The Multiple Vitamin Status of Chinese Pregnant Women with Anemia and Nonanemia in the Last Trimester

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Summary Iron-deficiency or anemia in pregnancy is a major public health problem in China. This cross-sectional study was carried out to observe the association between iron status and multiple vitamin levels of Chinese pregnant women in the third trimester. We measured iron, ascorbic acid, retinol, folate and vitamin B12 in serum, and riboflavin in urine specimens of 1,163 pregnant women in four sites throughout rural and city areas in China. Based on hemoglobin concentrations (Hb), the subjects were divided into an anemia group with Hb <110g/L or Hb <100g/L as severe anemia group, and nonanemia group with Hb >110g/L. Results showed that 41.58% of the population with serum iron <700µg/L and 51.04% of the population with ferritin <12µg/L in the anemia group, percentages that were much higher than those in the nonanemia group. Relationships between five vitamins and hemoglobin concentrations of all subjects were observed. There was a lower level of serum ascorbic acid (291.05µg/dL) in the Hb <100g/L group than in the Hb ≥120g/L group (487.79µg/dL) (p<0.001). Serum levels of vitamin B12 and folate were 445.67pg/mL and 5.94ng/mL in the Hb <100g/L group, whose levels were much lower than the levels of 502.01pg/mL (p<0.012) and 8.07ng/mL (p<0.010) respectively in the Hb ≥120g/L group. Further, cross-sectional analysis showed positive correlations between abnormal hematological results and prevalences of vitamin deficiencies. The subjects with iron-deficiency anemia had much higher rates of vitamin C, folate and vitamin B12 deficiencies than those in the nonanemic subjects, and especially in the deficient rates of ascorbic acid and folate in the anemia (Hb <110g/L) group, which reached 64.04% and 22.70% respectively. Moreover, we observed that the decreasing trends of hemoglobin concentrations were accompanied by the decreases of serum levels of vitamin A, ascorbic acid, folate and vitamin B12. In conclusion, multiple vitamin deficiencies, especially ascorbic acid, retinol and folic acid, may be associated with anemia or iron deficiency in pregnant women in the last trimester. The study suggested that anemic pregnant women in China should be supplemented with iron and multiple vitamins simultaneously.

Key Words anemia, pregnancy, iron, multiple vitamin, China

The World Health Organization estimates that 58% of pregnant women in developing countries are anemic (1). Anemia is still a nutritional problem for pregnant women in China. Many studies showed that the incidence of iron-deficiency anemia was 42% among pregnant women in the third trimester in the Xi’an area (2) and 55% of pregnant women with anemia were found in Jilin’ city of North China in 1997 (3). It is estimated that the prevalence of anemia may still remain high in Chinese pregnant women, especially in rural areas.

Anemia has many possible causes during pregnancy. It has not only been attributed to increased iron requirements, but also associated with deficiencies of micronutrients, especially iron and vitamin C (4). Deficiency of iron in the diet is regarded as the most important factor by far in the etiology of nutritional anemia. Several studies in humans and animals have shown that abnormality of iron metabolism is associated with other relative nutrients. The relationship between vitamin A and anemia among Chinese pregnant women has been analyzed. The serum levels of vitamin A, serum iron, ferritin and transferrin in 40 anemic pregnant women supplemented with the vitamin A for 3 mo were much higher than those in the placebo group (5). A low intake of ascorbic acid was considered to be a factor in accelerating anemia or iron deficiency (6). Supplementation with vitamin C and retinol may increase
iron status as measured by hematological indices (7). The low intakes of iron and vitamin B2 were found in Chinese pregnant women in the second and third trimester, which only accounted for 79.6% and 77.2% of Chinese RNI (recommended nutrition intake) (8). The prevalence of riboflavin deficiency was 36% in anemic pregnant women, which was higher than that in the non-anemic group (9).

However, the limited data above were involved in the relationship between iron status and serum multiple vitamin levels. Some of the studies were carried out in small sample sizes, and mainly based on the investigation of iron and single vitamin intake. The objective of this study was to investigate the multiple vitamin status of pregnant women with anemia or iron deficiency and non-anemia by measuring biochemical indices in the populations of China.

