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# Effect of single-dose albendazole and vitamin A supplementation on the iron status of pre-school children in Sichuan, China

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## Abstract

The aim of this study was to explore the effect of single-dose albendazole and vitamin A intervention on the anaemic status and Fe metabolism of pre-school children. This study was a randomised, placebo-controlled and double-blinded intervention trial. All eligible anaemic pre-school children were randomly divided into three groups: group 1 received no intervention, which served as the control group, group 2 received 400 mg single-dose albendazole administration and group 3 received a 60000 μg vitamin A capsule combined with 400 mg single-dose albendazole at the beginning of the study. The follow-up period was for 6 months. Anthropometry and biochemical index about Fe metabolism were measured before and after intervention. A total of 209 pre-school anaemic children were randomly divided into three intervention groups (sixty-four, sixty-two and sixty for groups 1, 2 and 3, respectively). The mean age of the children in the study was 4·4 (SD 0·7) years and 50·5 % of the children were female (94/186). After a follow-up period of 6 months, the levels of serum retinol, ferritin, transferrin receptor-ferritin index and body total Fe content of children in group 3 were significantly higher compared with children in groups 1 and 2 ($P<0·05$). Moreover, the proportion of vitamin A deficiency, marginal vitamin A deficiency and Fe deficiency among children in group 3 were markedly lower compared with children in groups 1 and 2 ($P<0·05$). Albendazole plus vitamin A administration showed more efficacy on the improvement of serum retinol and Fe metabolic status.

**Key words:** **Anaemia: Albendazole: Vitamin A: Iron metabolism: Children**

Anaemia is a worldwide public health problem. In May 2002, the General Assembly of the UN[1] re-emphasised that control of nutritional anaemia should be one of the global development goals achieved in the early years of this millennium. Despite this goal, the global prevalence of anaemia has declined little in the past decade[2]. The reasons for this lack of improvement of anaemia are multifactorial. Some of the largest problems include[2–4] under-funding and poor programme implementation, designed on the assumption that anaemia is solely caused by Fe deficiency (ID). Potential other causes of anaemia include infections such as hookworm, malaria, chronic diseases and other nutritional deficiencies[5]. In this study, we present an intervention for anaemia that was implemented in pre-school children living in Sichuan, China. The plan focuses on hookworm control and vitamin A supplementation.

Human hookworm infection is a leading cause of anaemia and under-nutrition in developing and underdeveloped countries. An estimated 576 million people are affected worldwide[6] with the highest incidence in sub-Saharan Africa followed by Southeast Asia, India and the Americas. It has been shown[7] that school-based anthelmintic treatment offers a number of health-related benefits to children, including improvements in Fe and Hb status.

Other nutritional deficiencies may also adversely affect haematopoiesis and lead to anaemia. In particular, the impact of vitamin A deficiency (VAD) on Fe metabolism has been well recognised[8]. Our previous studies showed a correlation between vitamin A supplementation and improvement in Fe-deficiency anaemia (IDA) in pre-school children in China[9–12]. VAD is also a major public health issue worldwide. It is the leading cause of paediatric blindness and a major nutritional determinant of severe infection and mortality among children in the developing world[13,14]. Therefore, supplementation that reduce VAD have the potential to decrease anaemia induced by either malnutrition or infection.

Current WHO and UNICEF guidelines recommend that supplementations for the prevention and control of anaemia should follow an integrated, long-term approach[15]. Anaemia must be addressed through a multidisciplinary approach, including increased Fe intake, infection control and improved nutritional status[15]. In addition, one of the key elements in developing a fortification programme is exploring an intervention

**Abbreviations:** CRP, C-reaction protein; ID, Fe deficiency; MVAD, marginal vitamin A deficiency; SF, serum ferritin; SNK, Student–Newman–Keuls; sTfR, serum soluble transferrin receptor; TBIC, total body Fe content; TFR-F index, transferrin receptor-ferritin index; VAD, vitamin A deficiency.

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that is cost-effective and sustainable[16]. This can be a challenge, especially in countries with large rural populations, a small food industry with limited technology and limited access to processed foods.

In order to reduce the morbidity of anaemia in China, food fortification, supplementation and other means to increase micronutrient intake have been proposed. However, cost-effectiveness remains a difficult challenge for the Chinese government. Sichuan province, located in Western China, contains highly impoverished areas with a high prevalence of hookworm infection and VAD[17]. To our knowledge, no public health strategy targeting has been performed in this area. In present study, we supplemented anaemic pre-school children with vitamin A and administered anthelmintic treatment in an impoverished region – the Sichuan province. Our study demonstrates a cost-effective, safe and efficacious public health strategy to manage anaemia in impoverished pre-school children.

