Original Article

Effects of vitamin A, vitamin A plus zinc, and multiple micronutrients on anemia in preschool children in Chongqing, China

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This study is to clarify the impact of vitamin A or vitamin A combined with other micronutrients supplementation on anemia and growth in preschoolers. In the present study, a total of 290 preschoolers, aged 36-72 months old were randomly assigned to 3 treatment groups: vitamin A (A group), vitamin A plus zinc (AZ group), and vitamin A combined with additional multiple-micronutrient (AMM group). After 6-month supplementation, the height and height-for-age z-score gains of the AZ group were significantly higher than the other groups; the weight gain of the AMM group was greater than the other groups. Compared with baseline values, the concentrations of hemoglobin, and zinc at the end significantly increased in all 3 groups. The incremental concentrations of hemoglobin in the AMM group were significant higher than in the other two groups. Furthermore, the incremental concentrations of serum retinol in the AMM group, and the increase in serum zinc concentrations in the AZ group were significantly higher, respectively, than in the other groups. These 3 kinds of supplements in the present study are effective in enhancing height gains and are effective in reducing the prevalence of anemia. Supplementation of zinc plus vitamin A is a better way for improving children's height and height-for-age z-score. Vitamin A combined with multiple-micronutrient is more effective in improving the hemoglobin concentrations in preschool children.

Key Words: anemia, vitamin A, zinc, multiple-micronutrient, supplementation

INTRODUCTION

Hidden hunger (contain micronutrient malnutrition) are caused by poor dietary intake, and lead to poorer health.[1] Vitamin A deficiency and zinc deficiency are regarded by WHO as global childhood malnutrition risk factors that, among other things, exacerbate a variety of communicable diseases. Vitamin A deficiency and zinc deficiency, also contribute to anemia, ocular disorders and growth retardation.[2-4]

Anemia is a major health concern especially in developing countries. Children and women of reproductive age are especially susceptible. In China, the incidence rate of anemia is between 9.7-16.3% among preschoolers. Iron deficiency disorder is regarded as the major cause of anemia. Nevertheless, it has been recently reported that nutritional anemia can be caused, and exacerbated by deficiencies in other micronutrients except for iron, such as zinc, vitamin A, copper, B-vitamins (such as vitamin B1, vitamin B2, vitamin B6, vitamin B12, folic acid, niacinamide). [5] Intervention trials have confirmed that supplementation with vitamin A can elevate hemoglobin (Hb) concentrations.[6-8] Our investigation showed that the prevalence of anemia was 26.6% in 2002, and 23.5% in 2005 among preschoolers in Chongqing, China. Our studies in 2000 showed that 37.9% of preschoolers in Chongqing, China suffered from vitamin A deficiency disorder (VADD), and this reduced to 6.3% in 2005. Our previous studies demonstrated that supplementation with vitamin A could elevate Hb concentrations in preschool children in the area.[9] In addition, it was reported that the incidence rate of zinc deficiency disorder (ZDD) was about 39% among 0 to 6 years old children in China in 2005. Our previous studies also showed that supplementation with zinc could also reduce the prevalence of anemia in preschool children; and that the impact of zinc supplement on preschool children's hemoglobin was similar to supplementation with vitamin A.

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A few experimental trials showed that zinc supplementation alone or in combination with vitamin A successfully improved the levels of Hb and serum retinol.[6,10] Furthermore, in the treatment of anemia, it was suggested that the combination of vitamin A and zinc in addition to iron could increase hemoglobin levels more than supplementing iron alone.[11] It was hypothesized that vitamin A and zinc should play important roles in increasing hemoglobin levels and reducing the prevalence of anemia.

Zinc and vitamin A are both antioxidants. There is a conspicuous relationship between vitamin A and hematopoiesis, and increasing serum zinc can induce the release of vitamin A from the liver to serum. It was reported that some other micronutrients such as zinc, and B-vitamins could enhance the absorption of vitamin A. Zinc is important for both intra- and intercellular transport of vitamin A. It affects synthesis of the transport protein, which transports retinol from the liver to the blood and other target tissues, and also participates in the synthesis of cellular retinol binding protein (cRBP). Researchers thought that there was synergy between zinc and vitamin A, which caused the improved circulating retinol concentrations.

