Co-assessment of iron, vitamin A and growth status to investigate anemia in preschool children in suburb Chongqing, China

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Background: **Anemia is a widespread public health problem, which is due to many factors, nutritional or non-nutritional. Iron, vitamin A and growth status were assessed to investigate anemia of preschool children in suburb Chongqing, China.**

Methods: **A descriptive, cross-sectional survey was performed on 459 preschool children aged 2 to 7 years randomly chosen from the kindergartens in 6 suburban districts of Chongqing. Weight and height levels, hemoglobin, erythrocyte protoporphyrin, serum retinol, and ferritin concentrations were measured to evaluate the anthropometric and nutritional status.**

Results: **The rates of stunt, underweight, overweight, wasting, obesity, anemia, iron deficiency, vitamin A deficiency (VAD), and marginal VAD were 6.3%, 3.9%, 3.7%, 1.5%, 3.1%, 23.5%, 15.0%, 6.3% and 25.9%, respectively. Serum retinol concentration was significantly lower in children with anemia than in those without anemia (*P*=0.003), and the retinol concentration was associated with hemoglobin (Pearson's correlation coefficient, *r*=0.22, *P*<0.01). Children with VAD had a significantly increased risk for anemia (odds ratio, 2.56; 95% confident interval, 1.15-5.70). In all 108 children with anemia, only 42 were related to VAD and 12 related to iron deficiency, suggesting that almost half of the anemia children cannot be explained solely by iron deficiency or VAD.**

Conclusions: **Vitamin A and iron deficiency are still public health problems in some localities of China. Public health interventions in anemia control should be used to eliminate deficiencies of vitamin A, iron, and other micronutrients by deliberate supplementation. Attention must be paid to such deficiencies in high-risk groups, especially in preschool children.**

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Key words: **anemia; iron; micronutrient deficiency; preschool children; vitamin A**

Original article

Introduction

Anemia is associated with multiple causes, nutritional (vitamin and mineral deficiency) or non-nutritional (infection). In developing countries, the causes of anemia in preschool children have been inadequately studied, and the children are at a high risk for vitamin A or iron deficiency. Vitamin A deficiency (VAD) is related to high risks of xerophthalmia and blindness, growth failure, anemia, depressed immunity, and increased morbidity and mortality of some infectious diseases.[1,2] Iron deficiency is assumed as one of the most common contributing factors of anemia. The consequence of iron deficiency is related to some metabolic processive alterations such as neurotransmitter synthesis and degradation, which affect brain function, physical activity, and motor and mental development.[3-5] Nutritional surveys have shown a close relationship between vitamin A and iron metabolic indicators, and vitamin A is considered to influence anemia by modulating erythropoiesis and iron metabolism and enhancing immunity to infectious diseases.[2,6-9]

A national nutrition and health survey[10] in 2002 revealed marginal VAD (45.1%) and mild VAD[11] (9.3%) in Chinese children aged 3-12 years. The total prevalence of anemia was found to be 17% in children of less than 6 years old.[10] The co-existing status of VAD and iron deficiency has rarely been investigated in preschool children in suburb Chongqing. The residents

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in this locality are experiencing a living environmental and economic transition, as well as changes of their dietary habit, lifestyle and nutritional concept. In 2002 we found that the micronutrient status of preschool children in the Banan District of Chongqing was not optimal,[12] and later, we conducted a series of educational programs about nutritional intervention and dietary instruction among inhabitants in the locality.

Nutritional surveys in several countries have shown an inadequate intake of some micronutrients. A possibility to challenge this situation is the fortification of selected foods with micronutrients.[13-15] As we aimed to provide a safe and effective fortified food for preventing iron deficiency and VAD in local preschool children, it is also necessary to investigate their basic levels of the developmental and nutritional status.

We conducted a community-based cross-sectional study to assess the nutritional status of iron and vitamin A and additionally, to evaluate the anemia status by co-assessment of iron deficiency and VAD among preschool children in suburban Chongqing.

