Original Article

Micronutrient status in anemic and non-anemic Chinese women in the third trimester of pregnancy

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Background: Anemia is a major nutrition related problem in China. In addition to iron deficiency this may be due to deficiencies of other micronutrients. Objective: To describe the micronutrient status of anemic and non-anemic pregnant women in China. Subjects and Methods: 734 clinically normal pregnant women in the third trimester aged 20-35, were randomly recruited from the population of pregnant women regularly receiving pregnant examination in community medical centers. Serum concentrations of vitamins A, B_{12} and C, iron and zinc status parameters, and vitamin B_2 in urine were determined. Subjects were categorized according to the presence or absence of anemia and compared according to micronutrient status. Results: Serum concentrations of iron and micronutrients were significantly lower in anemic women than non-anemic women: serum iron 909 µg/L versus 1109 µg/L, ferritin 13.8 µg/L versus 19.6 µg/L, vitamin C 308.9 µg/L versus 388.1 µg/dL, and retinol 50.0 µg/dL versus 59.3 µg/dL. Zinc concentrations were also lower in anemic women. Subnormal serum iron (<700 µg/L) and iron depletion (ferritin <12 µg/L) were 39.7% and 52.6%, significantly more frequent in anemic than 23.9% and 35.0% in non-anemic subjects, as were subnormal vitamin A and ascorbic acid. Subnormal vitamin B_2 and B_{12} were frequent in both anemic and non-anemic groups. Conclusion: Subnormal concentrations of iron and micronutrients in combination may contribute to this situation. Further studies on food-based or supplement-based approaches trying to increase intake of iron and certain vitamins are warranted to decrease anemia in pregnant Chinese women in the third trimester.

Key Words: anemia, pregnancy, micronutrient, vitamin, iron

INTRODUCTION

Anemia in pregnancy is a common and worldwide problem that deserves more attention. In many developing countries, its prevalence is reported even as high as 75%. Often, anemia is severe in these situations, contributing significantly to maternal mortality and morbidity [1] and to low birth weight as well. [2] Anemia is also a major nutrition related problem among pregnant women in China. Prevalence of anemia differs in different areas of China. Some studies show that the prevalence of anemia during pregnancy is 10% to 20%. Others suggest anemia prevalence to be 42% among pregnant women in the third trimester in Xi'an city,[3] and 55% in Jilin city in 1997. It was hypothesized that the main probable cause was an unbalanced diet that lacks protein, iron and certain vitamins.[4]

Anemia during pregnancy has been attributed not only to increased iron requirements during the second and the third trimester of gestation,[5] but also to micronutrient deficiency. Deficiencies of iron and vitamin A were among the major contributory factors. [6] Several studies in humans and animals have shown that iron deficiency is accompanied with other micronutrient deficiencies like vitamin A and ascorbic acid.[7,8] Studies also have shown that supplementation with these vitamins may improve iron status as measured by hematological indices.[9] However, data on iron status and multivitamin levels in pregnant women with anemia in China are insufficient. In this study, we assessed and compared the micronutrient status of pregnant women with anemia and those without anemia.

SUBJECTS AND METHODS

Subjects

This cross-section study was designed and conducted between November 1999 and April 2001. Seven hundred

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and thirty four clinically normal pregnant women aged 20-35 and in the third trimester of pregnancy were randomly recruited from four sites in Gansu, the northwest of China; Guangxi, the southwest, Shandong, the northeast and Fujian, the southeast of China for hematologic and micronutrient measurements. The subjects were healthy pregnant women who did not experience abnormal bleeding, did not smoke or drink any alcoholic beverages, and had taken no dietary supplements for the past 2 months.

This study was approved by the Research and Ethics Committee of the Institute of Human Nutrition, Medical College of Qingdao University. The informed consent was obtained from all subjects prior to the trial.

Sample collection and biochemical analysis

Approximately 5ml of venous blood and 10 ml of urine samples were taken on the day of the prenatal examination, and stored in ice for transport to the local laboratories of the four sites. Hematocrit and hemoglobin concentrations were measured in heparinized blood. Serum was separated from the remainder of the blood by centrifugation at 2000 × g for 15 min at room temperature upon arrival. Serum samples were stored separately at -80 °C in the dark and transported by air or train to the laboratory of the Institute of Human Nutrition, Medical College of Qingdao University for analyses of ferritin, vitamin A, ascorbic acid, riboflavin, vitamin B_{12} and folate.