In this study, we conducted a randomized and controlled trial with 3 intervention groups: a vitamin A group, a vitamin A plus zinc group and a group given vitamin A combined with other multiple micronutrients (vitamin A plus vitamins B-1, B-2, B-6, B-12, vitamin C, vitamin D, folate, niacinamide, and calcium). One of the aims of this study is to confirm whether vitamin A plus zinc supplementation is effective in preventing and treating anemia. At the same time, this study is to compare the effectiveness between vitamin A alone, vitamin A plus zinc and multiple micronutrients fortified food in increasing erythropoietin (EPO), hemoglobin concentrations, and retinol status. At the same time, we observed the change of subjects' health. The effect of supplementation on growth was also evaluated.

MATERIALS AND METHODS

Study design, participants and ethical approval

The study was designed as a randomized trial with 3 intervention groups. It was carried out from November 2008 to June 2009 in Banan District, a suburb of Chongqing. This is an area of median socioeconomic status in southwest China

Three kindergartens were randomly selected out of 7 in this region; and 3 classes were chosen from each of them. More than 300 children from 9 classes, aged between 36-72 months old, were enrolled from the selected kindergartens. The eligibility criteria for participation were as follows: 1) not having any chronic infectious diseases; 2) hemoglobin concentration ≥60 g/L; 3) C-reaction protein (CRP) <5 mg/L; 4) parental/ guardian agreement to avoid additional supplementing vitamin and mineral during the investigation. Children with evidence of recent acute or chronic illnesses and/ or hemoglobin concentration <60 g/L were excluded and referred to a local medical center for treatment. Informed consent was obtained from each child's parent or guardian with the aid of an assistant after a detailed explanation of the purpose and procedures of the study. The study was approved by the Ethical Review Committee of the Children's Hospital of Chongqing Medical University in Chongqing, China.

Intervention

The supplementation phase of the study began within one month after the screening. A total of 361 children from the selected classes who met the inclusion criteria and with parental consent were enrolled in the trial. The selected classes in each kindergarten were randomly assigned to receive vitamin A (A group), vitamin A plus zinc (AZ group), or vitamin A combined with multiple micronutrients (contain vitamins B-1, B-2, B-6, B-12, C, D, folate, niacinamide, and calcium) (AMM group) (Table 1). The content of the micronutrient supplements provided 100% the Chinese Dietary Reference Intakes (DRIs, Chinese Nutrition Society, 2000, China Light Industry Press). For the mean daily intake of each supplementation agent in this study (Figure 1), vitamin A was supplemented once per 14 days, on the first day (Xiamen, China, Code: H34020246; vitamin A 2,5000IU per 14 days); zinc in the form of zinc gluconate tablets was given 5 days a week, through Monday to Friday (Hainan, China, Code: H46020030; zinc 10mg per day, and for 5 days per week); while vitamin A plus multiple micronutrients in the form of chewable tablets were given 5 times per 14 days, and the mixed micronutrients were provided by Wyeth Company (China,

Table 1. Micronutrients list in the three groups

Micronutrient	A group	AZ group	AMM group
Vitamin A (IU)	25000	25000	5000
Zinc (mg)	0	10	0
Vitamin B-1 (mg)	0	0	1.5
Vitamin B-2 (mg)	0	0	1.7
Vitamin B-6 (mg)	0	0	2
Vitamin B-12 (μg)	0	0	4
Vitamin C (mg)	0	0	50
Vitamin D (IU)	0	0	400
Folic acid (μg)	0	0	100
Niacinamide (mg)	0	0	20
Calcium (mg)	0	0	162

The vitamin A soft capsule was in the form of retinyl acetate, provided once per 14 days, on the first day; zinc was given as zinc gluconate tablets, 5 times per week, through Monday to Friday; while vitamin A combined with multiple micronutrients were given as chewable tablet, provided 5 times per 14 days.