SUBJECTS AND METHODS

Subjects. The study was carried out in November 1999 and April 2000 and involved the examination of 1,163 clinically normal pregnant women aged 20–35 y in the third trimester of pregnancy. The subjects were selected in 4 centers, two rural areas and two developing cities in China. They might geographically differ in variable diets, food supply, living habits and climate and socioeconomic status between different locales. The stages of gestation and estimated date of delivery were calculated from the first day of their last period of menstruation and compared with results of the obstetric examination. All subjects enrolled were healthy pregnant women with no abnormal bleeding and refrained from iron supplementation, smoking and drinking alcohol.

The research was carried out following the ethical standards of the local authorities and informed consent was obtained from every subject.

Blood collection and analysis of iron and vitamins. Specimens of about 5 mL venous blood and 10 mL urine from each subject were taken on the same day as the antenatal examination, and stored on ice for transport to the laboratory. Hemoglobin concentration was measured and serum was separated from blood by centrifugation at 2,000 × g for 15 min at room temperature on arrival in each center. Serum samples were separately stored at −80°C in the dark and then transported to one center by plane for analysis of serum iron (SI), serum ferritin (SF), serum vitamin A, ascorbic acid, riboflavin, vitamin B12 and folate.

Blood hemoglobin concentration was analyzed by using a semi-automatic biochem-analyser (Sysmex F-820, China). A standard hemoglobin-cyanide solution was used in four centers as the quality control of hemoglobin measurements. Measurements of serum ferritin were performed by radioimmunoassay (10), as described by the manufacturer (The North Biol. Tec. Institute, Beijing, China). The criteria for a diagnosis of anemia was Hb <110 g/L. SF <12 µg/L and SI <500 µg/L, which can be considered abnormal.

Vitamin A in serum was determined by the fluorescent spectrometry method (RF-540, Japan) (11) and ascorbic acid in serum by the 2, 4-dinitrophenylhydrazine method (12, 13), and deficient ranges were vitamin A <20 µg/dL (0.70 µmol/L) and ascorbic acid <400 µg/dL respectively; serum level of riboflavin was determined by a ratio of riboflavin/creatinine (Cr) and marginal deficiency of riboflavin/Cr was <80 µg/g in urine (14). Folic acid and vitamin B12 in serum were measured by radioimmunoassay method (Diagnostic Products Corporation DPC, USA) and the deficiency of folic acid and vitamin B12 were <3.0 ng/mL and <200 pg/mL (15).

Statistical analysis. The significance of differences was determined by Independent Samples t-test and by the Chi-square (χ²) test. The SPSS (10) of statistical software was used.

RESULTS

Levels of the five vitamins in serum and urine were measured and compared between the two groups of Hb

| Indices          | Hemoglobin concentration (Hb) of the groups |
|------------------|---------------------------------------------|
|                  | ≤100 g/L          | 101–119 g/L  | ≥120 g/L     | p<   |
| Retinol (µg/dL)  | 51.9±17.4a        | 62.3±16.1    | 64.1±14.8    | <0.001|
| (n)              | (328)             | (393)        | (379)        |      |
| Vitamin C (µg/dL)| 291.1±246.8      | 360.3±292.6  | 487.8±369.2  | <0.001|
| (n)              | (346)             | (401)        | (416)        |      |
| Vitamin B12 (pg/mL) | 445.7±257.8   | 465.7±264.7  | 502.0±326.6  | <0.05 |
| (n)              | (311)             | (343)        | (367)        |      |
| Folate (ng/mL)   | 5.9±4.2           | 6.8±6.5      | 8.1±7.2      | <0.01 |
| (n)              | (318)             | (401)        | (406)        |      |
| Vitamin B2/Cr (µg/g) | 126.8±153.78   | 161.3±163.8  | 182.5±215.4  | >0.05 |
| (n)              | (281)             | (334)        | (402)        |      |

