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Original Article

Effects of iron and zinc fortified milk supplementation on working memory of underweight poor-urban school children: A randomized double blind controlled trial

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Abstract

Background Undernutrition associated micronutrient deficiencies in children are still prevalent in most developing countries. Iron and zinc deficiencies are the most common micronutrient deficiency globally, which significantly contribute deficits in cognitive function. Fortification with iron and zinc has proven successfully in reducing certain cognitive impairments like memory.

Objective To determine the effects of milk fortified with iron and zinc on memory of underweight poor-urban schoolchildren.

Methods A double blind randomized controlled trial was conducted on 218 underweight poor-urban schoolchildren aged 7-9 years old; 113 children were supplemented twice daily with 27 gram of milk fortified with iron pyrophosphate (12.15 mg) and zinc sulfate (4.4 mg) for three months, 105 children served as controls. Anthropometry measured including body weight, height, sitting height and middle upper arm circumference. Memory was measured using digit span backward test in Wechsler Intelligence Scales III. Statistical analyses were performed with SPSS for Windows, version 11.0.

Results Baseline data of digit span backward score in iron and zinc group was 2.4 (SD 1.2), and control group was 3.0 (SD 1.8). After intervention, mean digit span backward score in iron and zinc group was 3.1 (SD 1.2) and in control group 3.0 (SD 1.3). There was no improvement digit span backward score in control group after intervention, compared with 0.7 point improvement in iron and zinc group ($P=0.009$).

Conclusion Milk fortified with iron and zinc improves working memory of underweight poor-urban schoolchildren. **[Paediatr Indones. 2010;50:92-95]**.

Keywords: *iron, zinc, underweight, memory*

Micronutrients deficiencies are still prevalent in most developing countries with low income population. Iron and zinc deficiencies are the most common micronutrient deficiency globally, affecting between 1.5 and two billion people especially children with undernutrition.[1-4] The main causes of these micronutrients deficiencies are low dietary intake and low bioavaibility in the diet.[4]

Iron and zinc are essential micronutrients for human health.[5] There is evidence that iron and zinc are physiologically linked to lead metabolism.[6] Zinc is necessary for the synthesis of nucleic acids and proteins which are important for brain maturation and function.[7] Iron deficiency, whether resulting from inadequate intake or decreased absorption, is the primary cause of nutritional anemia.[5] In many previous studies, zinc and iron deficiencies significantly contribute deficits in certain cognitive function like memory.[5,7] Iron and zinc fortification has proven successful in reducing

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certain cognitive impairments that are associated with these micronutrients deficiency.[6] However, a study conducted in Purworejo, Central Java, shows that combined iron and zinc supplementation is not optimal.[4] Therefore, milk fortification is an attractive and easy strategy to combat iron and zinc deficiencies because it could increase the iron and zinc intake of the entire population.[1] We hypothesized that milk fortified with iron and zinc improves memory of underweight poor-urban schoolchildren.

Methods

Design

This community based double blind randomized controlled trial study was conducted from July to September 2007 in poor-urban elementary school in Solo. Poor-urban areas in Solo were located using data from Provincial Central Bureau of Statistics. Elementary schools located in that area were selected based on economical indicator using parental income and school's fee as indicators. Using an estimation that there were around 50 of 2nd and 3rd grade elementary school children in each schools and 50% of them were underweight, 10 elementary schools from the school pool were randomly selected. After obtaining the schools, 10 elementary schools were randomly assigned into two groups of intervention as iron and zinc group and control group.

All 2nd and 3rd grade elementary school children in selected schools were screened for anthropometric measurements. All children received albendazole 450 mg as anti helminthic treatment. They were prohibited to use vitamins and minerals supplementation during the study. Subjects were considered eligible for the study if they were underweight (weight for age 5-10th percentile CDC 2000), 7-9 years old (2nd – 3rd class of elementary school), at the time of recruitment did not suffer from chronic illness including renal, congenital, thyroid disease, diarrhea, thalassemia and have provided informed consent. Children with anemia (Hb < 8 g/dl) were excluded. Participants who were eligible for clinical trials were recruited to the study after providing informed consent. The Research Ethics Committee of the Moewardi Hospital Surakarta, Indonesia approved the investigation.

Intervention

All subjects consumed two glasses of milk each day for 3 months. Subjects in iron and zinc group were allocated to receive milk A which contained iron pyrophosphate 12.15 mg/serving and zinc sulfate 4.4 mg/serving, and subjects in control group were allocated to receive milk B which contained iron pyrophosphate 0.479 mg/serving and zinc sulfate 1.63 mg/serving. Products served in single serving sachet. The children were given milk twice a day (27 g milk diluted with 180 ml water/serving). Drinking milk was done in the morning before the school begins, while the other sachet were given for home consumption. Mothers had been shown how to reconstitute the powdered milk.

Assessment and statistical analysis

Trained observers measured anthropometry indicators before intervention. Weight was measured to nearest 0.1 kg with an electronic scale (SECA Corporation, Colombia, MD) and length or height to the nearest 0.1 cm with *Microtoise*. Mid upper arm circumference was measured to the nearest 1 mm by using a non stretchable plastic measuring tape. All measurements were done in duplicate, and the mean value was used in the analysis. Z-scores for weight and height (weight-for-age, height-for-age and weight-for-height) were calculated using EPI-Info version 3.4. Memory has been measured using digit span backward test in Weschler Intelligence Scales III before and after intervention.