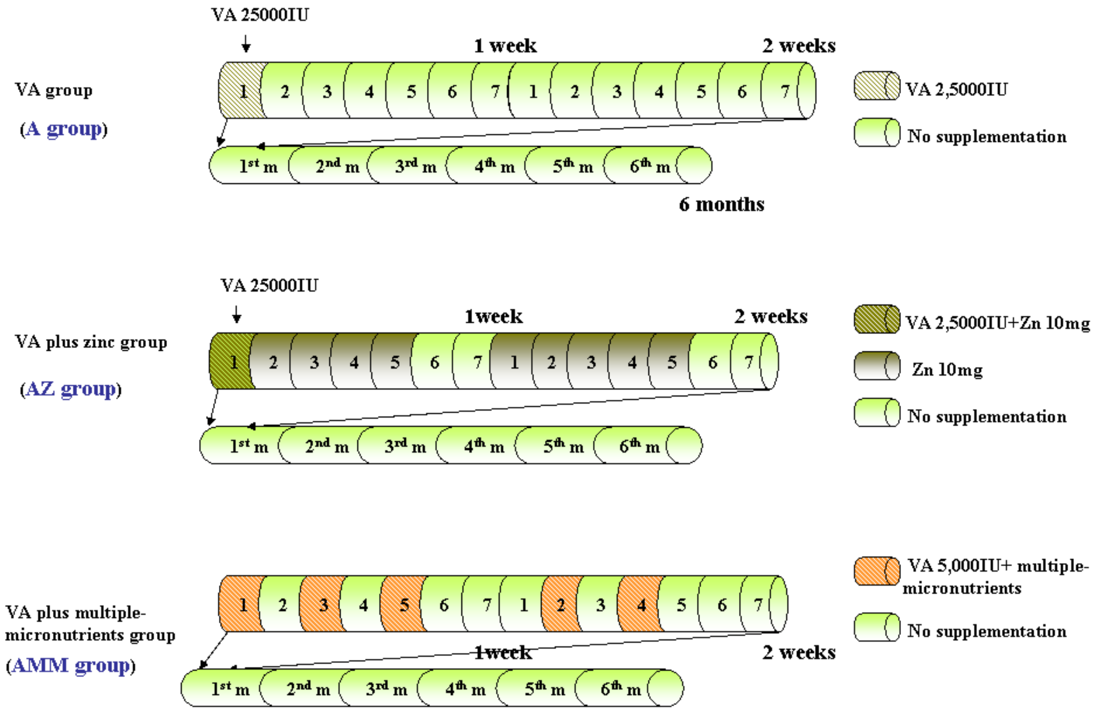

Figure 1. Methods of supplementations. The vitamin A was supplemented twice a month; zinc in the form of zinc gluconate tablets was given 5 days a week, through Monday to Friday; while multiple micronutrients in the form of chewable tablets were given every three days

Code: H10950027; subjects gained vitamin A 2,5000 IU per 14days; and gained 100% DRIs others multiple micronutrients per day, by supplementing chewable tablets 5 times per 14 days). The supplementation lasted 6 months.

Measures

Questionnaire

There were two parts in the questionnaire. First, there was a brief socioeconomic survey to be completed at the beginning of the treatment. This part was performed only once at baseline. Second, there was a 24-h dietary recall questionnaire for all the participants to be conducted except on Saturday, Sunday or any festival days. The questionnaires were performed for a total of seven times (at the beginning, and end of each month). A Wednesday was chosen for interviews for each time. All the children stayed in the kindergartens from 08:00-17:00, and they had their breakfast and lunch there. Therefore, we were able to collect information on the food and drink given to them there. On that Wednesday, the quantities of food eaten by the children measured with utensils currently used in the kindergartens (bowl, spoons, cup etc.) were recorded in detail. The workers would call the mothers the next day for information about the food, especially snacks, eaten by the children in the previous night as measured with utensils used in their household (bowl, spoons, cup etc.). If any child was sick, the 24-hour dietary recall would be delayed until the next Wednesday. Intakes were then calculated using energy, protein and micronutrient contents of ingredients given in food composition tables (set by China Food Composition 2004).

Anthropometric measurements

Anthropometric measurements of the children were done at the start and the end of the supplementation period. To rule out individual variations in the measuring process, all anthropometric measurements were taken by the same researchers who were from the Children's Hospital. All measurements were performed in duplicate. Weight was recorded to the nearest 0.1 Kg with the children minimally clothed with bare feet and the same weighing scale (100 Med, China) was used for all children. Height was marked to the nearest 0.01 cm and with the same standing scale (100 Med, China) for all children. Anthropometric data were assessed as z-scores for HAZ, weight-for-age (WAZ) and weight-for-height (WHZ) using the Epi Info program (version 2002, CDC) and the U.S. National Center for Health Statistics data 2000. A cut-off point of < -2 SD was used to define low WAZ (underweight), low HAZ (stunting) and low WHZ (wasting).

Sample collection

At both the start and the end (1 week after supplementation) of the study, about 4 mL of blood was collected by venepuncture using aseptic technique, and another aliquot 200 μL was put in tubes with EDTA for the estimation of hemoglobin. Then serum was obtained by centrifuging the blood at 3000×g for 6 min at room temperature. It was then stored at -80℃ in the Nutrition Research Center until analysis.