Methods

Subjects and ethical approval

Six pre-school institutions were randomly chosen from kindergartens in each of 6 suburban districts of Chongqing, respectively in 2005. All children in one institution were recruited into the study. Children with infection such as pneumonia, diarrhea, febrile illness and parasite infection were excluded from the study and subjected to treatment at a local health care center. Finally, 459 preschool children aged 2-7 years (229 boys and 230 girls) from 36 pre-school institutions were included randomly in a community-based cross-sectional study. Demographic and socioeconomic information including children's sex and age, passive smoking exposure, family monthly income, guardians' educational level, self-reported anemia of parents were also collected. Based on 95% confidence interval and at 0.05 level of 0.5 g/L desired accuracy with an assumed 5 g/L standard deviation of hemoglobin, the sample size was estimated.[10]

Anthropometric measurements

Anthropometric examinations were conducted by a trained anthropometrist from Chongqing Children's Hospital using standard techniques. Duplicate measurement was used for all participants and the inter-examiner coefficient of variation of weight and height for each examiner was <5%. Weight was recorded using a weighing scale (Wuxi Weigher Factory Co., Ltd., China) to the nearest 50 g with subjects in minimum clothing and bare feet. Height (more than 3 years old)/length (less than 3 years) was measured in a standard position by the same standing scale (Wuxi Weigher Factory Co., Ltd., China) or in supine position by the supine scale (Haode, China) to the nearest 0.1 cm. Reference data from the WHO (2005) was used to calculate Z-scores for height-for-age, weight-for-height, and weight-for-age. A cut-off of less than minus two standard deviations (-2SD) was used to define stunt (height-for-age Z-score), wasting (weight-for-height Z-score), and underweight (weight-for-age Z-score), while a cut-off of more than +2SD was used to define obesity (weight-for-height) and overweight (weight-for-age).[16] All indexes were computed using the ANTHRO software (Anthro 2005 PC) recommended by the WHO (http://www.who.int/childgrowth/software/en/).

Blood sampling and biochemical assessment

A fasting blood sample (about 3 ml) was collected by venepuncture of an antecubital vein before breakfast. One ml of blood was drawn into a container with heparin to measure hemoglobin by hemiglobincyanide (Maker, Chengdu, China) and erythrocyte protoporphyrin by hematofluorometer immediately (Maker, Chengdu China).[17,18] The results of inter-assay for hemoglobin and erythrocyte protoporphyrin were ≤10% and those of the intra-assay were ≤5%. The remaining blood was centrifuged at 3000 g for 5 minutes at room temperature. The serum and blood specimens were immediately frozen at -20ºC until analysis. The concentrations of serum ferritin were measured in duplicate by the method of enzyme-linked immunosorbent assay (ELISA) (Sunbiote, Shanghai, China).[19] The accuracy of the ELISA method for serum ferritin was monitored in each batch by including control samples of low and high concentrations. The inter-batch precision with use of these control samples was <8%.

Serum retinol concentration was determined by high-performance liquid chromatography (HPLC) according to the method of Miller and Yang with slight modification.[20] Briefly, retinol was extracted with hexane after deproteinization with ethanol containing retinyl acetate as the external standard, and evaporated to dryness with nitrogen gas. The residue was dissolved in 0.1 ml methanol. 20 μl of the sample was injected into the column (Symmetry Shield RP_{18} 3.9×150 mm) installed with the HPLC apparatus (Waters 1525 Binary HPLC Pump, Waters Breeze, USA). All procedures were performed in dark room to protect the serum from light. The mobile phase was a methanol-DH_2O mixture (95:5). The concentration of retinol was determined by spectrophotometry (Waters 2487 Dual λ Absorbance Dector, USA) at 315 nm. Duplicate analyses were made on one tenth of the samples and the estimated variability was 0.02 μmol/L. Three control samples with low (0.70 μmol/L), medium (1.40 μmol/L) and high (2.79 μmol/L) concentrations of retinol were provided by retinol standard solution (Sigma, USA) with pooled serum. The between-day coefficient of variation for low, medium and high concentration was 5.7%, 3.2% and 1.9%, respectively. All biochemical indices were measured by expert examiners in the Pediatric Laboratory of Chongqing Medical University, China.