*a Means±SD. b Number of subjects tested. c p values are the comparisons between the five groups of Hb ≤100 g/L and ≥120 g/L by analysis of variance.
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In Table 1, the serum retinol levels were lower in the Hb ≤100g/L group (291.05 μg/dL) than in the Hb ≥120g/L group (487.79 μg/dL) (p<0.001). Serum levels of vitamin B12 and folate were 445.67 pg/mL and 5.94 ng/mL respectively in the Hb ≤100g/L group, which were much lower than the levels of 502.01 pg/mL and 8.07 ng/mL in the Hb ≥120g/L group (p<0.012, p<0.01). However, there was no significant difference of vitamin B2/Cr in urine between the Hb ≤100g/L group and the ≥120g/L group (p>0.05).

Abnormal hematological values associated with the prevalence of vitamin deficiencies were shown in Table 2. There were 41.58% of the subjects with serum iron<700 μg/L in the anemic (Hb <110g/L) group, which was higher than that in the nonanemic group (Hb ≥110g/L). The rate of iron depletion in the pregnant women was also much higher in the anemic group than the nonanemic group. The higher deficiencies of serum vitamin C, folate and vitamin B12 were found in the anemic group than those in the nonanemic group, and especially the deficient rates of ascorbic acid and folate in the anemia population reached to 64.04% and 22.70%, respectively.

Changeable trends of serum levels of five vitamins associated with hemoglobin concentrations were shown in Fig. 1. The curve of serum vitamin C in Fig. 1(a) was gradually rising accompanied by increasing hemoglobin concentration. The serum levels of retinol, vitamin B12, folate and riboflavin/Cr increased along with increasing hemoglobin concentration from 70g/L to 150g/L as shown in Fig. 1(b), (c), (d), (e), respectively.

**DISCUSSION**

Anemia is a public health problem and its common type is iron-deficiency anemia both in Chinese pregnant and nonpregnant women. It was reported that there were 28.25% and 25.82% of nonpregnant women in cities and rural areas respectively (16), while a prevalence of anemia in pregnant women is even more severe. There was a higher incidence of anemia in pregnant women and 44.3% subjects tested with iron-deficient anemia in this study. The reasons for the prevalence of anemia in pregnant women are not only related to a greater requirement of iron due to an expanded red blood cell volume, the needs of the fetus and placenta and blood loss at delivery (17), but also poor iron intake or low bioavailability (<10%) from a traditional Chinese diet (65-80% vegetables) (18). The bioavailability of nonheme iron was considered to be dependent on the interaction of the promotors and the inhibitors of iron absorption from diets (19, 20). The two most common inhibitors of nonheme iron absorption are polyphenoles and phytates, whose presence in foods such as cereals and legumes have shown a marked decrease in the bioavailability of dietary iron. Iron absorption decreased progressively when the intake of phytate phosphorous (from 10 to 58 mg) from maize bran was increased. However, the inhibitory effect was overcome by 30 mg of ascorbic acid, and so it was suggested that more than 50 mg of ascorbic acid would be required to overcome the inhibitory effects on iron absorption of any meal containing >100 mg tannic acid (21). In our study, low levels of serum vitamin C occurred both in the anemia and nonanemia populations, and vitamin C marginal deficiency was more severe in pregnant women with anemia than nonanemia. During pregnancy, serum vitamin C progressively decreased by 50%, partly because of the extra consumption by the fetus and partly because of hemodilution (22). Moreover, our investigation was carried out during the winter from September to April in order to limit the subjects’ intake of green vegetables and ascorbic acid. In addition, the standard Chinese diet contains a lot of the inhibitors of iron absorption and is commonly stir-fried, and fresh fruits are seldom eaten with a meal (18). As the enhancing effect of ascorbic acid on the absorption of nonheme iron is well known (23), iron supplementation with ascorbic acid is expected to be more effective (24).

Vitamin A deficiency occurs when the intake of dairy products and carotene-rich vegetables and fruit is limited. The prevalence of vitamin A deficiency in this study was very high and about 14% of pregnant women had a serum retinol concentration of <20 μg/L.

