

## Original Article

# Comparison of serum levels of iron, zinc and copper in anaemic and non-anaemic pregnant women in China

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Zinc and copper deficiency is associated with anaemia or iron deficiency and affects fetus growth and pregnant women during pregnancy. To examine iron, zinc and copper status of Chinese pregnant women with and without anaemia in the third trimester, 1185 subjects were enrolled for measurements of Hb, ferritin, transferrin, soluble transferrin receptor (sTfR), and serum iron, zinc and copper. The results showed that there were lower levels of ferritin (14.1 µg/L) and transferrin (3.33 g/L) in subjects with Hb≤100g/L as compared with subjects with Hb≥101g/L. sTfR levels in subjects with Hb≤100g/L were significantly higher than those in subjects with Hb≥120 g/L (38.5 nmol/L vs. 25.04 nmol/L,  $P<0.001$ ). Serum iron was lower in subjects with Hb≤100g/L than those with Hb≥120 g/L (871µg/L vs. 990 µg/L,  $P<0.01$ ). Lower levels of serum iron and zinc were also found in anaemic (Hb<110g/L) as compared with non-anaemic women (Hb≥110g/L). Frequencies of marginal deficiencies in serum iron and zinc were 41.58% and 51.05% respectively higher in anaemic than in non-anaemic subjects. Distribution of serum zinc and iron showed a decreasing trend as Hb decreased. Few anaemic as well as non-anaemic subjects had copper deficiency although copper and Hb levels were found inversely correlated and the ratio of copper/iron was higher in anaemic than in non-anaemic group. In conclusion, a lower level of serum zinc in anaemic pregnant women might be related to anaemia and iron deficiency during pregnancy. Therefore, combined zinc and iron supplementation should be recommended to Chinese pregnant women, especially those with anaemia.

**Keyword:** anaemia, pregnancy, iron, zinc, copper, Qingdao, China

## Introduction

Minerals have important influences on the health of pregnant women and the growing fetus. Iron deficiency results in anaemia, which may increase the risk of death from hemorrhage during delivery.<sup>1</sup> Zinc deficiency is common in developing countries.<sup>2</sup> It has been estimated that 82% of all pregnant women worldwide suffer from zinc deficiency.<sup>3</sup> Maternal zinc deficiency is a public health problem because zinc has an important role in the expression of genetic potential, nuclear acid metabolism, and protein synthesis.<sup>4,5</sup> Zinc is therefore critical for fetal growth. Deficiencies in other minerals such as copper and calcium have also been associated with complications of pregnancy and abnormal fetal development.<sup>6</sup> Multi-mineral interactions of iron-zinc-copper have been reported. A high concentration of iron can interfere with zinc uptake when no dietary ligands are present. High iron intakes may also interfere with copper absorption when both are taken simultaneously. Modest increase in zinc intake may have positive effect on immune functions but higher amounts can interfere with copper and iron absorption, which in turn can adversely affect immune functions.<sup>7</sup>

A recent study examining serum copper, iron, and zinc levels in Chinese subjects has been reported.<sup>8</sup> However, the study involved only 70 gravidas and 45 unpregnant

women. To date, no large scale studies have been performed to investigate the mineral status of pregnant women in China. The present study was therefore carried out to determine serum iron, zinc, and copper levels in women with and without anaemia during the third trimester of their pregnancy.

## Subjects and methods

### Subjects

1185 clinically normal pregnant women, aged 20-35 y, were examined during the third trimester of their pregnancy. All subjects had no abnormal bleeding, no iron and other mineral supplements, and attended the clinics regularly for prenatal care. They were enrolled in a random manner in each clinic and can therefore be regarded as a random sample of all pregnant women attending the clinic. Subjects were from two rural areas and two developing cities in China. Pregnant women in the cities were mainly

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**Table 1.** Iron status of pregnant women with anaemia and nonanaemia in China<sup>1</sup>

