Zinc, Vitamin A, and Micronutrient Supplementation in Children with Diarrhea: A Randomized Controlled Clinical Trial of Combination Therapy versus Monotherapy

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Objective To compare the clinical efficacy of supplementation of zinc, zinc plus vitamin A, and zinc plus combination of micronutrients and vitamins (iron, copper, selenium, vitamin B_{12}, folate, and vitamin A) on acute diarrhea in children.
Study design This was a double-blind, randomized, placebo-controlled trial. Children aged 6 to 24 months with diarrhea and moderate dehydration were randomized to receive zinc plus placebo vitamin A (group 1), zinc plus other micronutrients plus vitamin A (group 2), zinc plus vitamin A (group 3), or placebo (group 4) as an adjunct to oral rehydration solution. Duration, volume of diarrhea, and consumption of oral rehydration solution were compared as outcome variables within the supplemented groups and with the placebo group.
Results The 167 study subjects included 41 in group 1, 39 in group 2, 44 in group 3, and 43 in group 4. All 3 supplemented groups demonstrated a significant reduction in outcome variables ($P < .0001$) compared with the placebo group. Group 3 had the lowest reduction of outcome variables and group 2 had a speedy recovery, but differences among the supplemented groups were not statistically significant.
Conclusions Supplementation with a combination of micronutrients and vitamins was not superior to zinc alone, confirming the clinical benefit of zinc in children with diarrhea. *(J Pediatr 2011;159:633-7)*.

Childhood malnutrition and diarrhea are common in developing countries and responsible for a high proportion of deaths in children.[1] Substantial progress has been made in the treatment of diarrhea in children, with the introduction of reduced-osmolarity oral rehydration solution (ORS) and zinc supplementation supported by strong scientific evidence.[2-8] At present, the World Health Organization (WHO) and United Nations Children's Fund (UNICEF) jointly recommended zinc supplementation for children with diarrhea.[9] Despite strong supportive evidence, zinc has not yet been globally accepted as a therapeutic agent.[10,11]

Vitamin A deficiency is a major public health problem in developing countries.[12] Vitamin A supplementation trials have documented reduced severity, duration, and even mortality due to diarrhea.[13,14] Deficiencies of copper, iron, folate, vitamin B_{12}, and selenium are also common in children of most developing countries and are responsible for increased severity of infection, inflammatory lesions, and reduced antibody response.[15-18] The therapeutic effect of combined supplementation of these micronutrients and vitamins has not yet been studied, however.

We evaluated the therapeutic impact of supplementation with zinc, zinc plus vitamin A, and a combination of micronutrients and vitamins (ie, zinc, iron, copper, selenium, vitamin B_{12}, folate and vitamin A) on diarrhea in children. Our primary hypothesis was that combined supplementation with micronutrients and vitamins might have a better therapeutic effect compared with supplementation with zinc alone. All micronutrient deficiencies could then be corrected simultaneously.

Methods

We conducted a hospital-based, double-blind, randomized, placebo-controlled clinical trial at the Dr B. C. Roy Memorial Hospital for Children, Kolkata, India between March 1999 and May 2001. The study was not registered in the clinical trial registry as the study was intra-mural in nature and at the time of initiation of the study registry as not mandatory. Male children were chosen for ease of separate collection of stool and urine samples. The children ranged in age from 6 to 24 months and had a history of acute watery diarrhea (more than 3 episodes within the last 24 hours) of less than 72 hours duration. All of the children had moderate dehydration, manifested by

HIV	Human immunodeficiency virus
ORS	Oral rehydration salt solution
RDA	Recommended daily allowance
UNICEF	United Nations Children's Fund
WHO	World Health Organization

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clinical signs and symptoms of thirst: irritability; sunken eyes; dry mouth, lips, and tongue; and loss of skin elasticity.

The sample size was calculated under the assumption that the average duration of diarrhea in the supplemented groups will be significantly lower (25%) compared with a mean duration of 66.4 ± 32.3 hours after initiation of standard treatment.[19] Considering a 5% level of significance, 80% power, and 10% dropout, the minimum sample size was calculated as 42 children in each of the 4 groups. However, for fear of more dropouts, we randomized 44 children in each group from a total of 176 children.