## Methods

### Subjects and ethics approval

This randomised, controlled and blinded cohort study was performed from March 2012 to September 2014 in Dayi, Pixian and Meishan counties of Sichuan Province, Western China, where the majority of the population is of a low socio-economic status. A total of 216 anaemic pre-school children aged 3–6 years were randomly recruited from nine kindergartens from the three different counties for the study during the follow-up period. The enrolment and research plan were reviewed and approved by the institutional ethics committee of the Chengdu Women's and Children's Central Hospital of Chongqing Medical University in Sichuan province, China, and comply with the code of ethics of the World Medical Association (Declaration of Helsinki).

### Eligibility and exclusion criteria

The eligibility criteria for participation were as follows: (1) apparently healthy, (2) Hb concentration <110 g/l but not <80 g/l, (3) C-reactive protein (CRP) <10 mg/l, (4) parental or guardian approval for participation in all aspects of the study and (5) parental or guardian agreement to avoid the additional use of vitamin and mineral supplements and anthelmintic treatments during the trial. Children with Hb <80 g/l and/or CRP >10 mg/l were sent to the hospital for further treatment. We conducted a census in each of the nine regional kindergartens to identify households with an eligible pre-school child. Field health workers explained the protocol, answered questions, obtained written informed consent from parents/guardians and then conducted a family survey to determine children who were eligible. All the surveys were performed at the local kindergartens. Parental consent was obtained before the Hb screening.

### Primary and secondary outcomes

The primary objective of the present study was to measure the change in serum Hb before and after supplementation. The secondary outcomes were to explore the effectiveness of intervention on other serum biochemical indices including serum retinol, serum ferritin (SF) and serum soluble transferrin receptor (sTfR).

### Sample size

A sample size of sixty anaemic pre-school children per group was required to detect an absolute difference of 10 g/l of Hb concentration among three intervention groups with 95 % power and $\alpha = 0{\cdot}05$ for a two-sided two-sample $t$ test. To allow for a 20 % dropout rate over the duration of the study, we planned to recruit seventy-two anaemic children per group for a total of 216 anaemic children altogether. The prevalence of anaemia in pre-school children in the locality was estimated to reach about 16 %[18]. Ultimately, 1350 children were screened in the initial Hb study and then divided into three groups. The 1350 children came from forty-one classrooms of the nine kindergartens, encompassing three counties.

### Randomisation and allocation concealment

Immediately after recruitment, each child ($n$ 209) within a class was randomly assigned a study number to one of the three treatment groups with fixed, equal allocation to each group prepared by a research secretary at the Chengdu Women's and Children's Central Hospital, who was not connected to the study. The RAND function of Excel (Microsoft) was used to generate randomly permutated codes with concealment to ensure that the allocation was not made before the parents of the subjects had given their consent and joined the study.

### Intervention

In total, 186 out of 209 eligible anaemic children participated in the study. They were randomly divided into three groups: group 1 ($n$ 64) received no supplementation, which was the control group; group 2 ($n$ 62) received 400 mg single-dose albendazole (helmintic treatment) and group 3 ($n$ 60) received a 60000 μg vitamin A capsule combined with 400 mg single-dose albendazole once initially. All the children in the control group were given a single-dose albendazole at the end of study for ethical purposes. The total duration of follow-up and evaluation was 6 months. Vitamin A capsule and albendazole tablet were provided by Sichuan Pearl Pharmaceutical Co. Ltd.

### Blinding

The physicians, nurses, field health workers, parents, children and laboratory personnel were blinded to the treatment assignment of each child throughout the study period. The data manager, statistician and all investigators remained blinded to group assignments until the end of data analysis.

### Compliance

Compliance was monitored using recording tables, where teachers recorded whether the child consumed 'all' or 'none' of

the albendazole tablet and vitamin A capsule. Distribution and consumption occurred under close supervision – children in group 2 and group 3 were not allowed to leave the classroom or return to their original seats until they had finished taking the medication. Nursery managers performed the distribution of tablets, but not healthcare workers or nursery teachers. Information on the acceptability of the albendazole tablet and vitamin A capsule was obtained from the records by teachers after administration.

### Questionnaire interview

A trained interviewer administered a 30-min questionnaire to the parents of anaemic children (*n* 209) after recruitment. This included questions on demographic information (children's age, sex), educational levels of main caregivers who were responsible for at least half of the care time of children, monthly family income, use of vitamin/mineral supplement before the study and food frequency recall (the frequency of deep-coloured vegetables and milk, liver and eggs). After Hb screening, only parents of 209 eligible anaemic children received the questionnaire interview.

### Anthropometric measurements

Anthropometric examinations were conducted by the same trained nurse at baseline and at follow-up using standardised techniques to eliminate intra-examiner error[19]. Duplicate measurements were performed for all children. The inter-examiner CV of weight and height for each examiner in each group was <5 %. Weight was recorded using a weighing scale (100Med) to the nearest 100 g with subjects in minimal clothing and bare feet. Similarly, height was measured in the standard position using a height scale (Haode) to the nearest 0·1 cm. By using reference data from the WHO (2005 database; http://www.who.int/childgrowth/standards/en/), *Z*-scores were calculated for height for age (HAZ), weight for height (WHZ) and weight for age (WAZ). All the indices were computed using WHO Anthro for PC (2005) as recommended by the WHO (http://www.who.int/childgrowth/software/en/). The anthropometric measurements were taken in each county's local hospital, only for anaemic children after Hb screening.