Definition of outcome

The prevalence of anemia was determined according to the WHO criteria, i.e., hemoglobin <110 g/L for 5 months to 6 year old, hemoglobin <115 g/L for 6 to 7 year old.[21] The concentration of hemoglobin between 90 and 110 g/L was defined as mild anemia, 60-90 g/L as moderate anemia, and <60 g/L as severe anemia.[21] Iron deficiency was defined as serum ferritin <12 mcg/dL and/or erythrocyte protoporphyrin >7 mcg/L.[22] Iron-deficiency anemia was defined as iron deficiency concurrent with anemia. According to the WHO criteria, a serum retinol concentration <0.7 μmol/L was classified as VAD, values 0.70-1.05 μmol/L were taken as marginal VAD.[23]

Statistical analysis

Kormogorov-Smirnov goodness-of-fit test was used to determine the distribution of each set of data for normality before analysis. Because the distribution of serum ferritin was skewed, values were natural-log transformed in these analyses. Data were presented as mean (geometric mean for serum ferritin), standard deviation (SD) (geometric SD for serum ferritin) and median. The Chi-square test was used to compare categorical variables between the groups. Differences in continuous variables between the two groups were examined using Student's *t* test with normal distribution and homogeneous variance, or using Wilcoxon's sign-rank test for non-normally distributed data. Pearson's correlation test was performed to examine the association between concentrations of serum retinol and hemoglobin for normally distributed data. One-way analysis of variance was used to compare the variance of multiple-samples and the Kruskal-Wallis test was performed to compare the variance of skewed distribution multiple-samples.

Moreover, the following covariables were examined: gender of participating children, guardians' educational level, family monthly income per person, passive smoking exposure, and self-reported anemia of parents. The unadjusted odds ratio (OR) between status of vitamin A and anemia/iron deficiency was determined by univariate logistic regression analysis, respectively and then adjustment was made for children's age and sex, as well as other factors including children's passive smoking exposure, guardian's educational levels, family income and self-reported anemia of parents. The assignment of vitamin A status was: 1 = sufficient vitamin A, 2 = marginal VAD, 3 = VAD.

All statistical analyses were performed using SAS 8.0 software for Windows. Two-tailed *P* values <0.05 were considered statistically significant.

Results

The anthropometric and biochemical indices are presented in Table 1. The age of children (mean±SD) in the study was 4.0±0.85 years, and 50.1% of the children were female. Of all children in the present study, the prevalence of stunt, underweight, overweight, wasting and obesity were 6.3% (29/459), 3.9% (18/459), 3.7% (17/459), 1.5% (7/459) and 3.1% (14/459), respectively. All of them were not significantly different between boys and girls (Chi-square test, *P*>0.05). The values of height-for-age, weight-for-age and weight-for-height were lower than those of the WHO criteria.[16] Weight-for-height was normally distributed but height-for-age and weight-for-age had double-hump distributions.

Hemoglobin concentration (mean±SD) was 115.70 ±9.03 g/L with a range of 72.2-154.0 g/L. The total prevalence of anemia was 23.5% (108/459) while mild anemia accounting for the main part (20.3%, 93/459). The prevalence of moderate and severe anemia were 2.8% (13/459) and 0.44% (2/459), respectively. The prevalence of anemia was not significantly different between boys and girls (Chi-square test, *P*>0.05). When grouped by weight-for-age, height-for-age and weight-for-height (Table 2), the prevalence of anemia in overweight and obesity children (35.3% and 42.9%) were higher than those in underweight and wasting (16.7% and 14.3%) respectively, but there was no significant difference (Chi-square test or Fisher's exact test, *P*>0.05); these prevalences were higher than those in children with normal weight-for-age and weight-

Table 1. Anthropometric and biochemical indices of preschool children in suburb Chongqing

Variables	Total (*n*=459)		
	Mean	SD	Median
Height-for-age Z-score	-0.30	1.31	-0.32
Weight-for-age Z-score	-0.30	1.15	-0.37
Weight-for-height Z-score	-0.01	1.22	-0.09
Hemoglobin (g/L)	115.70	9.03	116.00
Serum ferritin (mcg/L)*	20.90	1.84	21.30
Erythrocyte protoporphyrin (mcg/L)	0.34	0.43	0.27
Serum retinol (μmol/L)	1.21	0.35	1.18

*: expressed as geometric mean and geometric standard deviation.

for-height (23.3% and 23.3%) respectively (Chi-square test or Fisher's exact test, P>0.05) but there was no statistical significance. No significant difference was found in the prevalence of anemia between stunted children and those with normal height-for-age values (Chi-square test, P>0.05). A cut-off value of 12 μg/L for serum ferritin concentration showed that 15.0% (69/459) of the children had iron deficiency and there was no significant difference in the prevalence of iron deficiency between boys (15.7%) and girls (14.5%) (Chi-square test, P>0.05). None of the children had an elevated concentration of erythrocyte protoporphyrin (>7 μg/L) and 88.9% (96/108) of the children could not be classified into iron-deficiency.

