Combined iron and zinc supplementation among school children in Galle District: a follow-up study

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Abstract

Introduction: Deficiencies of iron and zinc are major public health problems in many developing countries. Combined iron and zinc supplementation may be beneficial, but the potential interactions observed in functional outcomes such as growth and development need to be verified.

Methods: Adolescent school children (n=821) of 12-18 years of age were randomized into one of four groups on a double-blind approach at class room level. Children in each group received two capsules daily containing either iron 50 mg/d (Group 1) or zinc 14 mg/d (Group 2) or iron and zinc combined (Group3) or a placebo (Group 4) for a period of 36 weeks. Their anthropometric assessment was made and a medical examination carried out prior to study and after the intervention.

Results: Mean change of weight and height in the placebo group was 0.53kg and 0.73cm. Iron alone group had 0.89kg gain in weight and 1.0cm in height. Zinc alone group had a higher gain in weight (2.27 kg) and height (2.37cm) whereas 1.52 kg and 1.63 cm gain was observed with combine supplemented group. The body mass index (BMI) of the supplemented groups significantly increased from their respective baseline status (0.32 in supplemented, 0.04 in placebo; $p < 0.001$). The increase in z-scores of weight-for-age and height-for-age in zinc supplemented groups was marginally significant when compared with the placebo group ($p < 0.05$).

Conclusions: After correcting for confounding effects of age and the respective baseline values of weight, height and BMI, the zinc alone supplemented group had the best anthropometric improvement. It appears therefore, that long term zinc supplementation has positive impact on the growth of children.

Key words: Iron supplementation, Zinc supplementation, Growth, School children

Introduction

Undernutrition is manifested in most developing countries by early growth stunting and high prevalence of micronutrient deficiencies. National surveys in Sri Lanka showed prevalence of underweight and stunting as 47.2% and 28.5%, respectively and 2.2% of overweight among adolescents (1). In a previous study done in 2003 we reported the prevalence of underweight and stunting as 31.1% and 21.5% in adolescent school children (aged 12-16 years) in Galle district with 14.9% of severe malnutrition (2). Even though national anaemia prevalence was 11.1% as reported in 2006 (1), a recent study done in Galle revealed that it was 54.8% (3) with a 55.7% deficiency of zinc.

The causes of early growth stunting are not yet understood (4), but may include a nutritionally inadequate diet as well as clinical (5) or sub clinical infections. Although growth stunting has been traditionally attributed to protein-energy malnutrition, it is widespread even where protein and energy intakes are adequate (4,6). Stunting is associated with habitual consumption of a diet that is low in animal products (and accompanying micronutrients) and high in plant constituents such as phytate that inhibit the absorption of minerals (7,8). We showed previously that the dietary intake of both macro and micronutrient are less than 50% of recommended amount (9) and 62% had usually not eaten breakfast before going to school. At national level 10.4% subjects missed breakfast during schooling (1).

Because zinc supplementation has improved the growth and/or body composition of stunted children in countries such as the United States, Canada, Ecuador, China, and Guatemala (4,10), the present study was designed to measure effect of zinc supplementation on the growth and body composition of school children in Sri Lanka. Unlike most of the previous zinc-supplementation studies reported in the literature, iron supplements were also provided because of the high prevalence of iron deficiency in this community (3).

If iron and zinc are to be provided together, it is important to determine whether they interact biologically, and if so, how. Because they have chemically similar absorption and transport mechanisms, iron and zinc have been thought to compete for absorptive pathways (11). New evidence based on cell culture studies has shown that iron may inhibit zinc absorption in some cells at very high ratios of iron to zinc, but not vice versa (12). However, evidence at low ratios of iron to zinc also is needed to assess any biochemical and functional effects of dual supplementation. A long term supplementation is needed for better understanding of the biological basis for potential interactions observed in functional outcomes such as growth and development. The experimental design of this study will provide some valuable information on effect of iron, zinc or combined supplements on adolescent growth.

Methods

The study received approval from the Ethical committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka. Schoolchildren of 12 to 16 years of age attending schools in Galle District constituted the study population. Three schools (1 girls' school and 2 mixed schools) were randomly selected. It was found that these schools have both urban and rural children in equal proportions. Principals of the selected schools were contacted after obtaining written permission from the Secretary, Ministry of Education, Sri Lanka. Then the class teachers were briefed about the study.