Laboratory analysis

Hb levels were determined by the cyanmethemoglobin determination method. Serum vitamin A concentration was determined by using high-performance liquid chromatography (HPLC) in a dark room according to the method of Miller and Yang while with some slight modifications.[4,9] Serum zinc concentrations were measured by flame atomic absorption spectrophotometry. CRP was measured by particle-enhanced immunoturbidimetry (Upper, China, Code No: 2400335).[12]

Definitions

Anemia was defined as Hb concentration <110 g/L for children aged 6-59 months, or Hb concentration <115 g/L for children aged 5-11 years.[13] Serum vitamin A concentration of <0.7 μmol/L was defined as vitamin A deficiency (VAD), and values between 0.7-1.05 μmol/L were defined as marginal vitamin A deficiency (MVAD).[14] Serum zinc concentration of <10.7 μmol/L was defined as zinc deficiency. Moreover, CRP levels of >5 mg/L indicated infection or inflammation as suggested by the manufacturer (DIGFA Diagnostics).

Statistical analysis

Data were analyzed by SAS software (version 8.1). The significance level was set at 5%. The Kolmogorov-Smirnov test was used to investigate whether the concentrations of hemoglobin, serum retinol and zinc, as well as the anthropometric indicators were normally distributed prior to analysis. Then data were presented as mean, standard deviation (SD) and median, as well as 95% confidence interval (CI). Analysis of pre-treatment data indicated that the children who received vitamin A supplementation alone were younger than those in the other groups. All statistical analysis on the anthropometrical and biochemical data were done after adjusting age, gender and nutritional status at baseline. The paired t-test was used to compare differences between the initial and final paired data with normal distribution while the paired Wilcoxon signed-rank test was used for non-normally distributed data. Performing multiple comparisons of the change of parameters over the 6 months of intervention, the Tukey-Kramer test was used to compare the different effects among the 3 intervention groups. Differences in prevalence were tested with a chi-square test.

RESULTS

Subjects

Among the 385 preschool children, 24 of them did not meet the eligibility criteria and thus excluded from the study. Of these, 3 children were excluded from the study because of having received additional vitamin A supplementation, 19 of these children had an initial CRP>5 mg/L, and 2 had initial Hb<60 g/L. They were referred to a health center for diagnosis and treatment for infection and serious anemia. The final sample consisted of 361 children, with 119 in the vitamin A group, 122 in the vitamin A plus zinc group, and 120 in the vitamin A combined multiple micronutrient group. After 6 months of supplementation, the number of children still in the 3 groups were 88, 93 and 109, respectively (total n=290).A total of 71 children dropped out (56 moved out of the study area during the trial, 10 withdrew from the study, and 5 with failure to draw blood). The baseline characteristics of these 71 children did not differ significantly from those of the 290 children who completed the trail (data not shown). As Table 2 showed, age of subjects was significantly different between the A group and AZ group, A group and AMM group, AZ group and AMM group (p<0.05). However, the baseline characteristics of the children such as gender and education status of the mother among the 3 groups were not significantly different. Furthermore, the percentages of anemia, MVAD and zinc deficiency at baseline also showed no significant differences among the three groups (Table 2, control for age, gender, and education status of the mother), that suggested that there were no significant differences in the nutrition status of vitamin A and zinc among the three treatment groups.

Diet

As shown in Table 3, the A group consumed significantly more energy and protein than the AMM group at baseline, while there were no significant differences between the AZ group and the AMM group, and consumption of diet in each group approximately met the requirement suggested by the Chinese DRIs. But the dietary intake of zinc and vitamin A was much lower in all three groups, being only about 60%-70% and 30-60% of the Chinese RDA, respectively. The intake of vitamin A, iron, calcium, vitamin C, vitamin B1, and vitamin B2 was not significantly different among the three groups (p >0.05), whereas the intake of zinc was significantly higher in the A group than in the AZ group and the AMM group (p <0.05). Among data collected from the seven questionnaires in this study, there were no significant differences for intake of energy, protein, vitamin A, iron, and zinc etc. in each group (control for age, gender, and education status of the mother).