	Hb≤100		101 – 119		Hb≥120		P
	N	Mean ± SD <sup>1</sup>	N	Mean ± SD	N	Mean ± SD	
Haematocrit (l/L)	342	29.8 ± 4.82 <sup>a</sup>	334	33.0 ± 3.76 <sup>b</sup>	385	37.8 ± 4.93 <sup>c</sup>	
Serum iron (µg/L)	301	871 ± 0.48 <sup>a</sup>	382	1080 ± 0.75 <sup>b</sup>	347	990 ± 0.54 <sup>c</sup>	0.000
Ferritin (µg/L)	316	14.1 ± 10.53 <sup>a</sup>	402	18.8 ± 13.52 <sup>b</sup>	407	19.34 ± 18.63 <sup>bc</sup>	0.000
Transferrin (g/L)	351	3.33 ± 0.44 <sup>a</sup>	406	3.37 ± 0.42 <sup>ab</sup>	428	3.46 ± 0.46 <sup>c</sup>	0.000
sTfR (nmol/L)	43	38.5 ± 22.60 <sup>a</sup>	90	25.19 ± 9.99 <sup>b</sup>	23	25.04 ± 7.87 <sup>bc</sup>	0.000

<sup>1</sup>Values (mean±SD) within rows with different superscript letters are statistically different at  $P < 0.05$  using one-way ANOVA.

from the middle socio-economic class whereas subjects in the rural areas were from the lower class. The study was carried out in accordance with the ethical standards of the local authorities. Informed consent was obtained from all subjects.

### Sample collection and methods

Fasting venous blood samples from 1185 subjects (5 ml/person) were taken at antenatal examination during 7 to 9 o'clock in the morning.<sup>9</sup> Samples were stored on ice for transport to the laboratory. Haematocrit and haemoglobin concentrations were measured. Serum was separated from blood by centrifugation at  $2000 \times g$  for 15 min at room temperature upon arrival. Serum samples were then stored separately at  $-20^{\circ}C$  in the dark and transported to the laboratory for analyses of ferritin, transferrin, serum iron, zinc, copper and sTfR.

Serum sTfRs were measured by enzyme-linked immunosorbent assay using a commercial kit (R&D Systems, Minneapolis, USA). The concentrations of test samples were determined in a Bio Rad Microplate Reader (model 3550: Richmond, CA) set at 450 nm. To test the reliability of sTfR kits, three control serum samples were assayed in each experiment.

Haemoglobin concentrations were measured with the cyanomethaemoglobin method and haematocrit with the micromethod. A standard haemoglobinocyanide solution was used for quality control of haemoglobin measurements. Measurement of serum ferritin was performed by radioimmunoassay,<sup>10</sup> as described by the manufacturer (The North Biol.Tec. Institute, Beijing, China). Transferrin (TRF) was determined by using a commercially available kit (Yadu Biotech Co. Shanghai, China). Levels of serum zinc, iron and copper were determined by atomic absorption spectrophotometry (PE 3100, USA). Iron status was assessed by using multiple criteria. Abnormal values are classified as: Hb < 110 g/L, HCT

(haematocrit) <33%, SF <12 µg/L and TRF <2.1 g/L. Marginal deficiency of serum nutrients was defined as serum iron <700µg/L, serum zinc <700µg/L, and serum copper <700µg/L.

### Statistical analysis

Data were cleaned by visual and logical checks and analyzed by using the SPSS program (10.0 version). Ratios of serum zinc/iron or zinc/copper were obtained by dividing individual serum zinc with iron or copper. Differences in biochemical parameters including haematocrit, serum iron, ferritin, transferrin and sTfR among three groups of subjects (Hb≤100g/L, Hb 101-119g/L, Hb≥120 g/L) were analyzed by one-way ANOVA (F-test); serum zinc, copper and iron in two groups of subjects (Hb<110g/L, Hb ≥110 g/L) were examined by Independent Samples *t*-test. Statistical significance was accepted at a probability level of 0.05. Data were expressed as mean ± SD.

### Results

1185 subjects were divided into three groups based on their haemoglobin concentrations. Iron status of pregnant women was evaluated using five parameters including haematocrit, serum iron, ferritin, transferrin and sTfR. Comparisons of these parameters among three groups of subjects (Hb ≤ 100g/L, 101-119g/L, and ≥120 g/L) are shown in Table 1. Levels of serum iron in Hb ≤ 100g/L and Hb ≥ 120 g/L were 871µg/L and 990µg/L ( $P < 0.05$ ) respectively. There were low levels of ferritin (14.1 µg/L) and transferrin (3.33 g/L) in subjects with Hb ≤ 100g/L as compared with other groups. sTfR levels of 156 subjects were examined. A higher value of sTfR with 38.5 nmol/L was found in Hb ≤ 100g/L than 101-119g/L and Hb ≥ 120 g/L groups ( $P < 0.001$ ), while there was no difference between 101-119g/L and Hb ≥ 120 g/L ( $P > 0.05$ ).