Screening

In selecting the sample, 845 children who presented with written consent from their parents were

subjected to a comprehensive physical examination, including measurements of height and body weight using a portable stadiometer and a beam balance with non-detachable weights, respectively. A sample of venous blood (3ml) was drawn for the initial assessment of haemoglobin and other parameters.

Enrollment

Children with a haemoglobin level $\geq 80\text{g/L}$ were eligible for the study. Others were referred for treatment and further investigations ($n=5$). Further, children suffering from acute or chronic diseases or inflammatory conditions and any drug consumption other than paracetamol or antihistamines for minor ailments ($n=15$), currently consuming nutrient supplementation ($n=4$) were also excluded from the study. A letter was given to their family physicians requesting notification if they wished to prescribe additional micronutrient supplements during the study period. They were treated for parasites with mebendazole 500mg as a single oral dose approximately two weeks prior to the start of the study.

Drug supplementation

Subjects were randomized into one of four groups and randomization was stratified by their classrooms. Therefore, all the subjects in one classroom were allocated to a one group. Four groups of children were supplemented with two capsules per day containing either iron (50mg/d) in the form of ferrous fumarate, zinc (14mg/d) in the form of zinc sulfate, iron + zinc (50 + 14mg/d) or a placebo made of anhydrous lactose. The study subjects received capsules daily on school days for a period of 36 weeks from their classroom teacher. These were consumed each morning at the time daily attendance was taken. Teachers were instructed to ensure that their students consumed the capsules each day. As this was a double blind procedure neither the subjects nor their teachers knew the contents of the capsules. Capsules were provided to the classroom teachers by the investigators every two weeks; the teachers were asked to sign a checklist when the doses were given. These checklists were randomly checked for accuracy and compliance. For the weekends and school holidays a separate pack of capsules was given to each child

with a letter to parents. Either mother or father was asked to sign the checklist once the child consumed the capsules. Supplementation was started on 20th January 2003 and continued till 07th November 2003. The final clinical examination was carried out one week after the end of the intervention (at 36 weeks) with an anthropometric assessment.

Statistical analysis

On the basis of the estimated prevalence of anaemia (anaemia defined as $Hb < 120.0 \text{ g/L}$) in this age group in Sri Lanka (12), the sample size was calculated to be 180 per group to demonstrate a 15% reduction in anaemia prevalence with an alpha error of 5% and beta error 10%. The calculated sample size was inflated to 200 per group with an assumption of a 25% dropout rate during follow-up period. A one-sample Kolmogorov-Smirnov test was used to investigate whether the anthropometry (weight, height and BMI) were normally distributed. The 1978 CDC/WHO growth reference curves were used from Epi-info version 3.0 (2003) to generate values for Z-scores of weight-for-age (WAZ; underweight indicators), and height-for-age (HAZ; stunting indicators).

Differences between groups in anthropometric indices at initial and post-intervention stages were investigated using multivariate analysis of variance (MANOVA) repeated-measures design with supplement type as a between-subject factor (four groups) and treatment effect (baseline compared with interim/ final) as a within-subject factor. Baseline values for WAZ (in 2 classifications: < -2.0 and > -2.0) and HAZ (in 2 classifications: < -2.0 and > -2.0) were also included in the analysis as between-subject factors to correct for their possible confounding influence on the change in weight and height. None of the 3-way interactions (treatment effect x supplement type x baseline value) were significant. All analyses were done using SPSS version 10.0 for WINDOWS (SPSS Inc, Chicago).

Results

When randomly grouped, 202 children were in the iron supplemented group, 213 in the zinc supplemented group, 216 in the iron and zinc combined group and 190 were in the control group who were treated with placebo capsules. Mean age of

iron group (170 months $\pm$ 19) and placebo group (166 months $\pm$ 16) was significantly different ($p < 0.05$) from zinc group (163 months $\pm$ 20) and combine supplemented (159 months $\pm$ 15) group. However, the initial anthropometry (weight and height; Table 1) and the initial prevalence of underweight (defined by $\leq -2SD$ of WAZ) and stunting (defined by $\leq -2SD$ of WAZ) were not different between these four groups.