Hemoglobin concentration was measured by the cyanomethemoglobin method and hematocrit by the micromethod. A standard hemoglobin cyanide solution was used for quality control of hemoglobin measurements. Measurements of serum ferritin were performed by radioimmunoassay[10] as described by the manufacturer (The North BiolTec Institute, Beijing, China). Transferrin (TRF) was determined by a commercially available kit (Yadu Biotech Co. Shanghai, China). Serum retinol concentrations were measured by reversed-phase high-performance liquid chromatography (HPLC) (Beckman 5000 with detector of 168, USA)[11] and the within-assay and between assay CVs were 3% and 8%, respectively. The nutritional status of riboflavin was determined by the ratio of urine riboflavin/creatinine, and the erythrocyte glutathione reductase activity coefficient (EGRAC) was measured for assessing riboflavin status.[12] Urinary riboflavin was measured by fluorometric procedures. Under conditions of adequate intake, the amount excreted per day is more than 80 μg per gram of creatinine. Folic acid and vitamin B_{12} were measured by radioimmunoassay method (Diagnostic Products Corporation DPC, USA).[13] Serum concentrations of iron, zinc and copper were measured by 710-ES ICP Optical Emission Spectrometer (Varian Medical System, USA).

Based on the report of the International Anemia Consultative Group,[14] the criterion for a diagnosis of anemia was Hb<110 g/L. The following results were considered abnormal: hematocrit <33%, ferritin <12 μg/L, transferrin <2.1 g/L. The following values were considered subnormal: serum iron <700 μg/L, vitamin A <30 μg/dL, ascorbic acid <400 μg/dL, riboflavin/creatinine <80 μg/g in urine, folic acid <3.0 ng/mL and vitamin B_{12} <200 pg/mL.[15]

Statistical analysis

The significance of differences was determined by independent samples t-test and the chi-square test. The Statistical Package of Social Sciences (SPSS) version10.0 was used for statistical analysis. The percentiles distributions of serum iron, ferritin, folic acid, retinol, ascorbic acid and vitamin B_2 were compared between anemic and non-anemic women. Two-sided p values <0.05 were considered statistically significant.

RESULTS

Results with respect to iron status in Table 1 shows that there were significant differences in terms of serum hemoglobin (-24%), ferritin (-30%), transferrin (-6%) and serum iron (-18%) between anemic and non-anemic pregnant women. Mean concentrations of serum vitamin C (-20%), zinc (-5%), retinol (-16%) and vitamin B_2 in urine (-21%) in the anemic group were significantly lower. There were no significant differences of serum copper levels, vitamin B_{12} and folate.

The frequency of subnormal serum iron and micronutrients is shown in Table 2. Prevalence of subnormal serum iron (<700 μg/L) and iron depletion (ferritin <12

Table 1. Iron status and micronutrient levels in anemic and non-anemic pregnant women

Items	Hb<110g/L		Hb≥110g/L		Difference (%)	p
	n	mean±SD	n	mean±SD		
Haemoglobin (g/L)	403	97.0±8.5	331	128±11.7	-24.0	0.000
Haematocrit (l/L)	403	30.9±5.1	331	37.1±5.3	-16.7	0.000
Ferritin (μg/L)	403	13.8±9.2	331	19.6±16.0	-29.6	0.000
Transferrin (g/L)	403	3.3±0.5	331	3.5±0.5	-5.7	0.000
Retinol (μg/dL)	403	50.0±15.6	331	59.3±13.9	-15.7	0.000
Vitamin C (μg/dL)	403	301±259	331	388±319	-20.4	0.001
Vitamin B_{12} (pg/mL)	403	440±274	331	433±256	1.4	0.749
Folate (ng/mL)	403	5.9±5.8	331	6.0±5.2	-1.7	0.914
VitaminB_2/creatinine (μg/g)	403	131±133	331	165±192	-20.5	0.005
Iron (μg/L)	403	909±480	331	1109±749	-18.0	0.000
Zinc (μg/L)	403	707±197	331	745±214	-5.1	0.012
Copper (μg/L)	403	1759±543	331	1815±499	-3.1	0.149

Table 2. Prevalence of subnormal micronutrients in anemic and non-anemic women

Items	Subnormal range	Anemia		Non-anemia		Total		p
		n	%	n	%	n	%	
Serum iron	<700 μg/L	160	39.7	97	29.3	257	35.0	0.003
Ferritin	<12 μg/L	212	52.6	116	35.0	328	44.7	0.000
Vitamin C	<400 μg/dL	279	69.2	202	61.0	481	65.5	0.020
Retinol	<30 μg/dL	91	22.6	24	7.3	115	15.7	0.000
Vitamin B_{12}	<200 pg/mL	56	13.9	37	11.2	93	12.7	0.271
Folate	<3.0 ng/mL	108	26.8	78	23.6	186	25.3	0.316
vitaminB_2/creatinine	<80 μg/g	159	39.5	121	36.6	280	38.1	0.421