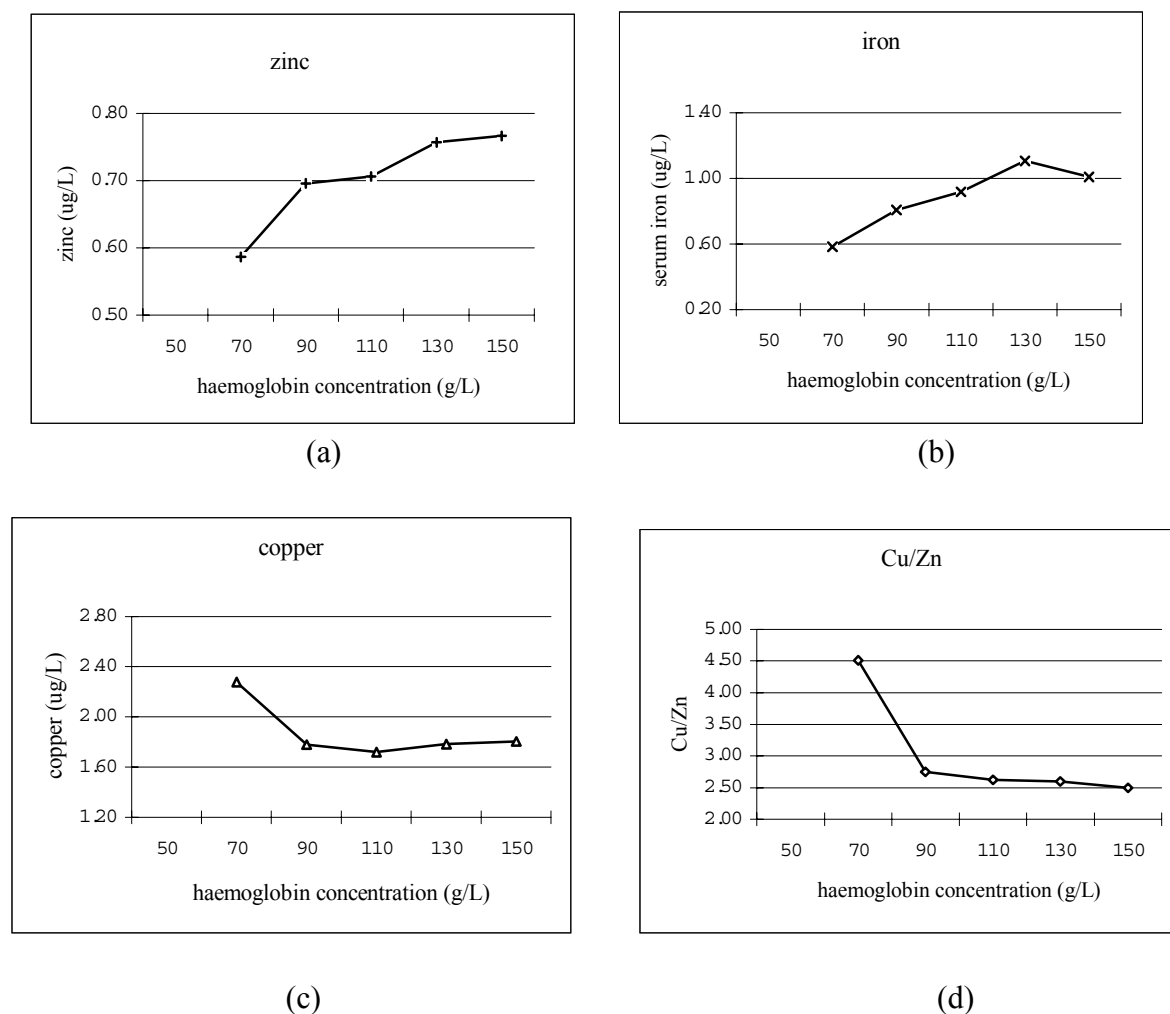
**Table 2.** Comparison of three serum minerals between Hb <110 and ≥ 110 g/L

	Hb<110		Hb≥110		P
	N	Mean ± SD <sup>1</sup>	N	Mean±SD	
zinc (µg/L)	523	701.8 ± 220.6	555	719.0 ± 252.0	0.000
iron (µg/L)	509	888.6 ± 498.2	521	1087.3 ± 700.1	0.000
copper (µg/L)	520	1733.3 ± 573.8	550	1788.3 ± 539.8	0.107
Cu/Zn	508	2.67 ± 1.16	545	2.57 ± 1.11	0.146
Cu/Fe	488	2.44 ± 1.51	510	2.15 ± 1.306	0.001