The iron group had a drop-out rate of 20.3% at the end of the intervention (36 weeks). Drop-out rate in zinc group was 22.1% whereas in combined supplement group it was 20.4%. There may have been a possibility of adverse side-effects of iron supplementation (i.e. gastric irritation) for the observed drop-out rate however, the placebo group also had a drop-out rate of 24.7% with an overall drop-out rate of 21.8% ($n=179$) by the end of intervention. Baseline parameters of the subjects who entered the final analysis were not significantly different from that of the subjects who did not enter the final analysis (data not shown). On the basis of compliance records, 78% of the subjects received possible 252 doses, 18% received 225-252 doses and only 4% of subjects received less than 225 doses. The mean number of capsules consumed by different groups were not significantly different ($p=0.36$).

Following 36 weeks of supplementation all four groups had a significant improvement ($p < 0.05$) in mean weight and height from their respective baseline values (Table 1). Iron supplemented group had mean improvement of weight to 38.3Kg from its baseline of 37.4 kg, mean height also improved to 151.1cm from 150.2cm. Zinc supplemented group improved to 39.4 kg from 37.1 kg in weight and 152.0cm of mean height from the baseline of 149.9cm. The combine supplemented group had showed an improvement in mean weight to 40.6kg from the baseline of 38.4 kg whereas mean height increased to 152.0cm from the baseline of 150.0cm. Placebo group had mean weight of 37.4 kg and 150.6 cm after the intervention

During the total intervention period (09 months), the body mass index (BMI) of the supplemented groups significantly increased from their respective baseline values when compared to the placebo group (0.32 in supplemented, 0.04 in placebo; $p < 0.001$). With nine months of supplementation, zinc supplemented groups (zinc and combined) showed a significant

improvement in weight and height over iron alone supplemented group ($p < 0.001$). Mean change in placebo group was 0.53 kg and 0.73 cm during the period of intervention. Iron alone group had 0.89 Kg gain in weight and 1.0 cm in height respectively. Zinc alone group had 2.27 kg gain in weight and 2.37 cm gain in height whereas combined supplementation had achieved 1.52 kg (MANOVA, $p < 0.001$) and 1.63 cm (MANOVA, $p=0.04$) gain in weight and height respectively (Figure 1 and Figure 2). These

effects contributed reflected in improvements in weight-for-age and height-for-age Z-scores in zinc supplemented groups and it was marginally significant when compared with the placebo group ($p < 0.05$). After correcting for confounding effects of age and the respective baseline values of weight, height and BMI, the zinc supplemented group had the best anthropometric improvement.

Table 1: Effect on anthropometry with micronutrient supplementation ⁽¹⁾

Group	n	Iron	n	Zinc	n	Combined	n	Placebo
Weight (Kg)								
Baseline	202	37.35 (7.45)	213	37.11 (8.42)	216	38.42 (8.91)	190	37.23 (8.77)
Final	161	38.31 (7.45) ^a	166	39.36 (8.23) ^b	172	40.58 (8.92) ^b	143	37.44 (7.54) ^a
Height (cm)								
Baseline	202	150.24 (8.49)	213	149.86 (9.61)	216	149.78 (8.12)	190	150.82 (9.14)
Final	161	151.11 (8.20) ^a	166	152.03 (9.18) ^b	172	151.98 (7.96) ^b	143	150.57 (8.72) ^a
BMI								
Baseline	202	16.39 (2.26) ^a	213	16.35 (2.44) ^a	216	16.95 (2.72) ^b	190	16.19 (2.53) ^a
Final	161	16.64 (2.25) ^a	166	16.87 (2.39) ^b	172	17.31 (2.98) ^b	143	16.38 (2.26) ^a
WAZ								
Baseline	202	-1.69 (0.76) ^a	213	-1.51 (1.23) ^a	216	-1.24 (0.93) ^b	190	-1.59 (0.96) ^a
Final	161	-1.70 (0.78) ^b	166	-1.52 (0.90) ^b	172	-1.24 (0.98) ^b	143	-1.94 (0.78) ^a
HAZ								
Baseline	202	-1.37 (0.94) ^a	213	-1.23 (1.07) ^a	216	-1.08 (0.95) ^b	190	-1.20 (1.05) ^a
Final	161	-1.33 (0.97) ^b	166	-1.27 (1.10) ^b	172	-1.08 (1.00) ^b	143	-1.66 (0.97) ^a

