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Effect of Zinc and Iron Supplementation on Indicators of Iron, Zinc and Vitamin A Status of Primary School Children

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Abstract: The aim of this study was to investigate the effects of supplementation of iron and zinc, alone or combined, on iron, zinc and vitamin A status in primary school children. The study was a randomized double-blind clinical trial in which 79 primary school children, 11 year of age, were randomly supplemented for 4 months with iron (20 mg day^{-1}), zinc (20 mg day^{-1}), or iron+ zinc (20 mg of each day^{-1}). Serum zinc significantly increased in all supplemented groups. Compared with iron alone, zinc supplementation and zinc plus iron were associated with higher serum zinc and plasma ferritin levels. Zinc supplementation resulted in a decrease in serum ferritin. Children deficient in zinc at the beginning of the study had a significantly greater increase in serum zinc than did children with adequate serum zinc. Four months after supplementation, hemoglobin remained unchanged in all supplemented group. Plasma retinol levels decreased in all supplemented groups. Supplementation with iron plus zinc improved serum zinc and plasma ferritin. However, since plasma retinol levels decreased as a result of supplementation, more studies are needed on the matter.

Key words: Supplementation, iron, zinc, vitamin A, primary school children

INTRODUCTION

Iron and zinc are essential micronutrients for human growth, development and maintenance of the immune system. Iron is needed for psychomotor development, maintenance of physical activity and work capacity and resistance to infection. Zinc is needed for growth and for maintenance of immune function, which enhances both the prevention of and recovery from infectious diseases (Walker *et al*., 2005).

The coexistence of multiple micronutrient deficiencies is increasingly recognized as a widespread public health problem in developing countries (Munoz *et al*., 2000). In many countries, major efforts are being carried out by governments, supported by international organizations, to reduce micronutrient deficiencies (Wieringa *et al*., 2003). Micronutrient deficiencies often occur together in populations, because the same dietary patterns and socioeconomic factors are associated with deficiency in numerous micronutrients. In many developing countries, diets are mostly cereal-based, low in animal products and high in phytate, which leads to a high risk for micronutrient deficiencies (Dijkhuizen *et al*., 2001).

Interactions between iron, zinc and vitamin A have of great consequences, especially in the context of micronutrient supplementation. Iron supplementation is widely used to combat iron deficiency, but high iron intake is known to impair zinc uptake (Wittaker, 1998). Furthermore vitamin A has a synergistic effect with iron supplementation in reducing anemia (Suharno *et al*., 1993). Severe zinc deficiency can impair vitamin A status (Christian and West, 1998) and zinc supplementation may be able to improve the uptake of β-carotene, its subsequent bioconversion to retinol and the mobilization of vitamin A from body stores in populations with marginal zinc status (Udomkesmalee *et al*., 1992). Previous studies indicated beneficial effect of zinc supplementation on vitamin A metabolism (Morrison *et al*., 1978; Hustead *et al*., 1988; Shingwekare *et al*., 1979). Other studies showed no such effect of zinc on serum indicators of vitamin A metabolism (Udomkesmalee *et al*., 1992; Palin *et al*., 1979).

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In the present study we investigated the effect of supplementation with iron, zinc, or both on iron status, serum zinc and plasma retinol concentrations.

MATERIALS AND METHODS

Subjects: A double-blind clinical trial was conducted in 2 schools in Khorramabad city, capital of Lorestan province, located in western of Iran. The schools ranged size from 415 to 516 persons. Subjects with renal failure, talassemia, tuberculosis, parasitic diseases, infections and those taking supplementary vitamins and minerals were excluded from the study. All children were in fifth grade (11±0.5 year of age) were considered as potential participants. According to a baseline census there were 221 children in this age group in the study area. After informing the parents about the design and potential risks and benefits of the study, the parents of 80 children agreed to their children's participation and signed consent forms. The protocol was approved by the Committee on Ethic, in Human Research of Lorestan University of Medical Sciences.

Zinc and iron supplementation: Children in each of the 3 groups received 1 capsule day^{-1} of a powder containing 20 mg Fe as ferrous sulfate, 20 mg Zn as zinc sulfate, or 20 mg Fe plus 20 mg Zn. Children in each group were visited at school from Saturday through Thursday each week by the field worker who gave the capsule to each child and ensured that it was consumed completely. Only one child dropped out of the study before the end of the 4 months.

Food intake: Food consumption survey was carried out by one 24 h recall and two days record of foodstuffs (2-week day and 1 weekend) for each subject.

