Folate Concentrations and Folic Acid Supplementation among Women in Their First Trimester of Pregnancy in a Rural Area with a High Prevalence of Neural Tube Defects in Shanxi, China

Le Zhang, Aiguo Ren,* Zhiwen Li, Ling Hao, Yihua Tian, and Zhu Li
Institute of Reproductive and Child Health, Peking University Health Science Center, Beijing, China

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BACKGROUND: Although an information campaign concerning periconceptional folic acid supplementation was launched in 1998 in Shanxi Province, China, the prevalence of neural tube defects in rural areas was reported as high as 140 per 10,000 births in 2002. The blood folate concentrations and the practice of folic acid supplementation among pregnant women in rural areas of the province are described. METHODS: A total of 483 pregnant women (mean gestation, 8.1 weeks) in a rural area of Shanxi were interviewed. Non-fasting blood samples and information on folic acid supplementation were collected. Folate concentrations in plasma and erythrocytes were determined by a microbiological assay. RESULTS: The mean concentrations of plasma and erythrocyte folate for pregnant women was 10.4 nmol/liter and 375.8 nmol/liter, respectively. Deficiencies of plasma and erythrocyte folate were observed in 20.9% and 47.6% of women, respectively. Seasonal variations were noted in the prevalence of folate deficiency, with significantly lower plasma folate concentrations in spring and summer and lower erythrocyte folate concentrations in seasons other than summer. Among pregnant women, <10% reported having taken or currently taking folic acid, and virtually no women (0.6%) took folic acid as recommended. CONCLUSIONS: Women in rural areas had low plasma and erythrocyte folate levels, and folate deficiency was highly prevalent in the area. Few women followed the recommendations regarding folic acid supplementation, and the information campaign in Shanxi was unsuccessful. These findings suggest the urgent need for combined strategies in rural areas to fortify grain with folic acid and promote folic acid supplements for childbearing-age women. Birth Defects Research (Part A) 76:461–466, 2006. © 2006 Wiley-Liss, Inc.

Key words: folate; folic acid; neural tube defects; women, pregnancy; supplementation
mation campaign to encourage women of childbearing age to consume 400 μg of folic acid daily to reduce the high prevalence of NTDs in the region. However, unpublished data from a population-based surveillance system established by the Shanxi provincial government showed that the prevalence of NTDs (including fetuses terminated before 28 weeks) in rural counties remained high (140 per 10,000 births). In China, farmers account for 70% of the total population and for a higher proportion of total NTD cases in the country since the prevalence of NTDs is higher in rural areas and women in rural areas usually have more children than do urban women. Therefore, the prevention of NTDs in rural areas is crucial to the success of the prevention of birth defects throughout the country. Therefore, we investigated the possible causes of the persistent high prevalence of NTDs in the rural areas of Shanxi. Specifically, the aims of the present study were to investigate the blood folate concentrations and the practice of folic acid supplementation among women in rural areas.

MATERIALS AND METHODS

We chose Pingding County in Shanxi Province as the project site on the basis of the following criteria: 1) the socioeconomic development was representative of the province; 2) the prevalence of NTDs was high; 3) the easy accessibility for blood sample shipment; and 4) the willingness of the county health authority to cooperate. The annual income of farmers in 2003 was $325, similar to that in most counties in the province. The prevalence of NTDs in Pingding County in 2002 was 230 per 10,000 births (annual health statistics, unpublished data), which was slightly higher than that in most counties in Shanxi.

The study protocol was reviewed and approved by the Institutional Review Board of Peking University Health Science Center. All participants provided written informed consent.

Subjects were women in their first trimester of pregnancy when they came for prenatal health care to the Maternal and Child Health Center in Pingding County, Shanxi Province. We used a convenience sample of the first 30 consecutive women, which accounted for ~85% of women in their first trimester who received health care in the health center each month. A total of 483 subjects were enrolled in the present study between December 2002 and March 2004, including 14 women (2 reported taking folic acid) who were married after October 1, 2003 when the new Marriage Registration Ordinance was enacted. Women with chronic liver and renal diseases were excluded.