Anthropometric Indices

The overall prevalence of underweight (WAZ<–2 SD),

Table 2. Baseline characteristics of the studied children

Characteristic	A group (n=88)	AZ group (n=93)	AMM group (n=109)
Age, mo	48±9†	55±10‡	51±8*
Boys, %	61.4%†	50%†	48.6%†
Mother's education, y	12 (9, 12)†	12 (9, 12)†	12 (9, 15)†
Anemia, %	24.4%†	25.0%†	24.3%†
Serum retinol <1.05 μmol/L, %	21.6%†	23.6%†	22.1%†
Serum zinc <10.7 μmol/L, %	25.6%†	28.3%†	23.4%†

Values are means±SD or median (range in 25th and 75th parentheses). $^{\dagger, \ddagger, *}$values within a row with unlike superscript letters are significantly different among the corresponding treatment groups, with p<0.05 (Chi-square test or Kruskal-Wallis test).

Table 3. Daily consumption of major nutrients in the three groups

Characteristic	A group (n=88)	AZ group (n=93)	AMM group (n=109)
Energy (Kcal)	1525 (1466-1585)†	1466 (1420-1512) †,‡	1428 (1395-1462) ‡
Energy (% Chinese RNI)	86%-122%	84%-116%	82%-112%
Protein (g)	48 (46-50) †	45 (43-47) ‡	45 (44-47) ‡
Protein (%Chinese RNI)	84%-111%	78%-104%	80%-104%
Vitamin A (µg RE)	284 (220-346) †	260 (195-326) †	284 (200-368) †
Vitamin A (%Chinese RNI)	37%-69%	33%-65%	33%-74%
Zinc (mg)	8 (7.6-8.3) †	7 (7.1-7.7) ‡	7 (7.4-7.9) ‡
Zinc (% Chinese AI)	63%-92%	59%-86%	62%-88%
Iron (mg)	15.0 (12.0-17.0)	14.0 (12.0-15.0)	14.0 (12.0-15.0)
Iron (%Chinese AI)	100%-142%	100%-125%	100%-125%
Calcium (mg)	455 (243-597)	439 (283-554)	475 (296-579)
Calcium (%Chinese AI)	30%-75%	35%-69%	37%-72%
Vitamin C (mg)	33.0 (30-37.0)	34.0 (28.0-40.0)	35.0 (30-38)
Vitamin C (%Chinese RNI)	43%-53%	40%-57%	43%-54%
Vitamin B-1 (mg)	0.55 (0.47-0.63)	0.54 (0.46-0.58)	0.61 (0.54-0.59)
Vitamin B-1 (%Chinese RNI)	67%-90%	66%-83%	0.77%-84%
Vitamin B-2 (mg)	0.74 (0.58-1.06)	0.69 (0.48-0.92)	0.7 (0.48-0.94)
Vitamin B-2 (%Chinese RNI)	83%-151%	69%-131%	69%-134%

RNI, Recommended Nutrient Intake.
AI, Adequate Intakes.
Values are median ((range in 25th and 75th-parentheses), †,‡ median values within a row with unlike superscript symbols are significantly different among the corresponding treatment groups, with $p<0.05$ (Chi-square test or Kruskal-Wallis test).

Table 4. Anthropometric indices in the three groups

Characteristic	A group (n=88)	AZ group (n=93)	AMM group (n=109)
Weight (Kg)			
Baseline	15.5±2.23	16.7±3.24	16.4±3.08
Final	17.1±2.65	18.3±3.47	18.5±3.01
gain	1.62±1.29 ‡,*	1.59±0.77 ‡,*	2.05±1.10 †,*
Height (cm)			
Baseline	99.9±5.17	104±6.72	104±5.84
Final	105±5.83	110±6.45	108±5.71
gain	5.19±1.29 ‡,**	5.51±1.11 †,**	5.00±1.25 ‡,**
WAZ score			
Baseline	-0.35±1.07	-0.44±1.11	-0.01±1.07
Final	-0.21±1.04	-0.35±1.02	0.10±0.98
change	0.14 (-0.05, 0.36) †,*	0.06 (-0.12, 0.27) †,*	0.05 (-0.14, 0.31) †,*
HAZ score			
Baseline	-0.28±0.90	-0.25±1.00	0.11±0.95
Final	-0.15±0.90	-0.01±0.93	0.26±0.95
change	0.17 (-0.01, 0.35) ‡,*	0.20 (0.06, 0.43) †,*	0.16 (-0.07, 0.33) ‡,*
WHZ score			
Baseline	-0.20±1.08	-0.43±1.09	-0.08±1.09
Final	-0.15±1.08	-0.56±0.98	-0.02±0.96
change	0.07 (-0.25, 0.38) †	-0.08 (-0.45, 0.26) †	0.03 (-0.36, 0.34) †