**Table 3.** Frequencies of marginal deficiency of iron, zinc and copper in anaemia<sup>1</sup> and nonanaemia

	Anaemia		Non-anaemia		Abnormal range	P
	N	%	N	%		
Haematocrit	536	77.99	555	19.62	<33%	0.000
Ferritin	531	51.04	521	38.18	<12µg/L	0.000
Serum iron	505	41.58	550	30.49	<700µg/L	0.000
Serum zinc	523	51.05	545	45.05	<700µg/L	0.048
Serum copper	520	4.23	510	1.64	<700µg/L	0.011

<sup>1</sup>Hb<110g/L as anaemia, Hb≥110g/L as nonanaemia



**Figure 1.** (a) distribution of serum zinc to haemoglobin concentrations; (b) distribution of serum iron to haemoglobin concentrations; (c) distribution of serum copper to haemoglobin concentrations; (d) distribution of serum Cu/Zn to haemoglobin concentrations.

Serum levels of iron in 1030 subjects, zinc in 1078 subjects, and copper in 1070 subjects were shown in Table 2. Mean serum zinc was 701.8µg/L and serum iron was 888.6µg/L in the anaemic group, which were much lower than those in the non-anaemic group ( $P < 0.001$ ). There was no difference in mean serum copper levels between anaemic and non-anaemic groups ( $P > 0.05$ ). The ratio of copper/iron was higher in anaemic than in non-anaemic subjects ( $P < 0.001$ ).

Frequency of abnormal haematologic results and serum mineral concentrations are shown in Table 3. Based on ferritin levels, iron depletion in anaemic subjects was more common (51.04%) than those without

anaemia (38.18%). Frequencies of marginal deficiencies of serum iron and zinc were higher (41.58% and 51.05%) in anaemic than non-anaemic (30.49% and 45.05%) women. Notably, few anaemic as well as non-anaemic subjects had copper deficiency.

Figure 1 showed the changeable trends of serum zinc, iron and copper with the distribution of haemoglobin concentrations. Serum zinc and iron levels were increased as Hb increased (Fig. 1a & 1b). The marginal deficiencies of iron and zinc kept below the level of Hb <90g/L. Nevertheless, serum copper levels and the copper/zinc ratio were decreased as Hb increased (Fig. 1c and 1d). There was a steep decrease of copper at the lower level of

Hb value from 70-90 g/L, while a steep increase was observed with zinc and iron.

### Discussion

The present study, which involved a large population of Chinese pregnant women, showed that there were iron and zinc deficiencies in anaemic women in the third trimester of their pregnancy. Consistent with reported findings<sup>11,12</sup> some biochemical indices for trace elements fall in parallel with red blood cell volume, and serum concentrations of zinc and iron were positively correlated with maternal haemoglobins. The positive correlation of haemoglobin concentrations with zinc and iron indicates that deficiencies of the two minerals were common and more severe in anaemic pregnant women. Iron and zinc deficiencies in pregnant women may result from an expanded blood volume, an increased need for zinc and iron, and also likely, poor intake and poor bio-absorption of iron and zinc. Although the average iron intake (23.52 mg/d) by anaemic women was close to the Chinese RDA (28 mg/d) for pregnant women, traditional Chinese vegetable diets may result in low iron bio-absorption.<sup>13</sup> The daily intake of zinc by anaemic subjects reached 19.83 mg/d. However, serum zinc levels in these people were very low. The high frequencies of marginal zinc deficiency (<700µg/L) in both anaemic (51.05%) and non-anaemic (45.05%) groups indicate that zinc deficiency is common in Chinese pregnant women. Several dietary factors are known to affect zinc absorption as a result of physico-chemical interactions in the intestine. Phytate, a component in plants with the highest concentration found in seeds (cereal grains/legumes/nuts), inhibits zinc absorption, as does calcium. Physiological states, such as pregnancy and lactation, increase the demand for absorbed zinc.<sup>14</sup> On the contrary, dietary proteins enhance zinc absorption. The molar ratio of phytate to zinc (P:Z) in diets has been used to estimate the absorption efficiency of zinc, with P:Z<5 being associated with relatively high absorption of zinc, P:Z 5-10 with moderate absorption, and P:Z >15 with low absorption.<sup>15</sup>