At enrollment, a trained interviewer administered a structured questionnaire to the women to collect information on sociodemographic and personal attributes and folic acid use. At that time, height and body weight in light clothing were measured to the nearest 0.1 kg and 0.1 cm, respectively, with a beam weighing scale and a height scale. In addition, subjects’ folate-related food patterns were obtained by a simplified food frequency questionnaire (semi-FFQ), which included meat, fresh vegetables, beans, fruits, milk, nuts and bean products.

A 4-ml nonfasting blood sample was collected from each consenting woman by venipuncture. Blood samples were drawn into k3EDTA-containing Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged (8000×g, 15 min) within 1 hr of collection. Plasma and erythrocytes were separated and frozen at −20°C. All specimens were transported on dry ice to our laboratory and stored at −70°C before nutritional analyses.

The folate concentrations in plasma and erythrocytes were determined by a microbiological assay (O’Broin et al., 1997). The intra- and interassay coefficients of variation (CV) were <10% across the range of folate concentrations. Erythrocyte folate data were corrected for the folate/hemoglobin ratio (nanograms of folate/grams of hemoglobin). Details of the laboratory procedures can be found elsewhere (O’Broin et al., 1997).

All data were analyzed by use of the SPSS package version 11.5 for Windows (SPSS, Chicago, IL). Before analysis, plasma and erythrocyte folate data were log-transformed to normalize the positively skewed distributions. Because there has been no research on the folate cutoff value for the Chinese population, we used the cutoff points for the U.S. population. Folate deficiency was defined as plasma folate <6.8 nmol/liter (3 μg/liter) (Herbert and Das, 1994) or erythrocyte folate <363 nmol/liter (160 μg/liter) (Saucerlich, 1995).

Summary statistics were calculated and used to describe the concentrations (geometric means) of plasma and erythrocyte folate and the percentage of women with folate deficiency. We also calculated the percent for use of folic acid supplementation. Because a survey conducted in China reported that the prevalence of NTDs varied seasonally with a peak in winter (Wu et al., 1995), we wanted to determine whether folate status reflected a similar seasonal trend. To characterize the folate status in different seasons during which blood samples were obtained, we defined 4 seasons: winter (December, January, and February), spring (March, April, and May), summer (June, July, and August), and fall (September, October, and November). SPSS ANOVA was used to identify seasons in which mean blood folate concentrations differed significantly, with Tukey’s adjustment for multiple comparisons. In addition, χ2 analysis was used to test for differences in the percent of women showing folate deficiency across seasons. To determine the effect of folic acid supplementation on plasma and erythrocyte folate levels, we compared plasma and erythrocyte folate geometric means in individuals who had taken and not taken folic acid supplementation, using the SPSS GLM program adjusted for age, body mass index (BMI, weight/height2), gestation time, educational level, and season. We also adjusted for food folate intake using the unmodified semi-FFQ to reflect the effect by folic acid supplementation.

RESULTS

The demographic characteristics of the study women are presented in Table 1. Their mean age was 28.7 (+SD 5.2) years, their mean BMI was 22.2 (+SD 2.9), and their mean gestation time was 8.1 (+SD 2.4) weeks. Most women (74.7%) completed elementary education, and few had an education of high school or above. Smoking (2.5%) and alcohol drinking (5.0%) were not prevalent in this population. Primigravidas comprised of about 1 of 4 (26.9%) and nulliparas comprised 38.9% of the study subjects.

The mean plasma folate concentration was 10.4 nmol/liter and the mean erythrocyte folate concentration was
375.8 nmol/liter. Plasma and erythrocyte folate deficiencies were observed in 20.9% and 47.6% of women, respectively (Table 1).

Seasonal variations were observed in folate levels. Mean plasma folate concentrations were lower in spring and summer than in winter and fall, whereas mean erythrocyte folate concentrations were lower in the seasons other than summer (Table 2). Seasonal trends for plasma and erythrocyte folate deficiency were similar to the folate concentrations but with a seasonally opposite direction (Fig. 1).

Of the 483 pregnant women, <10% reported having ever taken or currently taking folic acid supplements during the current pregnancy, ~2.0% began to take folic acid before their last menstrual period, and only 0.6% took folic acid for the advised period of 4 weeks before to 4 weeks after conception (Table 4).

Mean plasma concentrations for women who reported taking folic acid were about twice as high for those who reported not taking folic acid. Erythrocyte folate concentrations showed a similar pattern, although the differences were not as pronounced as for plasma folate. Adjusting for potential confounding factors (age, BMI, gestation, education, gravidity, parity, and folate-related dietary patterns) did not materially change the results (Table 5).