¹Results expressed as mean (SD)

^{a,b}Values with same superscripts in a given row are not significantly different

Figure 1 - Supplementary effect on mean weight gain during the follow-up period

Figure 2 - Supplementary effect on mean height gain during the follow-up period

Discussion

This study showed that micronutrient supplementation has a positive effect on weight and height gain during a certain stage of childhood development. The composition of the daily supplement is based on the daily recommended allowances published by the World Health Organization (14) for zinc and global guidelines for iron supplementation provided by the International Anemia Consultative Group/ World Health Organization / UNICEF (15). Using these recommendations resulted in a ratio of iron (mg) to zinc (mg) of 3.6 : 1. In a previous metabolic balance study among infants by Haschke *et al*, (16) iron to zinc ratios of 5.4 : 1 and 1.3 : 1 demonstrated no significant effect on zinc absorption (zinc absorption 15.6% and 20.35 respectively). In another study, Sandstrom *et al* (17) found that increasing molar ratios of iron to zinc from 1 : 1 to 2.5 : 1 did not affect absorption of zinc from water but iron to zinc ratio of 25 : 1 decreased zinc absorption.

While the essential role of several micronutrients in growth has been demonstrated clearly by clinical-based human trials, the positive impact of community-based supplementation trials indeed confirms that iron and zinc are common growth-limiting nutrients (18). However, iron appears to be limiting growth only when deficiencies of these nutrients are severe, whereas growth may be limited only by mild to moderate deficiency of zinc. This is consistent with the known metabolic and physiologic activity of these nutrients: zinc has direct effects on the primary hormonal system that controls growth in the postnatal phase when the stunting occurs. On the other hand, iron do not appear to influence this system directly, but more likely exert its effects on growth when their functional stores are depleted and/or when deficiency results in increased morbidity, which in turn contributes to growth faltering. Although single-micronutrient supplementation trials have been useful to confirm the effects of specific micronutrients on growth outcomes, programmes that provide supplements of only one nutrient may not be the most cost-effective way of preventing growth faltering and associated adverse health outcomes because of the co-existence of multiple-micronutrient deficiencies in many populations. Multiple-micronutrient supplements are expected to be more efficacious in preventing growth faltering in at-risk populations, as all possible growth-limiting micronutrient deficiencies may be

corrected simultaneously. Although the few available multiple-micronutrient supplementation trials have demonstrated positive effects on growth, in some cases these effects were limited to specific subgroups of the study population (18).

In schoolchildren significant gains in height and weight, as well as increased food intake, have been shown after 14 weeks of treatment with iron whereas an increased weight and weight-for-height but no increase in height was observed after 7 months of intermittent iron supplementation (19). A recent meta-analysis of 33 studies which investigated the effect of zinc supplementation on growth and serum zinc concentrations of pre-pubertal children (20) revealed a highly significant positive response in height and weight increments and a large increase in the children's serum zinc concentrations. The overall effect size (expressed in standard deviation units) in height reported was 0.350 (CI, 0.189-0.511; $p < 0.0001$) and weight was 0.309 (CI, 0.178- 0.439; $p < 0.0001$). The growth responses were greater in children with low initial weight-for-age Z-scores.

Although anthropometry improved in the supplemented groups in this study, there was no significant treatment effect on height-for-age after correcting for the confounding influences of between-group differences in initial height-for-age and age. This result is similar to those seen in other studies carried out among representative groups of pre-school children from countries such as Chile (21), Mexico (22) and the Gambia (23). A similar supplementation study with iron and zinc in Vietnamese children also resulted in an improvement of height-for-age, but the average initial height-for-age Z-scores of these children were already low at < -2.66 (24). The height of Guatemalan children who were stunted improved more than that of non-stunted peers after zinc supplementation (25). It appears therefore, that zinc supplementation influences the growth of children only when they are zinc deficient and having lower-than-average anthropometric measurements. This effect was observed in the present study too. In the subgroup of children who had an initial height-for-age Z-score < -2.0 , there was a significant treatment effect on the height-for-age of subjects in the zinc and the combined groups, whereas the values for the iron and placebo groups did not change significantly. Similar effects on weight-for-age were seen in the zinc and the combined groups. It can be concluded

that the linear growth of children with lower height and weight-for-age Z-scores is positively affected by zinc supplementation.

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