μg/L) was 39.7% and 52.6% in the anemic group, compared to 23.9% and 35.0% in the non-anemic group (p<0.003, p<0.001). Subnormal vitamin A (23% vs 7%) and subnormal ascorbic acid (69% versus 61%) were also significantly more frequent in anemic pregnant women (p<0.001, p<0.02). Nevertheless, with 38% and 25%, frequencies of subnormal riboflavin and folate were not significantly different.

The percentiles distributions of iron status and vitamin concentrations according to presence or absence of anemia is presented in Figure 1 and show a differentiated pictures of serum ferritin, serum iron, serum ascorbic acid, serum retinol, and vitamin B_2/creatinine in urine. Moreover, the curves of Hb <110 g/L and Hb ≥110 g/L in ferritin, serum iron, vitamin C and vitamin B_2/creatinine overlap in the lower end of the distribution, while the upper end is distinct. However, the retinol distributions were entirely distinct for non-anemic and anemic subjects, and there is no difference in serum folate between non-anemic and anemic subjects.

DISCUSSION

Results form this study that investigated in part of rural and low-economic towns in China showed that anemic pregnant women (Hb <110 g/L) had a lower serum iron concentration, ferritin and transferrin levels than non-anemic pregnant women; moreover, micronutrients were significantly lower in anemic women than non anemic women in terms of serum vitamin C, serum retinol and vitamin B_2 in urine. Serum subnormal vitamin A and ascorbic acid levels were significantly more frequent in anemic than in non anemic women.

This study has several advantages over previous studies that examined micronutrient status during pregnancy. Apart from having a large sample size, a wide range of micronutrients was examined simultaneously in Chinese rural areas. In addition, we reported the extent to which multiple deficiencies coexist, data from both rural developing country settings as well as towns are scarce. Results from this study also have the potential to provide valuable reference values for assessing nutritional status. However, the assessment of vitamin and mineral status during pregnancy is complicated because there is a general lack of pregnancy-specific laboratory indices for nutritional evaluation[16], and pregnancy itself may alter.

The subjects were from the population living in low or middle socio-economic levels and undeveloped areas, which was expected to be represent the average micronutrients and anemia status in parts of the nation. Information on the characteristics and rates of anemia as well as iron deficiency of the subjects have been prepared for publication in another paper; while the stratified study may need a larger population by area, which will be designed on the larger scale.

Although the most common cause of anemia is iron deficiency, deficiencies of vitamin B_{12}, folate, vitamin A and even zinc contribute either singly or in combination to maternal anemia.[17] Women in developing countries have a high prevalence of iron deficiency but also tend to be deficient in other micronutrients such as zinc,[5] vitamin A, folate and vitamin B_{12}. Iron deficiency rarely occurs in isolation and is often accompanied by other micronutrient deficiencies.[18] Makola[19] confirmed that micronutrient deficiencies are prevalent in the female population of Tanzania and the prevalence of anemia (63%). In our study, low levels of serum vitamin C, retinol, riboflavin, occurred both in the anemic and non anemia groups, but the three marginal vitamin deficiencies were more severe in pregnant women with anemia than those without, which should be resulted in part by the additional requirements of the fetus and the pregnant women themselves, as well as low intakes. The subjects in the investigation could not get enough green vegetables and animal foods.[20] Unlike Western societies, food is not routinely fortified with iron in rural areas of China. Moreover, green vegetables were scarce in the subjects' diets during the winter season. Therefore, the low intakes and shortage of heme iron and fresh vegetables may contribute to low average serum levels of retinal, ascorbic acid and low iron in anemic women in our study. Retinol status is a putative factor for improved iron status or iron absorption. Vitamin A deficiency may also result in anemia in humans and animals that can be reversed only by vitamin A supplementation.[21] Vitamin A and β-carotene may form a complex with iron, keeping it soluble in the intestinal lumen as well as preventing the inhibitory factors on iron absorption.[22] Ascorbic acid is considered a promoter of on non-heme-iron absorption. In the general Chinese diet, vegetables are commonly stratified, and fresh fruits are seldom eaten with a meal. Therefore, the amount and availability of vitamin C present in the diet are even more compromised by heat susceptibility, explaining low serum concentrations of vitamin C both in anemic and non anemic women. Simultaneous occurrence of both vitamin C and iron in the gut is necessary for effective interaction.[23] In this study, percentage of subnormal vitamin B_{12}