The concentration of serum retinol was 1.21±0.35 μmol/L. Children with retinol concentration below 0.7 μmol/L (VAD) and between 0.7-1.05 μmol/L (marginal VAD) was 6.3% (29/459) and 25.9% (119/459), respectively. The prevalence of VAD and marginal VAD between boys and girls was not statistically different (Chi-square test, P>0.05).

The indices of anemia, iron status, and vitamin A were analyzed. Children were grouped according to the presence or absence of anemia (Table 3) and the status of serum retinol (Table 4). The concentration of serum retinol in children with anemia was significantly lower than that in children without anemia (P=0.003) and the concentration of serum retinol was associated with hemoglobin (r=0.22, P<0.01). Serum retinol was not significantly correlated with ferritin concentration. There was no significant difference in concentrations of serum ferritin and erythrocyte protoporphyrin between anemic and non-anemic groups (P>0.05). There was marked difference in prevalence of anemia (Chi-square test, P=0.044) at different serum retinol status (<0.7 μmol/L, 0.7-1.05 μmol/L, and >1.05 μmol/L). However, the difference in hemoglobin, serum ferritin, and erythrocyte protoporphyrin was not significant in these three groups (P>0.05). With the increase of retinol concentration, hemoglobin level tended to increase while serum ferritin and erythrocyte protoporphyrin to decrease. The overall prevalence of VAD, anemia, iron deficiency, combined serum retinol concentration <1.05 μmol/L and anemia, and combined retinol concentration <1.05 μmol/L and iron deficiency were 6.3%, 23.5%, 15.0%, 9.2% and 4.6%, respectively.

Table 2. Values for biochemical indices grouped by growth status of preschool children in suburb Chongqing

	Z-score	Nutritional status	n	Anemia, n (%)	P value
Weight-for-age	<-2SD	Underweight	18	3 (16.7)	0.3925*
	≥+2SD	Overweight	17	6 (35.3)	
	-2SD~+2SD	Normal	424	99 (23.3)	
Height-for-age	<-2SD	Stunted	29	7 (24.1)	0.9364†
	-2SD~+2SD	Normal	430	101 (23.5)	(χ^2=0.0064)
Weight-for-height	<-2SD	Wasting	7	1 (14.3)	0.1901*
	≥+2SD	Obesity	14	6 (42.9)	
	-2SD~+2SD	Normal	438	102 (23.3)	

*: Fisher's exact test; †: Chi-square test.

Table 3. Values for biochemical indices grouped by anemic status of preschool children in suburb Chongqing

Variables	Anemic group (n=108)			Non-anemic group (n=351)			P value
	Mean	SD	Median	Mean	SD	Median	
Serum ferritin (mcg/L)§	22.62	1.76	23.20	20.40	1.85	20.60	0.121 (t=1.55)*
Erythrocyte protoporphyrin (mcg/L)	0.35	0.42	0.27	0.33	0.44	0.27	0.693 (χ^2=0.16)†
Serum retinol (μmol/L)	1.12	0.32	1.11	1.24	0.36	1.21	0.003 (t=2.98)‡

*: Student's t test based on natural log-transformed values; †: Wilcoxon's test; ‡: Student's t test; §: expressed as geometric mean and geometric standard deviation.

Table 4. Values for biochemical indices grouped by serum retinol status of preschool children in suburb Chongqing

Variables	Serum retinol (<0.7 μmol/L) (n=29)			Serum retinol (0.7-1.05 μmol/L) (n=119)			Serum retinol (>1.05 μmol/L) (n=311)			P value
	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	
Hemoglobin (g/L)	113.9	8.1	113.0	115.1	7.9	115.0	116.1	9.5	117.0	0.376 (F=0.98)*
Serum ferritin (mcg/L)‖	22.8	2.0	24.0	21.7	1.9	20.4	20.5	1.8	21.4	0.760 (F=0.46)†
Erythrocyte protoporphyrin (mcg/L)	0.3	0.2	0.3	0.4	0.7	0.3	0.3	0.3	0.2	0.248 (χ^2=1.33)‡
Anemia, n (%)	12 (41.4)			30 (25.2)			66 (21.2)			0.044 (χ^2=6.24)§

*: one-way analysis of variance; †: one-way analysis of variance based on natural log-transformed values; ‡: Kruskal-Wallis test; §: Chi-square test; ‖: expressed as geometric mean and geometric standard deviation.