### Blood sample collection and biochemical assessment

Three blood samples (about 1 ml) were collected by venepuncture of an antecubital vein from each subject before breakfast at time points before intervention and at 3 and 6 months after intervention. The concentration of Hb was measured by the haemoglobincyanide method (Maker) immediately after 25 µl was drawn into a heparinised container with the same type of instrument. The inter-assay variation was lower than 5 % and the intra-assay variation was lower than 10 %. The remaining blood samples were immediately stored at 4°C to prevent micro-haemolysis and were centrifuged at 3000 ***g*** for 5 min at room temperature within 5 h. The centrifuged serum samples were divided into aliquots and immediately transported to the laboratory and stored at −20°C. The concentrations of SF were measured using a commercial ELISA (Sunbiote). sTfR was measured by microparticle-enhanced immunoassay (Sunbiote). CRP was measured by particle-enhanced immunoturbidimetry (Sunbiote). Serum retinol concentration was measured using HPLC at the Pediatric Laboratory of Chongqing Medical University. All other biochemical indices were measured at the Clinical Laboratory Center of Chengdu Women & Children's Central Hospital. Hookworm detection was carried out using the Kato–Katz technique at each local hospital before and after intervention.

### Definition of outcomes

Prevalence of anaemia was determined according to World Health Organisation criteria[20] – that is, <110 g/l Hb for 6 months to 6 years. VAD was defined as a serum retinol concentration of <0·7 µmol/l, with 0·7–1·04 µmol/l defined as marginal vitamin A deficiency (MVAD)[21]. ID was defined as SF of <12 µg/l. IDA was defined as the simultaneous existence of anaemia and ID[22]. CRP levels of >10 mg/l indicated infection or inflammation as stated by the manufacturer[23]. Transferrin receptor-ferritin index (TFR-F index) was estimated with sTfR (mg/l)/log SF (µg/l), and total body Fe content (TBIC) (mg/kg) was estimated with −(log (sTfR/SF) − 2·8229)/0·1207 (sTfR, µg/l; SF, µg/l)[10].

### Statistical analysis

Using the Kolmogorov–Smirnov goodness-of-fit test, the distribution of each set of data was tested for normality before analysis. Data were presented as the mean and standard deviation for normally distributed variables (age, height, weight, CRP, serum retinol, Hb, SF, sTfR, TFR-F index and TBIC) or as median (25th, 75th) for skewed distribution variables (HAZ, WAZ and WHZ). Two-tailed tests were performed with $P$ value <0·05 considered as statistically significant. Paired Student's $t$ tests were used to compare paired data with normal distribution and homogeneous variance in the before–after intervention pairs of each group, whereas paired Wilcoxon's signed-rank test was used for skewed distribution data. The $\chi^2$ test was used for categorical variables, with a Bonferroni correction for multiple comparisons among multiple groups. The Student–Newman–Keuls (SNK) test (normal and homogeneous data) and the SNK rank test (skewed data) were used to compare the different effects of the three interventions, with multiple comparisons of parameters at the 3-month and 6-month follow-up. The SNK grouping letters were provided for SNK and SNK rank test. Data were analysed using SAS for Windows statistical software package, version 8.1 (SAS Institute Inc.).

## Results

A total of 1350 pre-school children completed the initial Hb screening, and 209 pre-school children with defined anaemia without inflammation were randomly divided into three intervention groups (seventy for group 1, seventy-two for group 2 and sixty-seven for group 3). A total of 11·0 % (23/209) of the