Indicators of iron, zinc and vitamin A status: An 8-mL sample of fasting venous blood was collected from each child at baseline and after 4 mo of supplementation. After separating the needle, 2 mL of the blood was poured in an acid-washed tube for serum zinc measurement by flame atomic absorption spectrophotometer. The remaining blood was transferred to a mineral-free evacuated tube and keep in an acid-washed tube containing 5% EDTA as an anticoagulant. Hemoglobin (Hb) was measured within 3 h (Coulter Electronic, Cobas micros). Plasma was separated by centrifugation at 1000×g for 10 min at 20°C. Portions of plasma and serum were frozen immediately and maintained at -70°C until analyzed. Plasma ferritin was measured by radioimmunoassay method. Plasma retinol was measured by HPLC method (Zanuto *et al*., 2003). C-reactive protein (CRP) was measured with the Chem. Enzyme-CRP kit (Tehran, Iran).

Statistical analysis: Biochemical data were analyzed as changes between basal and 4 mo values by using SPSS (version 11.5; SPSS Inc, Chicago). For comparison of differences between the groups one-way ANOVA was used. Means were compared by using Tukey's range test. Basal and final values were compared by paired t-test. For ferritin, since there was no normal distribution, non-parametric tests were used (Wilcoxon for basal-final comparison and Kruskal-Wallis for between groups' comparison). For calculating nutrient intake, Food Processor software was used. Values of $p<0.05$ were considered to be significant.

RESULTS AND DISCUSSION

The characteristics, nutritional status and nutrients intake of the children at the beginning of the study are shown in Table 1.

Hemoglobin, plasma ferritin, serum zinc and plasma retinol concentration are shown in Table 2. As expected supplementation with zinc alone or in combination with iron resulted in significantly higher serum zinc levels than in the Fe group.

In this study, supplementation with zinc, iron, or both was associated with a significant increase in the serum zinc concentrations of the primary school children. This effect was much more evident in children who were initially deficient in zinc (no data show). Compared with iron alone, zinc alone and in combination with iron were associated with significantly higher serum zinc.

Zinc supplementation also produced a significant decrease in the serum ferritin.

Lind *et al.* (2003) showed that supplementation with iron and zinc was less efficacious than were single supplements in improving iron and zinc status. Furthermore, the negative effect of iron supplementation on zinc status was shown. However, double supplementation with iron and zinc raised ferritin levels over placebo.

In this study, iron alone and in combination with zinc had a positive effect on iron status by higher serum ferritin concentration. So, our results suggest that a combination of the 2 was effective in improving iron and zinc status. Dijkhuizen *et al.* (2003) showed that zinc supplement resulted in ferritin decrease. They also showed that, iron supplementation did not negatively affect plasma zinc concentration and zinc supplementation

Table 1: Characteristics of subjects in each group at the beginning of the study[1]

Characteristics	Fe (n = 10 M,17F)	Zn (n = 10 M,18F)	Fe+Zn (n = 10 M,16 F)	All groups (n = 30 M,51 F)
Age (year)	11±0.5[2]	11±0.3	11.1±0.5	11.1±0.5
Anthropometric measurements				
Weight	32±4.8	34.5±7.4	34.2±7.9	33.8±6.8
Height	139.6±7.3	139.8±6.8	139.6±5.8	139.7±6.6
Energy and micronutrients intake [3]				
Energy (kcal day^{-1})	1522±747	1666±465	1442±344	1545± 546
Protein (g day^{-1})	59±49	63±30	51±15	58±34
Vitamin A (μg day^{-1})	1040±235	1090±320	1105±389	1070±352
Vitamin C (mg day^{-1})	67.6±40	83.3±60	61.9±53	71±52
Zinc (mg day^{-1})	4.5±2.8	5.8±3.2	4.3±1.8	4.9±2.7
Iron (mg day^{-1})	15.5±8.3	20.9±15	16.7±4.9	17.7±10.5
Biochemical indicators (% deficient)[4]				
Hemoglobin	0	0	0	0
Plasma ferritin	0	3.6	7.7	3.7
Serum zinc	70.4	70.6	73.1	71.3
Plasma retinol	33.3	42.9	46.2	40.2

There were no significant differences among groups, [2]X±SD, [3]n = 26 in the Fe and Fe+Zn groups 27 in the Zn and 79 in all groups [4]Deficiency defined as <11.7 g L^{-1} for hemoglobin, <12 μg L^{-1} for ferritin,<10.7 μmol L^{-1} (<70 μg dL^{-1}) for zinc and <0.70 μmol L^{-1} (20 μg dL^{-1}) for retinol

Table 2: Biochemical indicators of zinc and iron status in primary school children at baseline and after 4 mo of supplementation with zinc, iron, or both[1]