Table 5. Odds ratios (OR) of anemia by vitamin A status with logistic regression analysis

Vitamin A status	Unadjusted OR (95% CI)	Multiple variables-adjusted OR (95% CI)*	Wald Chi-square
Vitamin A sufficient	1.00 (Referent)	1.00 (Referent)	Referent
Marginal vitamin A deficiency	1.39 (0.87, 2.21)	1.24 (0.77, 2.01)	0.7539
Vitamin A deficiency	2.47 (1.13, 5.43)	2.56 (1.15, 5.70)	4.2218

CI: confident interval. *: adjusted for children's age and sex, as well as other factors including children's passive smoking exposure, guardian's educational levels, family income and self-reported anemia of parents.

Table 6. Odds ratios (OR) of iron deficiency by vitamin A status with logistic regression analysis

Vitamin A status	Unadjusted OR (95% CI)	Multiple variables-adjusted OR (95% CI)*	Wald Chi-square
Normal	1.00 (Referent)	1.00 (Referent)	Referent
Marginal vitamin A deficiency	0.89 (0.49, 1.60)	0.91 (0.50, 1.65)	0.2043
Vitamin A deficiency	0.62 (0.18, 2.13)	0.57 (0.16, 1.98)	0.6639

CI: confident interval. *: adjusted for children's age and sex, as well as other factors including children's passive smoking exposure, guardian's educational levels, family income and self-reported anemia of parents.

Vitamin A insufficiency (VAD and marginal VAD) with anemia, vitamin A insufficiency with iron deficiency, and iron-deficiency anemia were analyzed, showing that the proportion with serum retinol concentration <1.05 μmol/L was higher in children with anemia (42/108) than in those without anemia (106/351) (Mantel-Haenszel Chi-square test, P=0.09). In the logistic multiple variables model, VAD (OR=2.56; 95% CI, 1.15-5.70) was associated with increased prevalence of anemia, but not for marginal VAD (OR=2.56; 95% CI, 1.15-5.70) (Table 5). Neither VAD (OR=0.57; 95% CI, 0.16-1.98) nor marginal VAD (OR=0.91; 95% CI, 0.50-1.65) was associated with the prevalence of iron deficiency regardless of the univariate or multiple variables model (Table 6). In addition, in the 108 children with anemia, 42 were due to insufficient vitamin A (42/108), and 12 due to iron deficiency.

Discussion

Growth status

Anthropometry is widely used to assess the nutritional status of an individual or population because of its high sensitivity in detecting under-nutrition.[24] Compared with the national health survey in China in 2002, the prevalence of stunt, underweight, overweight and obesity of preschool children in the present study was lower than the national levels of 14.3%, 7.8%, 17.6% and 5.6%, respectively.[10] The negative values of Z score indicated that the development of preschool children in the Banan district of Chongqing fall behind the WHO level.

The present study showed a higher prevalence of anemia in overweight and obese children although it was not significant. Despite their excessive dietary and caloric intake, these children may be not at risk for iron deficiency because they tend to consume unbalanced meals, particularly those rich in carbohydrates and fat but low in many micronutrients.[25-27] Although the distribution of weight-for-height was normal, height-for-age and weight-for-age presented double hump distributions. Possibly a country undergoing rapid nutritional transition has a separate frequency distribution for those underfed or those prosper, with a large gap between them.