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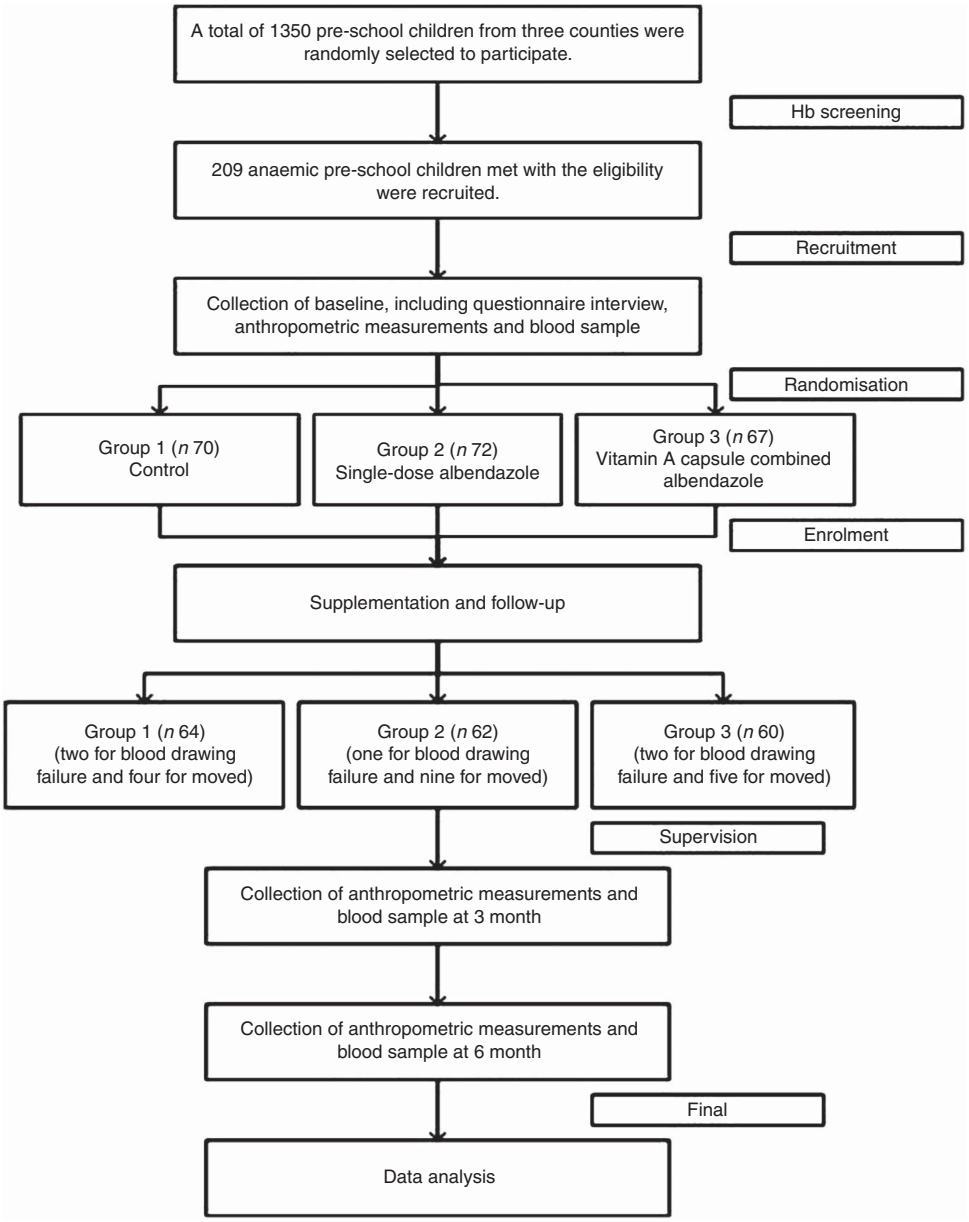


**Fig. 1.** The flow diagram of the study design.

participants dropped out during the course of the study. The dropout number was six for group 1 (two due to failure of blood sample collection and four as they moved out during the trial), ten for group 2 (one due to failure of blood sample collection and nine as they moved out during the trial) and seven for group 3 (two due to failure of blood sample collection and five as they moved out during the trial) (Fig. 1). Thus, primary outcome measures including analyses of micronutrient status of blood and anthropometric indices at the end of intervention, which was on the 6th month after drug administration, were obtained from 186 pre-school children (sixty-four, sixty-two and sixty from groups 1, 2 and 3, respectively). Retrospective data about the administration of the vitamin A capsule and albendazole tablet collected and summarised by caregivers indicated 98 % compliance.

### *Baseline characteristics*

The mean age of the children in the present study was 4·4 (SD 0·7) years and 50·5 % of the children were female (94/186). The demographic and biochemical characteristics of the children studied were not significantly different among the three intervention groups (Tables 1 and 2).

### *Effect of supplementation on serum biochemical indices*

There was no significant difference in the biochemical indices of pre-school children in group 1 at baseline and at the 3-month and 6-month follow-up ($P > 0·05$) (Tables 2 and 3). The serum retinol levels of children in group 3 significantly increased at 3 months (1·79 μmol/l) compared with baseline (1·14 μmol/l)

**Table 1.** Socio-demographic, anthropometric and biochemical characteristics of the three intervention groups at baseline (Mean values and standard deviations; medians and 25th, 75th percentiles; numbers and percentages)