Data were analysed using SPSS statistical software package version 11.0. We used Chi-square for categorical data and independent t-test for numerical data. The level of statistical significance was $P<0.05$.

Results

Baseline characteristics

Out of total of 222 underweight children, five children dropped out (two moved to other school and three children refused drink milk). Two hundred and eighteen children (48.2% female and 51.8% male) whose age ranged from 7 to 9 years participated in our study. The baseline characteristics of the subjects in both groups are depicted in **Table 1**. There were no clinical differences between groups in terms of demographic charcteristics.

Memory

In both groups, minimum digit span score was zero before intervention. Maximum score before intervention in iron and zinc group was seven, and in control was eight. After intervention, minimum and maximum digit span score was same in both groups, minimum score was zero, and maximum score was eight. Before intervention mean digit span backward score in control group were significantly higher than in iron and zinc group (P = 0.007). However after intervention mean digit span backward in iron and zinc group were higher than in control group (P = 0.562). After supplementation mean digit span backward score in control group was not improve, compare with 0.7 point improvement in iron and zinc group (P= 0.009) (**Table 2**). In intention to treat analysis, we calculated event rate in control group (CER) which was 0.569 and event rate in iron and zinc group (EER) which was 0.368, so absolute risk reduction in this study (ARR) was 0.201. The inverse of absolute risk reduction or number needed to treat for this study was 5.

Discussion

Digit span backward is one of several subtests in Wechsler Intelligence Scales III. Digit span backward is used as measures on an individual's working memory. In digit span backward, a series of digits must be repeated in the reverse order. Several studies have challenged the assumption that because of the increased complexity of the backward test, it is more sensitive to impairments associated with aging and various neurological conditions.[8] This is the reason why we use digit span backward in Wechsler Intelligence Scales III.

Middle upper arm circumference was the only one characteristic of subjects that significantly different between two groups. The other characteristics were not significantly different. The main result of this study shows that after 3 months intervention the

Table 1. Baseline characteristics

Characteristics	Iron and zinc (n = 113)	Control (n = 105)	Total (N = 218)
Age, mean (SD) yrs	8 (0.6)	8 (0.6)	8 (0.6)
7-8 years, %	49.6	48.6	49.1
>8-9 years, %	50.4	51.4	50.9
Sex			
Male, n (%)	61 (54)	52 (49.5)	113 (51.8)
Female, n (%)	52 (46)	53 (50.5)	105 (48.2)
Nutrition status			
Height, mean (SD) cm	117.2 (4.4)	117.1 (4.6)	117.1 (4.5)
Sitting height, mean (SD) cm	62.9 (2.3)	63.2 (2.4)	63 (2.3)
Weight, mean (SD) cm	19.2 (1.6)	19.0 (1.8)	19.1 (1.7)
MUAC, mean (SD) cm	16.3 (0.8)	16.9 (0.7)	16,6 ± 0,8

SD = standard deviation; MUAC = middle upper arm circumference

Tabel 2. Digit span backward result

Digit span backward score	Iron and zinc group	Control group	P
Before supplementation			
Mean (SD)	2.4 (1.2)	3.0 (1.8)	0.007
Minimum score	0.0	0.0	
Maximum score	7.0	8.0	
After supplementation			
Mean	3.1 (1.2)	3.0 (1.3)	0.562
Minimum score	0.0	0.0	
Maximum score	0.8	0.8	
Delta mean before and after	0.7 (1.7)	0.0 (1.9)	0.009

Independent sample t-test

mean digit span backward score in iron and zinc group increased, while mean digit span backward in control group did not. There is no definite explanation for this. Several biological mechanism potentially link iron deficiency with impaired cognitive performance. Iron deficiency results in decreased body iron stores, including decreased iron in the central nervous system, even before red blood cell production is affected. Cerebral oxidative metabolism disorder attributable to low levels of heme containing and iron-dependent enzymes results in certain impairments cognitive function.[6,9-11] Zinc, on the other hand, plays an important role in the modulation of some CNS functions, because it is one of the most prevalent trace elements in brain. Zinc is also necessary for the synthesis of nucleic acids, brain maturation and brain function. When applied at high concentration it has neurotoxin effects on neuron cultures, or, when present at concentration below physiological values, it causes certain cognitive impairments.[7,12-13] The main causes of these micronutrients deficiencies are low dietary intake and low bioavaibility in the diet,[4] so that like many previous studies, milk fortification of these micronutrients seems can prevent certain cognitive impairments associated iron and zinc deficiencies.[1,6]

Our subjects may get iron and zinc as micronutrients from other diets they have taken besides our milk intervention, because we can not fully control of our subjects diets. Other physical conditions of subject during digit span backward test also may influence their test score.

To sum up, milk fortified with iron and zinc can improve working memory of underweight poor-urban schoolchildren in Solo. Other study with other method to measure memory is needed to ensure that milk fortified with iron and zinc can improve memory.

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