WAZ, Weight-for-Age z-score; HAZ, Height-for-Age z-score; WHZ, Weight-for-Height z-score.
Values are means ±SD or median (range in 25th and 75th-parentheses), †,‡ mean or median values within a row with unlike superscript letters are significantly different among the corresponding treatment groups w, with * $p<0.0001$, ** $p<0.05$ (Chi-square or Kruskal-Wallis test).

stunting (HAZ<–2 SD) and wasting (WHZ<–2 SD) at baseline was 5.17%, 2.41% and 5.52%, respectively. After 6 months of supplementation, the prevalence percentage decreased to 3.79%, 1.72% and 5.17%. The baseline and final anthropometric indices, as well as the z-scores are shown in Table 4. WAZ and HAZ increased significantly in all groups during the intervention period ($p<0.05$, $p<0.0001$), and no significant effect was observed in WHZ after the 6 months of supplementation in all groups ($p>0.05$). The height increment and the HAZ increase in the AZ group was significantly greater than in the other two groups ($p<0.05$). The weight increment was significantly greater in the AMM group than in the other two groups ($p<0.05$), whereas the children in the AMM group tended to have a smaller increase in WAZ than those in the other two groups, but this difference was not significant.

Biochemical indicators

In our study, only 5-6% of the participants had an elevated CRP concentration, and no change in CRP concentrations was observed during the course of the study. The baseline and post-supplementation serum retinol, serum zinc and hemoglobin concentrations are presented in Table 5. The mean serum retinol concentration increase in the AMM group was significantly higher than that in the other groups. While the post-supplementation serum retinol concentration tended to have a higher numerical value in the AZ group than in the A group. But such differences were not statistically significant. The mean concentration of hemoglobin increased significantly within each group over the 6 months ($p<0.0001$). The concentrations incre-

Table 5. Changes on Hb, serum vitamin A, and zinc concentrations in all groups

Characteristic		A group (n=88)	AZ group (n=93)	AMM group (n=109)
Hb (g/L)	Pre-	115±8	117±9	118±9
	Post-	120±7	122±8	124±10
	Change	5.07 ‡	5.31 †, ‡	6.35 †
	95% CI	3.62-6.53	3.91-6.84	5.03-7.74
	p-value	<0.0001	<0.0001	<0.0001
Serum retinol (µmol/L)	Pre-	1.25±0.26	1.20±0.23	1.27±0.24
	Post-	1.28±0.22	1.25±0.20	1.35±0.27
	Change	0.03 ‡	0.05 ‡	0.09 †
	95% CI	-0.03-0.08	0.01-0.11	0.03-0.15
	p-value	0.0693	0.0313	0.0018
Serum zinc (µmol/L)	Pre-	12.0±2.32	12.6±2.60	12.1±2.06
	Post-	15.0±3.14	16.3±2.80	13.8±2.08
	Change	2.52 ‡	3.67 †	1.63 ‡
	95% CI	1.91-3.14	3.04-4.29	1.13-2.12
	p-value	<0.0001	<0.0001	<0.0001

Hb, Hemoglobin.
Values are means ±SD or median (range in 25th and 75th-parentheses), †, ‡ values with different superscript character are significantly different with another groups, with $p<0.05$ (Chi-square or Kruskal-Wallis test).