Supplement	Fe	Zn	Fe+Zn
Serum zinc(μ mol L^{-1})[2]			
Baseline	9.9±1.8	9.8 ±1.6	9.7±1.4
Post treatment	13.5±1.3[c]	14.8±1.6[c]	14.2±2.1[c]
Change	3.6±2.2	5±2.1[a]	4.5±2.7[a]
Plasma ferritin (μg L^{-1})[3]			
Baseline	43.5±33.2	34.5±16.1	41.4±21.2
Post treatment	46.7±21.2	25.1±11.6[c]	41.6±24.2
Change	0.07±0.3	-0.12±0.2[b]	0.01±0.3
Hemoglobin (g L^{-1})[3]			
Baseline	137.1±9.8	137.7±7.7	135.1±8.6
Post treatment	135.8±9.6	135.5±6.7	135.4±8.6
Change	-1.2±4.6	-2.1±5.9	0.04±5.9
Plasma retinol (μ mol L^{-1})			
Baseline	0.81±0.2	0.74± 0.1	0.70±0.2
Post treatment	0.7± 0.1[c]	0.62± 0.1[c]	0.60±0.1[a, c]
Change	-0.1±0.2	-0.1± 0.1	-0.1±0.1

[1]X±SD, [2] n = 25 in the Fe group and 24 in the Zn and Fe+Zn groups, [3]n = 27 in the Fe group, 28 in the Zn group and 26 in the Fe+Zn group, [a]Significantly different from Fe group, $p<0.05$, [b]Significantly different from Fe and Fe+Zn groups, $p<0.05$, [c]Significantly different from the baseline, $p<0.05$

did not increase the prevalence of anemia or iron deficiency anemia. However, iron supplementation combined with zinc was less effective than iron supplementation alone in reducing prevalence of anemia and increasing hemoglobin and plasma ferritin concentrations. Our data did not show any significant effect on hemoglobin.

Previous studies reported that zinc supplementation did not change in hemoglobin (Christian *et al*., 2001; Osendarp *et al*., 2000; Shankar *et al*., 2000). In our study, the initial mean of Hb was high. It is expected that in persons with low Hb, iron is absorbed well and iron indices will improve fast (De-Oliviera and Scheid, 1996). Supplementation with multiple micronutrients would be an appealing strategy for the prevention and treatment of anemia and common morbidities that affect women and young children. However, drawing definitive conclusions regarding the potential benefit or harm of joint supplementation, based on a variety of study designs, target populations and outcome measures has proven challenging (Walker *et al*., 2005). Three trials (Lind *et al*., 2003; Dijkhuizen *et al*., 2003; Schultink *et al*., 1997) found that zinc may reduce the beneficial effect of iron supplementation on iron status, but this negative interaction does not appear to be great enough to discourage joint supplementation. Even in the presence of zinc, the benefit of iron supplementation on iron indicators was significant and important. Iron dose not appear to have a negative effect on serum zinc concentrations and the effect, if any, is small.

In this study plasma retinol levels decreased in all 3 groups. Wieringa *et al*. (2003) showed that iron supplementation in infants with marginal vitamin A status led to markedly lower plasma retinol concentrations and simultaneously to higher liver vitamin A concentrations. Their results confirm our results. Of course in our study liver vitamin A concentration was not measured. This is a shortcoming in our study. They surmised that iron supplementation leads to a redistribution of retinol from the plasma to the liver. Udomkesmalee *et al*. (1992) studied the effect of 6 mo of supplementation with 25 mg Zn/day on the vitamin A status of preschoolers in Thailand. They found no effect of zinc supplementation on plasma retinol or RBP.

Interaction between iron, zinc and vitamin A are of great importance, especially in the context of micronutrient supplementation. Iron supplementation is widely used to combat iron deficiency, but high iron intake is known to impair zinc uptake (Wittaker, 1998). Furthermore, vitamin A supplementation affects iron metabolism, decrease anemia prevalence and has a synergistic effect with iron supplementation in reducing anemia (Suharno *et al*., 1993).

A redistribution of retinol from the plasma to the liver reduces the amount of circulating retinol, which could reduce the availability of retinol to target cells and thus induce a state of functional vitamin A deficiency. No effect of either iron or zinc supplementation on plasma retinol concentrations was found in children in Mexico (Munoz *et al*., 2000).

Observations of the interaction between vitamin A and iron metabolism can be absorbed by the acute phase response, which leads to increased plasma ferritin concentrations and to decreased plasma retinol concentrations. In the present study children with positive CRP were excluded from the study, from the beginning.

An explanation for the lower plasma retinol concentrations after iron supplementation may be that vitamin A requirements are increased as a result of accelerated erythropoiesis (Wieringa *et al*., 2003).

All previous studies have focused on mothers, infants and preschoolers, whereas our study showed that school children may be at high risk of zinc and vitamin A deficiency and that supplementation with iron and zinc in this age group may be different than others.

In general, supplementation programs that combine iron and zinc together are an efficient way to supply both micronutrients, provided the benefits of individual supplementation are not lost. Of course, in view of the possible adverse effect of iron and zinc supplementation on vitamin A status found in this study, supplementation with iron or zinc alone and jointly in school children may compromise serum retinol. Further studies are needed to confirm these results and clarify precise mechanism by which these interactions occur.

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