| | Groups* | | | | | |
|---|---|---|---|---|---|---|
| | Group 1 (*n* 64) | | Group 2 (*n* 62) | | Group 3 (*n* 60) | |
| Variables | Mean | SD | Mean | SD | Mean | SD |
| Age (years)† | 4·3 | 0·5 | 4·5 | 0·7 | 4·4 | 0·6 |
| Proportion of sex (%female) (boys/girls)† | 48·4 (33/31) | | 53·2 (29/33) | | 50·0 (30/30) | |
| Height (cm)† | 101·9 | 7·6 | 100·4 | 7·3 | 101·3 | 7·8 |
| Weight (kg)† | 16·4 | 4·6 | 15·8 | 4·4 | 16·1 | 4·3 |
| Height for age (*Z*-score)† | | | | | | |
| Median | −0·88 | | 0·02 | | −0·89 | |
| 25th, 75th percentiles | −1·16, 0·21 | | −0·92, 0·15 | | −1·14, 0·06 | |
| Weight for age (*Z*-score)† | | | | | | |
| Median | −0·92 | | 0·09 | | −0·96 | |
| 25th, 75th percentiles | −1·08, −0·08 | | −0·91, 0·11 | | −1·14, 0·05 | |
| Weight for height (*Z*-score)† | | | | | | |
| Median | −0·68 | | −0·80 | | −0·63 | |
| 25th, 75th percentiles | −1·12, 0·12 | | −1·14, 0·22 | | −1·01, 0·47 | |
| C-reactive protein (mg/l)† | 5·4 | 0·8 | 6·1 | 1·2 | 5·7 | 1·1 |
| | *n* | % | *n* | % | *n* | % |
| Regular consumption of milk, liver and eggs†‡ | 33 | 51·6 | 35 | 56·5 | 37 | 61·7 |
| Regular consumption of deep-coloured vegetables†‡§ | 30 | 46·9 | 29 | 46·8 | 31 | 51·7 |
| Use of vitamin/mineral supplements before the trial† | 14 | 21·9 | 16 | 25·8 | 14 | 23·3 |
| High school or above education of main caregivers†‖ | 37 | 57·8 | 36 | 58·1 | 35 | 58·3 |
| Monthly family income <2000 yuan† | 20 | 31·3 | 22 | 34·4 | 18 | 30·0 |

* Group 1: control group; group 2: 400 mg single-dose albendazole; group 3: 60000 μg vitamin A combined with 400 mg albendazole once initially.
† No significant difference among intervention groups.
‡ Consumption of more than or equal to 3 d/week.
§ Indicated red amaranth, purple cabbage, Houttuynia, tomatoes, carrots, pumpkin, red peppers, spinach, rape, etc.
‖ Main caregiver who was responsible for at least half of the care time of the children.

**Table 2.** Changes in biochemical indices at baseline and after 3 and 6 months (Mean values and standard deviations)

| | | Groups* | | | | | |
|---|---|---|---|---|---|---|---|
| | | Group 1 (*n* 64) | | Group 2 (*n* 62) | | Group 3 (*n* 60) | |
| Variables | Time of after administration | Mean | SD | Mean | SD | Mean | SD |
| Serum retinol status (μmol/l) | Baseline | $A1{\cdot}13^{a}$ | 0·28 | $A1{\cdot}12^{a}$ | 0·30 | $A1{\cdot}14^{c}$ | 0·27 |
| | After 3 months | $A1{\cdot}15^{a}$ | 0·30 | $A1{\cdot}17^{a}$ | 0·29 | $B1{\cdot}79^{a}$ | 0·34 |
| | After 6 months | $A1{\cdot}14^{a}$ | 0·33 | $A1{\cdot}16^{a}$ | 0·31 | $B1{\cdot}39^{b}$ | 0·35 |
| Hb concentration (g/l) | Baseline | $A94{\cdot}8^{a}$ | 10·9 | $A96{\cdot}3^{c}$ | 9·8 | $A97{\cdot}1^{b}$ | 10·1 |
| | After 3 months | $A96{\cdot}4^{a}$ | 12·2 | $B105{\cdot}7^{b}$ | 13·4 | $C114{\cdot}2^{a}$ | 11·9 |
| | After 6 months | $A97{\cdot}9^{a}$ | 11·7 | $B115{\cdot}5^{a}$ | 13·9 | $B116{\cdot}8^{a}$ | 13·1 |
| Serum ferritin concentration (μg/l) | Baseline | $A33{\cdot}1^{a}$ | 8·9 | $A31{\cdot}5^{b}$ | 9·1 | $A32{\cdot}8^{b}$ | 8·6 |
| | After 3 months | $A32{\cdot}8^{a}$ | 8·8 | $A39{\cdot}1^{a}$ | 11·6 | $B46{\cdot}1^{a}$ | 11·5 |
| | After 6 months | $A33{\cdot}2^{a}$ | 8·1 | $B33{\cdot}7^{b}$ | 10·3 | $C44{\cdot}4^{a}$ | 13·4 |
| Serum transferrin receptor (mg/l) | Baseline | $A1{\cdot}66^{a}$ | 0·15 | $A1{\cdot}72^{a}$ | 0·16 | $A1{\cdot}70^{b}$ | 0·15 |
| | After 3 months | $A1{\cdot}68^{a}$ | 0·15 | $A1{\cdot}71^{a}$ | 0·12 | $B1{\cdot}85^{a}$ | 0·15 |
| | After 6 months | $A1{\cdot}69^{a}$ | 0·16 | $A1{\cdot}73^{a}$ | 0·12 | $A1{\cdot}71^{b}$ | 0·13 |
| Transferrin receptor-ferritin index | Baseline | $A1{\cdot}60^{a}$ | 0·31 | $A1{\cdot}61^{b}$ | 0·36 | $A1{\cdot}63^{c}$ | 0·33 |
| | After 3 months | $A1{\cdot}59^{a}$ | 0·30 | $B1{\cdot}79^{a}$ | 0·34 | $C1{\cdot}96^{a}$ | 0·35 |
| | After 6 months | $A1{\cdot}52^{a}$ | 0·33 | $B1{\cdot}64^{b}$ | 0·33 | $C1{\cdot}83^{b}$ | 0·28 |
| Total body Fe contents (mg/kg) | Baseline | $A9{\cdot}19^{a}$ | 2·43 | $A9{\cdot}02^{b}$ | 2·53 | $A9{\cdot}22^{c}$ | 2·51 |
| | After 3 months | $A9{\cdot}15^{a}$ | 2·39 | $B10{\cdot}1^{a}$ | 2·47 | $C13{\cdot}61^{a}$ | 2·88 |
| | After 6 months | $A9{\cdot}11^{a}$ | 2·26 | $A9{\cdot}12^{b}$ | 3·01 | $B11{\cdot}17^{b}$ | 2·76 |