**DISCUSSION**

Our survey found that blood folate deficiency among pregnant women in a rural area with a high risk for NTDs was quite common. Approximately 1 of 5 pregnant women had evidence of plasma folate deficiency, and 47.6% had erythrocyte folate deficiency. Because microbiological assays give higher plasma and erythrocyte folate values than radioassays (Gunter et al., 1996; Molloy et al., 1998; Hao et al., 2004), the levels of plasma and erythrocyte folate deficiency may be underestimated. A study conducted in another county in Shanxi province reported that 71% of the women who attended premartial examination had an erythrocyte folate deficiency (by radioimmunoassay) (Liu, 1992). Although erythrocyte folate deficiency decreased significantly in the past 10 years compared with that in the previous study (Liu, 1992), it was still high. The high percentage levels of folate deficiency among rural pregnant women in Shanxi is associated with an increased risk for NTDs.

In the present study, pregnant women in rural areas had very low mean levels of plasma and erythrocyte folate. Considering that blood folate values are higher when measured by microbiological assay rather than by radioassay, the mean plasma folate level in Pingding County might be lower than that (9.7 nmol/liter) for women aged 21–34 years in south China (Ronnenberg et al., 1996).
et al., 2000). In addition, folate levels among the subjects were much lower than those in other countries. A recently published study in the United States, where data from the semi-FFQ analysis were collected, found that the median serum folate values were 24.0 ng/ml (54.4 nmol/liter) and 16.7 ng/ml (37.8 nmol/liter) for nonfasting women early in pregnancy who were consistent multivitamin users or nonusers, respectively (Lawrence et al., 2006). The National Health and Nutrition Examination Survey (NHANES) in 1999 found that the mean erythrocyte folate concentration for all women ages 15–44 years was 315 ng/ml (713.8 nmol/liter) (CDC, 2000). Another survey conducted in 2000 in Chile reported that mean levels of plasma and erythrocyte folate among childbearing-aged women of low socioeconomic status were 37.2 ± 9.5 and 707 ± 179 nmol/liter 1 year after enactment of folic acid fortification, respectively (Hertrampf and Cortés, 2004). Whether samples in the survey in Chile were fasting samples was not indicated, but if our samples were drawn after a 1-night fast, the difference between plasma folate levels in our study and those in the Chilean study might be even greater because serum folate for nonfasting samples is higher.

We found that there were distinct seasonal trends in the concentrations and prevalence of deficiencies of plasma and erythrocyte folate levels. The mean concentration of plasma folate was nearly 30% lower in spring than in fall, and the mean concentration of erythrocyte folate was 25% lower in winter than in summer. Although our finding of a lower prevalence of plasma folate in winter and fall than in spring and summer is not consistent with a study in southern China (Ronnennen et al., 2000), it had a similar trend to that in another study in northern China (Hao et al., 2003). Moreover, it corresponds to the variations of the prevalence of NTDs, identified by last menstrual period, in rural women in Shaanxi in different seasons (Li, 2004). Our preliminary data from the semi-FFQ analysis suggest that although the women had a lower intake of vegetables in winter and fall, they consumed more of other folate-rich foods, such as legume products, peanuts, and sunflower seeds, than in spring and summer. Those folate-rich foods might contribute to the seasonal variations in plasma folate concentration, which is sensitive to recent folate intake (Shane, 1994). There was a longer time lag between the intake of more folate-rich foods in winter and fall and higher erythrocyte folate concentrations in summer, probably because erythrocyte folate levels are a good indicator of long-term folate status and intracellular folate stores are retained throughout the cell’s life span (Lindenbaum and Allen, 1995).

Our study found that <10% of pregnant women reported to have taken or to currently be taking folic acid, and few (0.6%) took folic acid for the advised period. Studies in several countries reported that campaigns aimed at informing women about folate and NTDs and encouraging them to use folic acid increased the use of folic acid supplements considerably. In 1994, <1% of women in the Netherlands took folic acid for the advised period (de Jong-van den Berg et al., 1995). However after the 1995 campaign aimed at women of low socioeconomic status, use of folic acid during the periconceptional period increased substantially, up to 52% (Bekkers and Eskes, 1999). In Western Australia, periconceptional intake of folic acid supplements among pregnant women with planned pregnancies increased from 19.1% before the promotion of folic acid to 43.1% 2.5 years after a health campaign (Bower et al., 1997). Compared to the effects of campaigns in other countries, the public health actions in Shaanxi appear to have been unsuccessful, and the provincial government has had limited success with promoting the use of folic acid supplements.