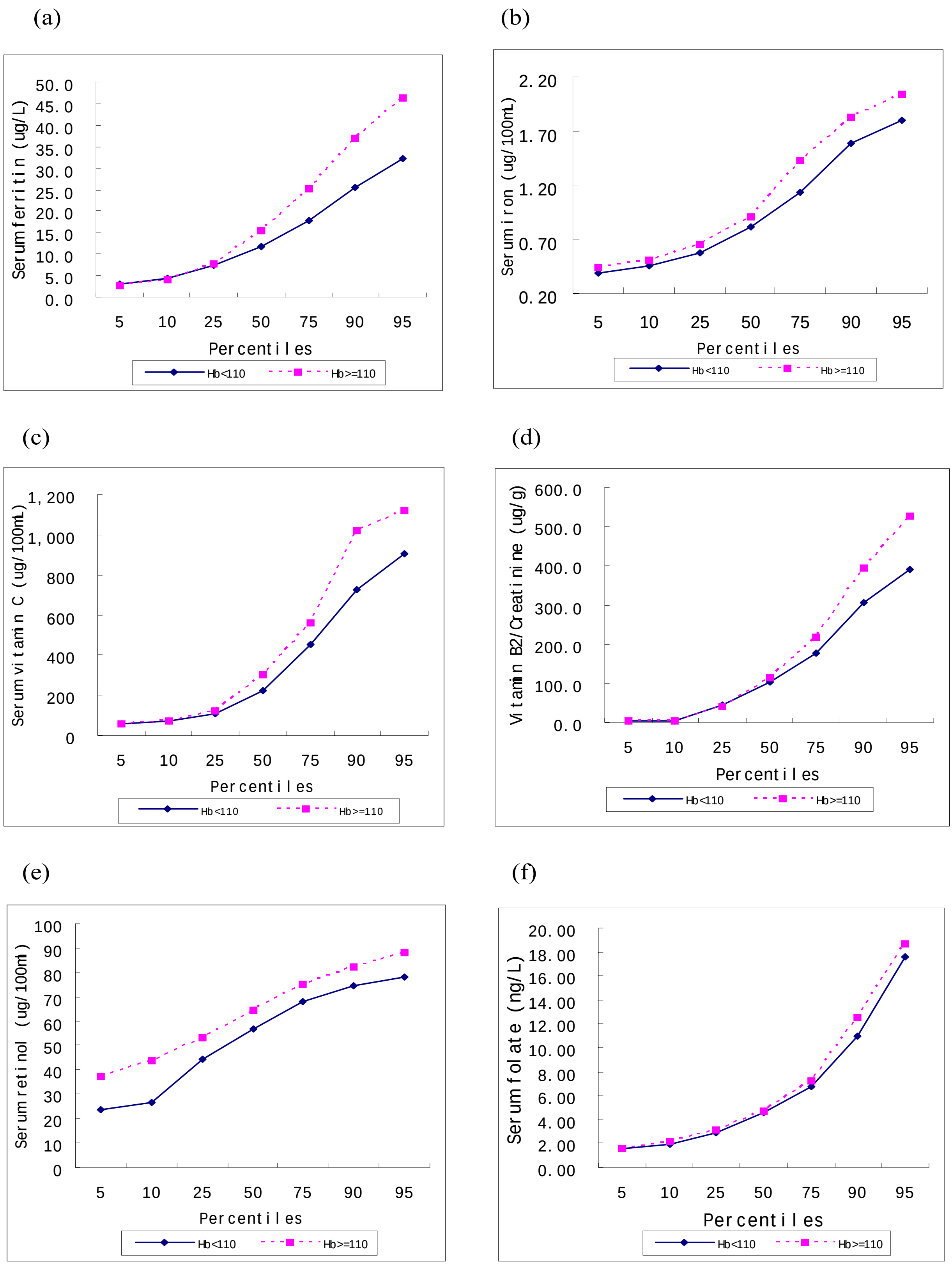

Figure 1. The distribution of concentrations of ferritin (a), serum iron (b), serum vitamin C (c), vitamin B_2 in urine (d), serum retinol (e) and serum folate (f) according to presence or absence of anemia as differentiated by Hb<110 and Hb≥110.