* Group 1: control group; group 2: 400 mg single-dose albendazole; group 3: 60000 μg vitamin A combined with single-dose 400 mg albendazole.
$^{A,B,C \text{ or } a,b,c}$ Student–Newman–Keuls test for multiple comparisons: A, B and C for multiple comparisons among different groups; a, b and c for multiple comparisons in one group at different intervention times.
$^{a,b,c}$ Mean values with unlike superscript letters were significantly different ($P<0{\cdot}05$) and the like superscript letters were not significantly different ($P>0{\cdot}05$).

**Table 3.** Changes in proportion of anaemia, vitamin A deficiency (VAD), marginal vitamin A deficiency (MVAD), iron deficiency (ID) and hookworm infection at baseline and after 3 and 6 months
(Numbers and percentages)

| | | Groups* | | | | | |
|---|---|---|---|---|---|---|---|
| | | Group 1 (*n* 64) | | Group 2 (*n* 62) | | Group 3 (*n* 60) | |
| Variables | Time of after administration | % | *n* | % | *n* | % | *n* |
| Proportion of anaemia | Baseline | A100 | 64[a] | A100 | 62[a] | A100 | 60[a] |
| | After 3 months | A92·2 | 59[a] | B67·7 | 42[b] | C56·7 | 34[b] |
| | After 6 months | A82·8 | 53[a] | B40·3 | 25[c] | B38·3 | 23[c] |
| Proportion of VAD | Baseline | A7·8 | 5[a] | A11·3 | 7[a] | A15·0 | 9[a] |
| | After 3 months | A9·4 | 6[a] | A8·1 | 5[a] | B1·7 | 1[b] |
| | After 6 months | A12·5 | 8[a] | B8·1 | 5[a] | C3·3 | 2[b] |
| Proportion of MVAD | Baseline | A32·8 | 21[a] | A30·6 | 19[a] | A31·7 | 19[a] |
| | After 3 months | A25·0 | 16[a] | A24·2 | 15[a] | B5·0 | 3[b] |
| | After 6 months | A26·6 | 17[a] | A24·2 | 15[a] | B3·3 | 2[b] |
| Proportion of ID | Baseline | A39·1 | 25[a] | A46·8 | 29[a] | A43·3 | 26[a] |
| | After 3 months | A37·5 | 24[a] | A24·2 | 15[b] | B18·3 | 11[b] |
| | After 6 months | A35·9 | 23[a] | A35·5 | 22[a] | B23·3 | 14[b] |
| Proportion of hookworm infection | Baseline | A9·4 | 6[a] | A12·9 | 8[a] | A6·7 | 4[a] |
| | After 3 months | A10·9 | 7[a] | B1·6 | 1[b] | B0 | 0[b] |
| | After 6 months | A10·9 | 7[a] | B3·2 | 2[b] | B1·7 | 1[b] |

* Group 1: control group; group 2: 400 mg single-dose albendazole; group 3: 60000 μg vitamin A combined with 400 mg albendazole.
[A,B,C or a,b,c] Student–Newman–Keuls test for multiple comparisons: A, B and C for multiple comparisons among different groups; a, b and c for multiple comparisons in one group at different follow-up times.
[a,b,c] Mean values with unlike superscript letters were significantly different ($P<0·05$) and the like superscript letters were not significantly different ($P>0·05$).