Vitamin A and iron status

VAD is a health problem in about 6.3% of preschool children in suburb Chongqing. The rate is significantly lower than that in children below 6 years in China.[28] However, the prevalence of marginal VAD in this region is 25.9%. In the present study, if the serum retinol value of <30 mcg/dL (1.05 μmol/L) was used as the cut-off value of VAD, the prevalence of VAD would increase to 32.2%.[29-31]

The prevalence estimates in the WHO Global Database on Anemia are largely based on the data from surveys conducted in developing countries of North America and Latin America.[32] The prevalence of anemia (23.5%) in the present study is lower than 42% according to the WHO database, but significantly higher than 16.8% according to the China Food and Nutrition Surveillance Project.[32,33]

Generally, there are three sequential stages for assessing the prevalence of iron deficiency.[34] In this study, 15.0% of the children had iron deficiency but only 2.6% was diagnosed as having iron deficiency anemia and most of the anemic children (88.9%) are not iron-deficient. Inflammation has effect on the biochemical indicators of iron and vitamin A, such as serum ferritin and retinol. However, the exclusion of children with obvious infectious disease alleviated the effect of inflammation on the estimation of iron deficiency and VAD. Dietary assessment was not performed among the children, hence the etiology of iron deficiency was not investigated in this study. Our data showed that none of the children had elevated erythrocyte protoporphyrin concentration more than 7 μg/dl.

The relationship among anemia, vitamin A and iron concentrations

A significant correlation was observed between concentrations of serum retinol and hemoglobin in this study. The mean serum retinol concentration and the prevalence of VAD in children with anemia were significantly lower and higher respectively than in those without anemia (Table 3). With increased serum retinol concentration, the concentration of hemoglobin increased (Table 4), but the concentration of serum ferritin and erythrocyte protoporphyrin decreased. In addition, VAD was associated with the increased prevalence of anemia after adjustment for other impact factors on anemia. These findings indicated a possible relationship between VAD and the mobilization of iron for hematopoiesis. In the present study, the increase of hemoglobin, the decrease of serum ferritin, and the increase of serum retinol concentration may indicate the effect of vitamin A on anemia because vitamin A can increase the iron mobilization from body stores and then increase erythropoiesis which corresponds to one of our previous supplementary trial.[35] A positive correlation between hemoglobin and serum retinol concentrations has been described in children in developing countries.[6,36-38]

Iron-deficiency anemia and VAD are predominant nutritional health problems in underdeveloped countries; both deficiencies would be prevalent in the same population.[2,31] In the present study, the prevalence of anemia and VAD was 23.5% and 6.3% respectively, which were not significantly different from the rates in the past 5 years.[35] These indicate that nutritional interventions are required in suburban Chongqing.

The results of the present study also suggest that synergistic relationship among vitamin A, iron and other micronutrients such as zinc, copper, folic, iodine, selenium and vitamin B_{12} partly contribute to anemia. In addition, anemia is also associated with maternal health, growth, dietary, eating habit, diseases and socioeconomic condition. This is why no exact reason can be found for the high prevalence of non-vitamin A and iron related anemia. Hence interventions for anemia control should meet the local conditions, for instance, the status of multi-micronutrients including vitamin A and iron.

Despite the improvement of controlling micronutrient deficiency which has been a by-product of socioeconomic development, the processes are too slow in the developing countries.[39] Elimination of micronutrient deficiency can be facilitated through deliberate supplement of micronutrients. The efficiency of nutritional interventions would be improved by targeting children with any of the conditions which should be simultaneously treated when designing supplementary programs.[40] In fact, the WHO workgroup on non-infectious diseases has noted multiple benefits in dealing with several micronutrient deficiencies simultaneously, one of the benefits is the efficiency of delivery.[41] Also, to reduce the prevalence of anemia, deficiencies of iron, zinc and vitamin A, preschool children in kindergartens of suburban Chongqing are given diets such as porridge, bean milk, soup or noodles fortified with iron, vitamin A, vitamin B1, vitamin B2, niacin, folate, zinc and calcium.

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Ethical approval: The research protocol was reviewed and approved by the Institutional Ethical Committee of Children's Hospital, Chongqing Medical University in Chongqing, China, and was in accordance with the Helsinki Declaration of 1964, as revised in 2000. Participating children's parents or guardians were well informed about the research and the written approval consents were obtained from parents or guardians.

Competing interest: The authors do not have any possible conflicts of interest.

Contributors: Chen K and Zhang X contributed equally to this paper and they contributed to project design, data acquisition, analysis and interpretation. Li TY contributed to project conception and design, data analysis and interpretation, article draft and revision, and general supervision. Chen L contributed to project design and data acquisition. Qu P contributed to project design, data analysis and interpretation, and technical assistance. Liu YX contributed to project conception and design, data analysis and interpretation, and general supervision.

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