Serum copper concentrations were in inverse relationship with maternal haemoglobin concentrations. Although frequencies of marginal copper deficiency were low in both anaemic (4.23%) and nonanaemic (1.64%) groups, copper deficiency has deleterious effects on pregnancy and therefore should not be neglected. On the other hand, high copper intake can decrease zinc absorption by competing with zinc at absorption sites.<sup>16</sup> Iron deficiency during pregnancy is common and has serious short and long term consequences such as fetal growth retardation and cardiovascular problems in the adult offsprings. In our study, we noted a higher ratio of copper/iron (2.44) in anaemic (Hb <110g/L) than in non-anaemic subjects (Hb ≥110 g/L). The higher copper/iron ratio in anaemic subjects may be attributed to the fact that iron deficiency increases copper levels in the maternal liver, serum and placenta, although it has much less effect in the fetal serum and liver. Apart from maternal ceruloplasmin, mRNA levels of copper-regulated proteins are not changed. Copper oxidase, which is believed to fulfil the function of hephaestin in placenta, is regulated by copper as well as by iron.<sup>17</sup> Our findings are consistent with those

of Huang *et al.*,<sup>18</sup> that hair concentrations of copper, zinc, iron and calcium in the gravidas in all three trimesters were significantly lower than those in non-pregnant women in Tianjin, China.

During pregnancy, nutrients are transferred from mother to fetus across the placenta. To compensate for iron deficiency, proteins involved in iron transfer, such as the transferrin receptor, are upregulated. Serum transferrin receptor (sTfR) has thus been used as an indicator for early tissue iron deficiency. Unlike ferritin, sTfR concentrations are not affected by conditions such as inflammation and are therefore considered a better parameter for determining the iron status.<sup>19</sup> sTfR concentrations have been shown to be associated with serum iron, serum ferritin and haemoglobin.<sup>20</sup> In our study, 50.5% of pregnant women were found anaemic (Hb <110 g/L), among whom 51.0% had depleted iron stores reflected by low ferritin concentration (<12 µg/L). sTfR values in severe pregnant women (Hb <100 g/L) were 1.53 times higher than other subjects (Hb 101-119g/L and ≥120 g/L). There was no difference in sTfR levels within the latter group. The findings suggest that severe anaemia may induce sTfR, whereas moderate anaemia (Hb 101-110g/L) had no such obvious effect. Elevation of sTfRs resulting from severe iron deficiency has also been reported in pregnant women in the later part of pregnancy.<sup>21</sup> Hence, sTfR assay may be a better test for ill and hospitalized patients in whom, despite having iron deficiency, ferritin levels are normal or elevated.<sup>22</sup>

Prophylactic iron supplementation is recommended in developing countries for all pregnant women in the second and third trimesters of pregnancy. In other countries, such as Great Britain, iron supplementation is recommended only to anaemic women with diagnosed iron deficiency.<sup>23</sup> Safety issues related to iron supplementation have been raised.<sup>24</sup> Zinc supplementation during pregnancy is recommended by the Institute of Medicine (IOM) in USA although IOM concluded that there was insufficient evidence to support the supplementation.<sup>25</sup> The high frequencies of marginal zinc deficiency (<700µg/L) in both anaemic (51.05%) and non-anaemic (45.05%) groups observed in our study indicate that zinc deficiency is common in Chinese pregnant women. Our findings thus provide strong evidence that necessitates zinc supplementation for pregnant Chinese women. Prophylactic doses of 20-25 mg elemental zinc/d have generally been used in pregnant women in developing countries.<sup>26</sup> The latest survey in China showed that the daily intake of zinc by anaemic pregnant women (19.83 ± 10.02 mg/d) and non-anaemic pregnant women (18.39 ± 8.49 mg/d) was lower than the generally recommended dosage. So zinc or combined iron and zinc supplementation for pregnant women should be considered in China.<sup>13</sup> Copper deficiency has not been observed, supplementation in pregnancy is therefore not recommended.<sup>25</sup> On the other hand, when zinc supplements are given to individuals with low copper intakes, a copper supplement should be given to compensate for the zinc-copper interaction.<sup>27</sup>

In conclusion, the present study revealed the unbalanced nutritional status of iron, zinc and copper in Chinese pregnant women. To decrease the adverse

outcomes of mineral malnutrition and to improve fetal growth and the health of pregnant women, further efforts should be made to develop and implement an appropriate mineral supplementation regime for Chinese pregnant women.

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