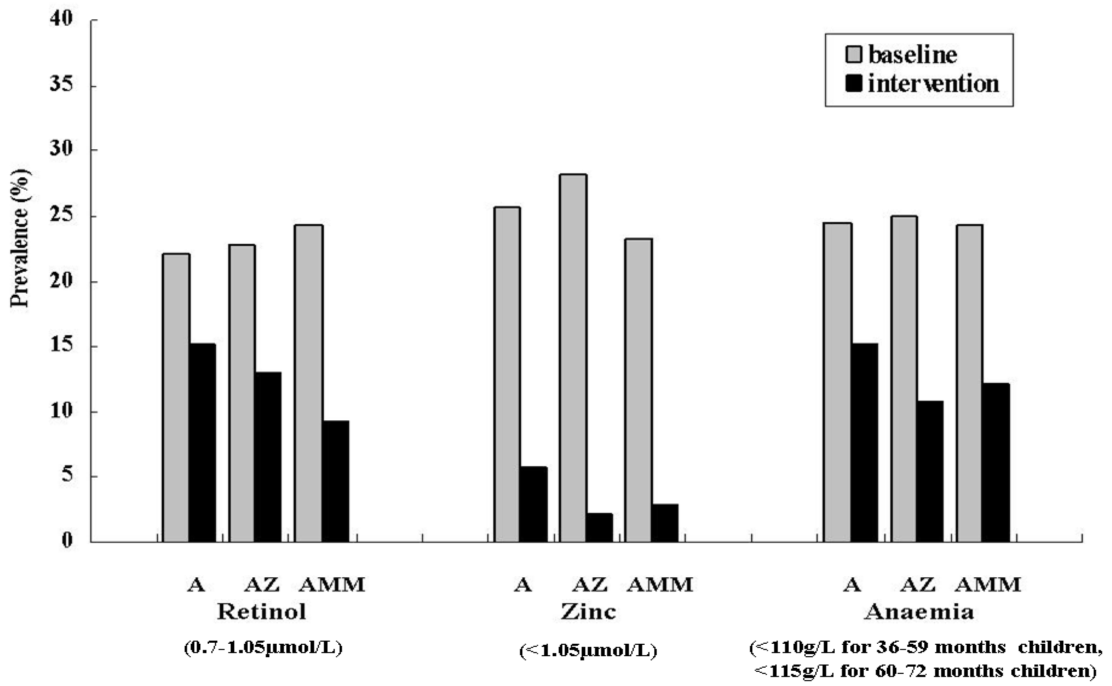

Figure 2. The prevalence of anemia, suspicious sub-clinical vitamin A deficiency and zinc deficiency for each group at baseline and at 6 months. After 6 months of supplementation, the prevalence of anemia, suspicious sub-clinical vitamin A deficiency and zinc deficiency decreased significantly in all three groups (*$p<0.05$).

ment of hemoglobin was in a decreasing order of AMM group, AZ group, and A group. And hemoglobin concentrations gain in the AMM group was significant higher than in the A group. Nevertheless, there were no significant differences in hemoglobin concentrations gain between A group and AZ group.

Serum zinc concentrations increased significantly during the 6 months in all groups ($p<0.0001$). The increase in serum zinc concentrations was significantly different between the AZ group and the other two groups ($p<0.0001$). However, there were no significant differences in the increment between the A group and the AMM group.

After 6 months of supplementation, the prevalence of anemia, MVAD and zinc deficiency decreased significantly in all three groups ($p<0.05$) (Figure 2**)**. The prevalence of children with anemia and zinc deficiency in the AZ group was significantly lower than those in the A group ($p<0.05$), indicating an improvement in hemoglobin and zinc status after taking the combined vitamin A and zinc supplementation. The children in the AMM group had significantly lower serum vitamin A concentration than those in the AZ group ($p<0.05$).

DISCUSSION

Effect of intervention on Anthropometric Indices

It is well known that zinc deficiency disorder (ZDD) can lead to bone growth retardation. Most zinc supplementation studies documented that zinc supplementation positively affects growth.[15-18] Moreover, micronutrients such as vitamin D and calcium are very important for bone growth. It is reported that there is no single lack of nutrient which limits a child's growth potential, but rather a combination of multiple, simultaneous micronutrient deficiencies are what limit the growth of a child.[19] A recent meta-analysis reports that multiple micronutrient interventions improve linear growth.[20] Our previous studies showed that zinc supplementation alone, or multiple micronutrient supplementation, which included zinc, could improve height gain in preschool children, and the different effects on height gain between the two kinds of supplementations were not significant. Our present results showed that HAZ increases in the AZ group was significantly greater than that of the other two groups. And there was a correlation between the changes in serum zinc concentration and height gains ($r=0.14$, $p=0.01$). We think that the results are correlated with the positive interaction of vitamin A on absorption and function of zinc, and with the inhibition of some other micronutrients (such as calcium) on zinc absorption.[21,22]

Effect of intervention on prevalence of anemia

It was reported that retinoids may stimulate erythropoiesis through their direct effect on the later stages of red cell development.[23] In our previous and present trials in preschool children, supplementation of vitamin A alone significantly enhanced the concentrations of hemoglobin and reduced anemia.[9] Several mechanisms about the effect of vitamin A status on anemia have been proposed: 1) increasing resistance to infection could decrease the prevalence of anemia due to infection; 2) directing modulation of erythropoiesis, such as retinoids regulating programmed cell death in erythroid progenitor cells.[24] A change in prevalence of anemia of infection as a result of vitamin A repletion is unlikely to explain our findings.

