinary retinol excretion and kidney function in children with shigellosis. *Am J Clin Nutr* **68**, 1095–1103.

Rahman MJ, Sarker P, Roy SK, Ahmed SM, Chisti J, Azim T *et al*. (2005). Effects of zinc supplementation as adjunct therapy on the systemic immune responses in shigellosis. *Am J Clin Nutr* **81**, 495–502.

Raqib R, Roy SK, Rahman MJ, Azim T, Ameer SS, Chisti J *et al*. (2004). Effect of zinc supplementation on immune and inflammatory responses in patients with shigellosis. *Am J Clin Nutr* **79**, 444–450.

Roy SK, Behrens RH, Haider R, Akramuzzaman SM, Mahalanabis D, Wahed MA *et al*. (1992). Impact of zinc supplementation on intestinal permeability in Bangladeshi children with acute diarrhoea and persistent diarrhoea syndrome. *J Pediatr Gastroenterol Nutr* **15**, 289–296.

Roy SK, Ireen S, Hossain MJ, Ara G, Ahmed S, Chakraborty B *et al*. (2006a). Impact of zinc supplementation to children with cholera admitted to an urban hospital in Bangladesh. 8th Commonwealth Congress on Diarrhoea and Malnutrition, CAPGAN, 15 (Abstract).

Roy SK, Tomkins AM (1989). Impact of experimental zinc deficiency on growth, morbidity and ultrastructural development of intestinal tissue. *Bangladesh J Nutr* **2**, 1–7.

Roy SK, Tomkins AM, Ara G, Jolly SP, Khatun W, Chowdhury R (2006b). Impact of zinc deficiency on v. cholera enterotoxin stimulated water and electrolyte transport in animal model. *J Health Popul Nutr* **24**, 42–47.

Roy SK, Tomkins AM, Haider R, Behren RH, Zaman SMA, Mahalanabis D *et al*. (1999). Impact of zinc supplementation on subsequent growth and morbidity in Bangladeshi children with acute diarrhoea. *Eur J Clin Nutr* **53**, 529–534.

Roy SK, Tomkins AM, Mahalanabis D, Zaman SMA, Haider R, Behrens RH *et al*. (1998). Impact of zinc supplementation on persistent diarrhoea in malnourished Bangladeshi children. *Acta Paediar* **87**, 1235–1239.

Roy SK, Tomkins AM, Zaman SMA, Behrens RH, Haider R, Mahalanabis D *et al*. (1997). Randomized controlled trial of zinc supplementation in malnourished Bangladeshi children with acute diarrhoea. *ArcDis Child* **77**, 196–200.

Roy SK, Tomkins AM, Akramuzzaman SM, Chakraborty B, Ara G, Biswas R *et al*. (2007). Impact of zinc supplementation on subsequent morbidity and growth in Bangladeshi children with persistent diarrhoea. *J Health Popul Nutr* **25**, (in press).

Sazawal S, Black RE, Bhan MK, Jalla S, Bhandari N, Sinha A *et al*. (1996). Zinc supplementation reduces the incidence of persistent diarrhoea and dysentery among low socioeconomic children in India. *J Nutr* **126**, 443–450.

Simer K, Khanum S, Carlson L, Thompson RPH (1988). Nutritional rehabilitation in Bangladesh – the importance of zinc. *Am J Clin Nutr* **47**, 1036–1040.

Speelman P, Kabir I, Islam M (1984). Distribution of and spread of colonic lesions in Shigellosis: a colonoscopic study. *J Infect Dis* **150**, 899–903.

The Zinc Investigators' Collaborative GroupZulfiqar AB, Sheila MB, Robert EB, Kenneth HB, Julie MG, Adi H *et al*. (2000). Therapeutic effects of oral zinc in acute and persistent diarrhea in children in developing countries: pooled analysis of randomized controlled trials[1–3]. *Am J Clin Nutr* **72**, 1516–1522.

World Health Organization (1983). *Measuring Change in Nutritional Status. Guidelines for Assessing the Nutritional Impact of Supplementary Feeding Programmes for Vulnerable Groups*. WHO: Geneva.