($P<0·05$), and then decreased at 6 months (1·39 μmol/l), remaining significantly higher compared with baseline ($P<0·05$). The retinol levels of children in group 2 showed no significant change from baseline at 3 and 6 months ($P>0·05$). Hb concentrations of children in groups 2 and 3 at 3 months (105·7 g/l and 114·2 g/l, respectively) and 6 months (115·5 g/l and 116·8 g/l, respectively) both showed significant increases when compared with baseline ($P<0·05$). Hb levels of children in group 3 at 3 and 6 months were statistically higher than those of children in group 2 ($P<0·05$). SF of children in group 2 markedly increased at 3 months (39·1 μg/l) compared with baseline ($P<0·05$). For children in group 3, SF markedly increased at 3 months (46·1 μg/l) and 6 months (44·4 μg/l) ($P<0·05$). SF levels of children in group 3 were statistically higher than those of children in group 2 at 3 and 6 months ($P<0·05$). There was no significant effect on sTfR levels of children in group 2 ($P>0·05$). However, sTfR levels of children in group 3 markedly increased at 3 months (1·85 mg/l) ($P<0·05$) and then decreased to baseline at 6 months ($P>0·05$). The TFR-F index and TBIC levels of children in group 2 at 3 months (1·79 and 10·1 mg/kg, respectively) and group 3 at 3 months (1·96 and 13·61 mg/kg, respectively) and 6 months (1·83 and 11·17 mg/kg, respectively) significantly increased compared with baseline ($P<0·05$). The TFR-F index and TBIC levels of children in group 3 were statistically higher than those of children in group 2 at 3 and 6 months (1·64 and 9·12 mg/kg, respectively) ($P<0·05$).

### *Effect of treatment on levels of anaemia, vitamin A deficiency, iron deficiency and hookworm infection*

Our data also indicated that there was no significant difference in the proportion of anaemia, VAD, MVAD, ID and hookworm infections at baseline levels among the three groups ($P>0·05$) (Table 3). As expected, no significant difference in these measurements of children in group 1 at 3 and 6 months was observed, because no intervention was performed ($P>0·05$). The proportion of anaemia and ID of children in group 2 significantly decreased after only 3 months ($P<0·05$) and for children in group 3 at 3 and 6 months ($P<0·05$). The significant decrease in MVAD and VAD after 3 and 6 months was only observed in children in group 3 ($P<0·05$) and not in those in group 1 or 2 ($P>0·05$). The proportions of hookworm infection of children in group 2 and 3 were significantly decreased at 3 and 6 months ($P<0·05$) when compared with baseline, but no statistical difference was found between children in groups 2 and 3 ($P>0·05$).

## Discussion

### *Effect of administration on serum Hb*

Anaemia is a multifaceted problem, and to treat it effectively multiple aetiologies need to be addressed, such as parasitic worms in lower socio-economic status areas. The combination of anthelmintic treatments and vitamin A resulted in increased vitamin A levels, Hb concentration, Fe levels and decreased levels of anaemia. Overall, our treatments in groups 2 and 3 significantly decreased the amount of anaemia. The combined intervention, however, showed dramatically decreased amounts of anaemia at 3 months. This supports the previous data by Carmona-Fonsec[24] and Tanumihardjo *et al.*[25], who performed similar studies in children with anaemia and parasitic infection supplemented with vitamin A and albendazole: an increase in Hb was observed.

One interesting finding was an overall decrease in anaemia and increase in Hb in the control group. This could be because

when pre-school-aged children grow, their levels of Hb increase as well, improving their incidence of anaemia based on the diagnostic criteria. Other similar studies[26,27] also observed that age can be an important influencing factor in anaemia. An alternate reason could be the reminder and education on nutrition at the initial interview, which could have influenced the healthier food choices and lifestyle adopted by the parents.

WHO and UNICEF have previously recommended that anthelmintic treatment be included in vitamin A supplementation programmes and administered every 4–6 months because of the practical benefits and cost-effectiveness of the combined supplementation[28]. Our results support this recommendation by demonstrating that, in addition to the efficacy of anthelmintic treatment, there were measurable health advantages to pre-school children by improving serum Hb and the proportion of anaemia when albendazole supplementation was combined with vitamin A supplementation.

### *Effect of administration on serum retinol*

The present study also showed that after sole administration of albendazole, the level of serum retinol of children in group 2 was slightly higher at 3 and 6 months; however, the difference was not significant. Only administration of vitamin A along with albendazole treatment significantly increased the level of serum retinol and improved the proportion of VAD and MVAD, an outcome that was in agreement with our previous findings[12]. Previous animal and human studies have shown that vitamin A and Fe status may affect each other's metabolism[29]. For example, normal Fe metabolism is interrupted during vitamin A depletion, and conversely vitamin A is sequestered in the liver during ID, resulting in depressed plasma retinol concentrations[30]. On this premise, we expected that improvement of Fe status should lead to the improvement of vitamin A status; however, the present data did not support this hypothesis.

There has been little research to explore the effect of albendazole administration on vitamin A status. Results of studies have shown that deworming, along with food-based supplementations, improved the vitamin A status of Filipino and Indonesian children[31,32]. However, the design of these studies did not address the question of whether anthelmintic treatment in the absence of added fat or dietary $\beta$-carotene would improve serum retinol. Haque *et al.*[33] found that anthelmintic therapy had a synergistic effect in improving the vitamin A status, which was given with low doses of $\beta$-carotene supplementation. Treatment only with anthelmintic therapy has an effect on serum $\beta$-carotene concentration but not on serum retinol.