Children clinically diagnosed with severe undernutrition (wasting) or another systemic illness (eg, septicemia, pneumonia, urinary tract infection, otitis media) or chronic underlying disease (eg, tuberculosis, liver diseases) or needing intensive care (eg, life support system, blood transfusion, total parental nutrition) were excluded from the study. Children who were exclusively breast-fed also were excluded. Children who had received antibiotics before enrollment or received vitamin A supplementation within the previous 6 months were excluded. The children's human immunodeficiency virus (HIV) status was not assessed, because HIV screening is not routinely done in children with acute diarrhea. Moreover, the HIV prevalence in the catchment population of this region did not warrant routine HIV testing of the study population.

Children who fulfilled the inclusion and exclusion criteria were randomized to 4 treatment groups according to a random number table. Randomization was done blindly and independently to allocate a patient to specific numbered bottle of supplementation or placebo. The serial code numbers were kept in a sealed envelope, and the groups were identified only after study completion. The children in group 1 received 20 mg elemental zinc (twice the Recommended Daily Allowance [RDA]) daily and a single oral dose of placebo of vitamin A on admission. Group 2 children received micronutrient combination (twice the RDA of all micronutrients and vitamins: zinc, 20 mg; iron, 10 mg; copper, 2 mg; selenium, 40 mg; vitamin B_{12}, 1.4 mg; folate, 100 mg) daily and a single oral dose of vitamin A on admission following national guidelines (age <1 year, 100 000 IU; >1 year, 200 000 IU). Group 3 children received 20 mg of elemental zinc (twice the RDA) daily and a single oral dose of vitamin A on admission according to national guidelines. Group 4 children received placebo of micronutrients and vitamins and a single oral dose of placebo of vitamin A on admission. Micronutrients, vitamins, or placebo were given in 2 daily doses for 14 days even after recovery. All of the supplemental micronutrients and placebo were in syrup form with a similar taste and appearance. They were prepared by Greenco Biologicals (Kolkata, India) according to our specifications and packaged in identical-looking bottles. Vitamin A and its placebo were prepared in same manner.

This study was approved by the Scientific Advisory Committee and Institutional Ethics Committee of the National Institute of Cholera and Enteric Diseases in Kolkata, India. Before enrollment, informed written consent was obtained from the parents of each child after the study procedure was described in detail. A complete clinical history was obtained from the parents, and a thorough physical examination was done. Children were weighed unclothed on a scale with a sensitivity of 1 g. Length and mid-arm circumference also were measured. Stool samples were collected on admission in sterile McCartney bottles, using sterile rectal catheters and were processed for detection of established enteropathogens, including bacteria, viruses, and parasites, using standard procedures. Children received the WHO-recommended reduced-osmolarity ORS for correction of initial dehydration and as maintenance therapy, matching the stool volume and loss in vomitus until cessation of diarrhea.[20] Intravenous fluid (ie, Ringer's lactate solution) was available for a child who developed severe dehydration or intractable vomiting during the hospital stay, in accordance with WHO guidelines.[20] No children received any drug therapy during the study period.

Immediately after correction of initial dehydration, feeding was resumed in all children. Breast-fed children were allowed to continue breast-feeding; others were allowed to take formula milk or animal milk. Older children continued on their normal diet before the onset of illness. Plain water was offered. Children were followed up in the hospital until recovery or for 5 days if recovery did not occur within that time period. Intake and output data were measured every 8 hours and recorded.

The duration of diarrhea was calculated as hours from the passage of the last liquid stool. Recovery was defined as passage of soft stool, formed stool, or no stool for 18 hours. Stool loss was measured using preweighed disposable diapers on a scale with a sensitivity of 1 g. Urine was separated from stool using a urine collection bag. Vomitus was weighed using preweighed gauze pads. Body weight was recorded after correction of initial dehydration and then every morning between 10 and 10:30 a.m. until recovery. The following measurements were recorded daily: number of stools, number of vomiting episodes, stool output (g), intake of ORS (mL), intake of plain water (mL), intake of liquid food (mL), intake of intravenous fluid (mL) if required for correction of dehydration, and body weight (g). Children were discharged from the study after the passage of formed stool. Mothers were advised at the time of discharge to bring their children to the hospital if they developed complications.

The checklist of treatment assignment was decoded for the experimental groups. The 4 study groups were compared based on clinical characteristics on admission and isolation of enteropathogens using the χ^2 test. The Mantel-Haenszel χ^2 test was used for testing recovery rates. One-way analysis of variance post hoc testing was carried out to compare the quantitative measures of duration of diarrhea, volume of stool output, and intake of ORS in the 4 treatment groups. Survival function analysis was used to assess the recovery status of the 4 groups.

Results

A total of 176 male children aged 6 to 24 months were initially recruited into the study; however, 9 children dropped