Although folic acid supplements could improve the blood folate level significantly for women who followed the recommendation, as shown in our survey and other studies (Heseker and Schmitt, 1987; Tsuji and Nordstrom, 1990), supplementation may not be an effective population approach to increase blood folate levels for all women of childbearing age since a large proportion of women do not take folic acid supplements as advised. A study in the United Kingdom found that even in a group of women who had recurrent miscarriages—a group highly motivated and knowledgeable about folic acid—only 51% took folic acid periconceptionally (Elkin and Higham, 2000). Four studies (de Jong-van den Berg et al., 1995; de Walle et al., 1998, 1999a, 1999b) in the Netherlands similarly found that the compliance of the subjects was always <50% for the entire advised period. A recent survey conducted in 2005 in the United States reported a decrease in the proportion of childbearing-aged women who reported taking folic acid supplements daily, from 48% in 2004 to 33% in 2005, returning to a level consistent with that reported during 1995–2003 (CDC, 2005). Those results supported Elkin’s proposition that compliance with recommended folic acid supplementation would not be likely to exceed 50% (Elkin and Higham, 2000). The supplementation rate among childbearing-age

### Table 4
Supplementation of Folic Acid among Women in the First Trimester Pregnancy in Pingding County, Shanxi Province, China

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ever took or currently taking folic acid during this pregnancy</td>
<td>37</td>
<td>7.7</td>
</tr>
<tr>
<td>No</td>
<td>446</td>
<td>92.3</td>
</tr>
<tr>
<td>Took folic acid before their last menstrual period</td>
<td>9</td>
<td>1.9</td>
</tr>
<tr>
<td>No</td>
<td>474</td>
<td>98.1</td>
</tr>
<tr>
<td>Took folic acid for the advised period</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>No</td>
<td>480</td>
<td>99.4</td>
</tr>
</tbody>
</table>

### Table 5
Comparison of Geometric Mean Plasma and Erythrocyte Folate Concentrations Between Folic Acid Users and Non-Users in Pingding County, Shanxi Province, China

<table>
<thead>
<tr>
<th></th>
<th>Plasma folate, nmol/liter (95% CI)</th>
<th>Erythrocyte folate, nmol/liter (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>User</td>
<td>37</td>
<td>19.9 (15.0–26.4)</td>
</tr>
<tr>
<td>Non-user</td>
<td>446</td>
<td>9.9 (9.4–10.4)</td>
</tr>
</tbody>
</table>

*P < .001 for the comparison with group of folic acid users.

**P < .05 for the comparison with group of folic acid users.

*Means were adjusted for age, BMI, gestation, education level, season, and folate-related dietary patterns.
women in rural areas in Shanxi is expected to be even lower after the enforcement of the new Marriage Registration Ordinance. The new Ordinance abolished mandatory health assessment before marriage, which was one of most important approaches used to disseminate information about folic acid (Zhang et al., 2005).

Our survey was conducted among women in their first trimester of pregnancy (mean gestation, 8.1 weeks). The information on folic acid use and blood folate levels was most relevant to the risk of NTDs. Moreover, chances of recall bias regarding folic acid should be smaller than when collected postpartum, as in other surveys.

There were some weaknesses in our study. Subjects were not sampled randomly. Women who came to seek prenatal health care in their first trimester of pregnancy might have been more conscious of their health and have been of higher socioeconomic status than those who did not. This would result in an overestimation of folic acid use and blood folate levels. However, because these women were living in a socioeconomically homogeneous geographical area, selection bias, if any, might not explain all of the results. Furthermore, although the subjects had typical lifestyles representative of the farmers in Shanxi province, it may be difficult to extrapolate the conclusion to other rural areas in China.

It is urgent to take more effective measures to increase the blood folate levels of rural women of childbearing age before fortified food is widely available. Folic acid supplementation will remain important approaches used to disseminate information about folic acid in China. N Engl J Med 20:1485–1490.

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