Some studies reported that zinc promote the absorption, mobilization, and transport of vitamin A.[21] Another studies reported that zinc supplementation could enhance hemoglobin concentrations.[6,10] Furthermore, some researchers showed that interaction of vitamin A and zinc tended to affect hemoglobin and anemia morbidity.[6,11] In the present study, at the endline, the prevalence of anemia in the AZ group was significantly lower than that in the A group.

The children who received vitamin A plus multiple micronutrients compared with those who received vitamin A plus zinc and who received vitamin A alone showed the highest numerical hemoglobin concentrations gain, which could be the result of two factors: firstly, riboflavin, vitamin B-12, and folic acid, which had been shown to have an enhancing effect on the metabolism of iron or synthesis of hemoglobin; secondly, vitamin A direct modulation for hematopoiesis, and the mean concentrations of vitamin A in the AMM group was significantly higher than in the other groups.[25,26]

It was suggested that supplementing with multiple micronutrients was more effective in improving the level of serum retinol in preschool children.

Effect of intervention on serum vitamin A and zinc concentration

Some other studies reports that supplementation of zinc alone or zinc combined with vitamin A led to a greater increase in serum zinc concentrations.[6] In our present study, serum zinc concentrations increased significantly in all the groups during the 6 months of intervention, with the greatest increase in the AZ group, and the smallest in the AMM group. There were some reasons: 1) it was reported that the concentrations of zinc were correlated with the intake of energy. In the present study, the children in the A group absorbed significantly more energy than the children in the AMM group.[27] 2) In the AMM group, lower serum zinc concentrations gain could be caused by minerals competing for the same receptors in intestinal tract; the calcium supplementation in the AMM group might interfere with the dietary zinc absorption.[22]

Some researchers concluded that that retinol status significantly improved in preschool children and school children who received supplementation of vitamin A only,[28-30] and it was reported that large supplements cause big spike in retinol levels, which then decline to baseline after 2 months. However, serum vitamin A concentrations in the present study, as in our previous trial, showed no significant improvements in the vitamin A alone group at endline, 4 weeks after the last vitamin A supplementation.[9] The research in Dhaka City reported that among the studied children who were initially deficient in vitamin A, 37.5% remained deficient after vitamin A supplementation.[31] And another study reported that 61% of the studied infants remained deficient in vitamin A despite having received 3 doses of 50000 IU vitamin A at a monthly interval for 3 months.[32]

Conclusion

This study demonstrates that supplementation of micronutrients, such as supplementation of vitamin A alone, vitamin A combined with zinc, and vitamin A plus multiple micronutrients, are effective in enhancing height gains, and effective in reducing the prevalence of anemia. Supplementation of zinc combined with vitamin A is a better way to improve children's height and HAZ, while vitamin A combined with multiple micronutrients is more effective to improve the levels of hemoglobin as well as weight in preschool children.

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AUTHOR DESCLUOSURES
All authors declared there is no conflict of interest.

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Original Article

Effects of vitamin A, vitamin A plus zinc, and multiple micronutrients on anemia in preschool children in Chongqing, China

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維生素 A、維生素 A 加鋅及微量营养素复合补充对中國重慶的学龄前儿童贫血及体格生长的影响

为进一步明确维生素 A（VA）、VA 与其他微量营养素复合补充对学龄前儿童贫血及体格生长的影响，本研究共纳入 290 名 36-72 个月的学龄前儿童，并随机分为 3 个强化补充组：VA 组（A 组）、VA 加锌组（AZ 组）以及 VA 与复合微量营养素组（AMM 组）。强化补充 6 个月后，AZ 组受试儿童的身高及身高 Z 评分（HAZ）增长显著高于其他两组；AMM 组体重增长优于其余各组；各组受试儿童血红蛋白（Hb）及血清锌浓度均较补充前明显增加，且 AMM 组受试儿童 Hb 浓度增长显著高于其余两组。此外，AMM 组血清 VA、AZ 组血清锌浓度增长均显著高于其他各组。3 种不同强化补充方式均能促进受试儿童身高增长并降低贫血患病率。补充 VA 加锌对受试儿童身高及 HAZ 的促进作用最优；而补充 VA 与复合微量营养素对改善学龄前儿童 Hb 浓度更加有效。

關鍵字：贫血、維生素 A、鋅、复合微量营养素、補充