(<200pg/mL) were 13.9% in the anemic and 11.2% in non-anemic pregnant women, respectively, but there was no significant difference between them (p=0.271), which may have confounded the enhancing effect of ascorbic acid on iron status.[24]

Vitamin B_2 in urine, estimated by a ratio of vitamin B_2 and creatinine, was also found to be lower in anemic women than in non anemic women. Subnormal folate was not prevalent in this study though it is associated with anemia and other micronutrient deficiencies. It may also be the result of low intake, decreased intestinal absorption, or increased demand.[25,26]

The serum zinc and iron concentrations were positively related with maternal hemoglobin.[27] The distributions of hemoglobin concentration to zinc and iron indicated that deficiencies of the two elements were common and more severe in anemic pregnant women. The possible reason is not only an expansive blood volume and an increasing need of zinc and iron by pregnant women, but also poor intake and low bio-absorption of zinc and iron. Zinc supplementation may improve pregnancy outcomes for chronically deficient pregnant women. Prophylactic doses of 20-25 mg elemental zinc per day have generally been used in pregnant women in developing countries.[28] So we should also pay attention to zinc supplementation during iron supplementation because iron can interfere with the absorption of zinc. Adverse effects on zinc metabolism were observed after ingestion of 100 mg Fe/d. An increase in the efficiency of zinc absorption was observed during late pregnancy.[29]

In conclusion, in this multi-center cross sectional study we observed a high prevalence of anemia in the third trimester of pregnancy in rural areas as well as sub-urban area. There was a high prevalence of anemia in Chinese pregnant women, and the prevalence of iron deficiency (ID) and iron deficiency anemia (IDA) was 42% and 19%, respectively.[30] These women often have a poor nutritional status, lacking sufficient dietary intake of multiple micronutrients. The present study indicated that anemia in pregnant women was still a severe and possibly nutrition related problem. It was hypothesized that the possible reasons for the difference in prevalence between the four sites were geographic factors, unbalanced diets and poor nutritional education.[4] Furthermore, concentrations of serum ferritin, iron, retinol, zinc and urinary excretion of riboflavin were lower in anemic women than in nonanemic women. This may be the consequence of an unbalanced diet with a low amount of iron and micronutrients. Therefore, supplementation with a combination of iron and other micronutrients should be encouraged for pregnant women and be more beneficial to anemic pregnant women in the third trimester as well.

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AUTHOR DISCLOSURES

Ma AG, Schouten EG, Wang Y, Xu RX, Zheng MC, Li Y, Wang QZ and Sun YY, no conflicts of interest.

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中国贫血与非贫血孕妇妊娠晚期的微量营养素状况

贫血在中国仍然是一个主要的营养问题。贫血的原因除了鉄摄入不足外，也可能与其他微量营养缺乏有关。本研究目的是对比分析贫血与非贫血孕妇机体鉄、维生素 A 等微量营养素的营养状况。研究对象的选择是在县、乡（镇）妇幼保健院（所）从常规孕情检查的孕妇中随机抽取年龄 20-35 岁，健康的孕晚期妇女 734 名，其中贫血孕妇（Hb < 110 g/L）403 名，非贫血孕妇（Hb ≥ 110 g/L）331 名。经知情同意后采集空腹静脉血并离心获得血清，分别检测血清维生素 A、C、B_{12}、叶酸、鉄、锌等，采集尿液分析维生素 B_2（/g 肌酐）的营养状况。结果显示血清鉄、鉄蛋白、维生素 C 和维生素 A 的浓度在非贫血孕妇中达到 1109 μg/L、19.6 μg/L、388.1 μg/dL 和 59.3 μg/dL，而贫血孕妇血清中相应的营养素水平明显较低，分别仅为 909 μg/L、13.8 μg/L、308.9 μg/dL 和 50.0 μg/dL（p 值均小于 0.05）；此外，血清锌的浓度在贫血孕妇中也较低。经过微量营养素边缘缺乏分析显示鉄、鉄蛋白、维生素 A 和维生素 C 的边缘缺乏率在贫血孕妇中明显高于非贫血孕妇。维生素 B_2 和 B_{12} 在贫血和非贫血孕妇中的边缘缺乏比例均高，但两组之间没有统计学差异。总之，本研究发现孕晚期妇女贫血可能与鉄、维生素 A、维生素 C 等缺乏有关，建议孕晚期孕妇应及早從飲食增加或額外补充鉄、维生素 A、维生素 C、维生素 B_2、B_{12} 等微量营养素，有利于预防和治疗贫血。

关键词：贫血，妊娠，微量营养素，维生素，鉄