### *Effect of administration on iron status index*

SF was significantly increased by albendazole administration alone after 3 months. However, SF is an Fe-storage and positive acute phase response protein whose concentration increases during inflammation regardless of true Fe status. This increase in SF and Hb levels and decrease in the proportion of anaemia and ID at 3 months after fortification could be attributed to the inflammatory process induced by alberdazole's anthelmintic effect, as well as increased intestinal absorption of Fe from lack of parasites. The result was consistent with several other studies[34,35]; however, other studies showed no significant effect of albendazole on SF[24,36]. We have no definite explanation for these varied findings, but methodological artefacts such as the mode of production of albendazole or the heterogeneity of the subjects could be the reason for these conflicting results. It is possible that the effect of albendazole on SF is dependent on the Fe status and the severity of hookworm infection, which remains to be investigated. However, the combined administration of one albendazole dosage and one mass vitamin A dosage showed a better SF increase and reduced the proportion of anaemia and ID for at least for 6 months.

Compared with sole albendazole supplementation, the present study showed that combined single-dose vitamin A with single-dose albendazole markedly increased the sTfR levels for at least 3 months. Transferrin receptor (TfR) is a transmembrane protein that mediates Fe delivery from the extracellular pool into erythroblasts by receptor-mediated endocytosis and is mostly located in the erythroid precursors in the bone marrow[37]. sTfR concentration is proportional to cellular expression of the membrane-associated TfR and increases with elevated cellular Fe needs and cellular proliferation. In our study, greater erythropoiesis was thought to be most affected by vitamin A, rather than by albendazole as in other studies[37,38].

The conventional markers, SF level and sTfR, are widely used to identify ID and its severity in epidemiological investigations. However, SF and sTfR levels are affected by inflammation and malnutrition status, respectively[39]. Therefore, more reliable methods to assess Fe status are needed to determine the prevalence of ID and the impact of Fe supplementation and fortification trials. Cumulative data[40] have described a new method for assessing Fe status based on the quantitative measurement of body Fe. A close linear relationship was demonstrated between the logarithm of the concentrations in micrograms per litre of sTfR:SF ratio (TFR-F index) and of body Fe expressed as milligrams per kilogram body weight. The latter was expressed as the Fe surplus in stores (positive value) or the Fe deficit in tissues (negative value).

Interestingly, our data indicated that when compared with the placebo-control group albendazole alone increased both TFR-F index and TBIC for at least for 3 months, whereas albendazole plus vitamin A supplementation had a greater effect on TFR-F index and TBIC, regardless of the increased Hb content. Decreased prevalence of anaemia, reduced prevalence of ID, MVAD and VAD and increased SF and TfR levels also occurred. Our previous research and other studies[10,38,41] have indicated that vitamin A administration alone has no significant effect on TFR-F index and TBIC, which suggests that vitamin A plays little role in Fe absorption in the small intestine. Sole administration of albendazole increased Fe absorption by its anthelmintic effect[42,43]; however, albendazole plus vitamin A showed greater effect on Fe absorption due to a complex interaction between albendazole and vitamin A[44,45]. In our study, there was no significant difference in the prevalence of hookworm infections at 3 and 6 months after treatment of children in groups 2 and 3 due to the small sample size of children with hookworm infection.

Another concerning problem is the recurrence of hookworm infection in all of the groups including the ones treated with anthelmintics. This shows that either there was a re-infection or that the parasites were not fully treated during the first treatment. This is a section that will be explored further – that is, on how to fully treat parasitic infection so as to maintain the increased Hb levels, decreased anaemia and raised Fe levels.

### *Strengths and limitations of the present study*

We believe that the present blinded, randomised-controlled trial had many strengths. The three intervention groups were comparable at baseline and the study was blinded, although the success of blinding was not assessed. Children were randomly assigned to an intervention group by an external investigator who had no information regarding potential covariate factors. Owing to low attrition, the results can probably be generalised to children in Western China living under similar socio-economic conditions and dietary hygienic environment.

However, there are some limitations to the present study. First, because of the different appearing styles of the vitamin A capsule, albendazole tablet and placebo, we did not perform a strictly blinded field study, which may have biased our results. However, most of the measured indices were objective indicators, which reduced the impact of bias as much as possible. Furthermore, because of the limited design of the original protocol and the inadequate size of blood samples obtained from these pre-school children, we did not evaluate the effect of the other micronutrients such as thiamine, riboflavin, niacin, folate, Zn or Ca on vitamin A and Fe status. Measuring these micronutrient levels will be an important addition in future studies. There was no overdose response report, because none of the participants took additional vitamin or mineral supplements during the investigation.

In summary, our data indicated that sole albendazole treatment and albendazole plus vitamin A administration showed improvement in anaemia, VAD and ID. Our results suggest that national programmers for anthelmintics treatment should be supplemented with vitamin A at least every 3–6 months. These data may be applicable to other pre-school-aged children living in similar socio-economic environments.

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