### Table 2. Abnormal hematological values and prevalence of marginal deficiencies of vitamins in pregnant Chinese women in the last trimester.

| Indices             | Abnormal range of index values | Hb <110g/L | Hb ≥110g/L | Total |
|---------------------|-------------------------------|------------|------------|-------|
|                     | (n)¹ | % | (n) | % | (n) | % |
| Serum iron          | <700 μg/L | (505) | 41.58 | (515) | 30.49 | (1,020) | 35.98 | <0.001 |
| Ferritin            | <12 μg/L | (531) | 51.04 | (584) | 38.18 | (1,115) | 44.30 | <0.001 |
| Vitamin C           | <400 μg/dL | (570) | 64.04 | (593) | 56.49 | (1,163) | 36.89 | <0.010 |
| Retinol             | <20 μg/dL | (545) | 13.76 | (555) | 3.06 | (1,100) | 8.36 | <0.001 |
| Vitamin B12         | <200 pg/mL | (507) | 12.62 | (512) | 8.40 | (1,019) | 10.50 | <0.030 |
| Folate              | <3.0 ng/mL | (551) | 22.70 | (573) | 16.90 | (1,124) | 19.80 | <0.003 |

¹ n values in parentheses are number of subjects. ² p values are comparisons between the anemic group (Hb <110g/L) and nonanemic group (Hb ≥110g/L).
The figures of (a)-(e) show distribution of mean levels of five serum vitamins to hemoglobin concentrations in all subjects tested. The rate of folate deficiency was found to be about 22.7% in the anemic pregnant women compared to 16.9% in the nonanemic pregnant women, and was accompanied by other vitamin deficiencies in this study (Table 2). The reason may be primarily a result of dietary folate insufficiency. Folate supplementation to iron was able to decrease iron deficiency anemia in pregnancy (32). Routine folate, iron and folate and iron supplementation results in a substantial reduction of women with hemoglobin levels <100 g/L in late pregnancy (33–35). In the case of pregnant women, a daily dose of one tablet containing 60 mg iron and 400 μg folic acid has been recommended for all except those who are severely anemic (36). Although vitamin B₁₂ deficiency was very uncommon (37), serum vitamin B₁₂ concentration of pregnant women with anemia was much lower than that in the nonanemic population in this study. The independent effect of vitamin B₁₂ deficiency had been examined on hematological indices in older Chinese vegetarian women using a cross-sectional study design. Vitamin B₁₂ deficiency defined by serum vitamin B₁₂ and meth-

Vitamin A binds iron liberated during the digestive process and forms a complex that acts as a chelating agent preventing the inhibitory effect of phytate and polyphenols on nonheme iron absorption (28). Vitamin A may inhibit erythropoiesis directly (30) or through inhibition of iron mobilization or transport (31).
ylmalonic acid (MMA) was associated with a decrease in hemoglobin concentrations by up to 0.9 g/dL, but it was not associated with an increase in mean corpuscular volume (MCV). Serum MMA but not vitamin B12 levels correlated inversely with hemoglobin and platelet counts and positively with MCV, after adjustment of confounding factors. So anemia associated with vitamin B12 deficiency was seldom macrocytic (38).

While iron deficiency is regarded as the major cause of nutritional anemia, changes in vitamins A, B12, C and E, folic acid and riboflavin status have also been linked to its development and control. Our study showed that iron status was related to levels of vitamins in serum, which suggested that vitamin C, retinol or carotene, folate, vitamin B12 might be main causes to promote iron absorption and decrease the rate of anemia in pregnant women in the last trimester. The anemic pregnant women should increase their daily intake of heme food or take multiple vitamins and iron supplementation. Multiple vitamin supplements may raise hemoglobin (Hb) concentration, but few studies have isolated the effect of multiple vitamins from iron on hematological status. Further research is needed to understand the roles of individual and combined vitamin deficiencies on anemia to design appropriate micronutrient interventions to prevent anemia (39).

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