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Folate and vitamin B_{12} status of women of reproductive age living in Hanoi City and Hai Duong Province of Vietnam

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Abstract

Objectives: To assess the folate and vitamin B_{12} status of a group of Vietnamese women of reproductive age and to estimate the rate of neural tube defects (NTD) based on red blood cell (RBC) folate concentrations.
Design and subjects: A representative sample of non-pregnant women (15–49 years) living in Hanoi City (n 244) and Hai Duong Province (n 245).
Measures: RBC folate, plasma vitamin B_{12} and plasma holo-transcobalamin (holoTC), a sensitive indicator of vitamin B_{12} status.
Results: Mean (95 % CI) concentrations of RBC folate, plasma B_{12} and plasma holoTC were 856 (837, 876) nmol/l, 494 (475, 513) pmol/l and 78 (74, 82) pmol/l, respectively. Only 3 % and 4 % of women had plasma B_{12} and holoTC concentrations indicative of deficiency. No woman had an RBC folate concentration indicative of deficiency (<317 nmol/l). Only 47 % of women had an RBC folate concentration ≥905 nmol/l. Accordingly, we predict the NTD rate in these regions of Vietnam to be 14·7 (14·2, 15·1) per 10 000 pregnancies.
Conclusion: There was no evidence of folate and vitamin B_{12} deficiency among this population of Vietnamese women. However, suboptimal folate status may be placing three out of five women at increased risk of NTD. Reductions in NTD rates are still possible and women would benefit from additional folic acid during the periconceptional period from either supplements or fortified foods.

Keywords
Folate
Vitamin B_{12}
Nutritional assessment
Vietnam
Women

Folic acid taken during the periconceptional period reduces the risk of neural tube defects (NTD) and possibly other adverse pregnancy outcomes[1–3]. Several countries have introduced mandatory folic acid fortification to reduce NTD rates[4–6]. The reduction achieved in these countries has depended on the background rate of NTD; the higher the background rate the greater the reduction[6]. In countries that lack information on the background rate of NTD, the folate status of women of childbearing age may be a surrogate and help identify countries or regions likely to benefit from additional folic acid[7,8]. In an Irish cohort study the risk of having an NTD-affected pregnancy was inversely associated with red blood cell (RBC) folate concentration in the first trimester and was lowest in women with RBC folate concentration above 905 nmol/l[7]. The relationship between blood folate and NTD appears to apply in other populations. In a northern region of China, the NTD rate was 50–60 per 10 000 births and mean RBC folate concentration in women aged 35–44 years was 508 nmol/l, whereas in a southern region where RBC folate status was higher (911 nmol/l), the NTD rate was much lower, approximately 10 per 10 000 births[8]. Furthermore, a public health campaign to promote periconceptional folic acid use led to a much greater reduction in NTD in the northern than in the southern region[2]. Vietnam is a country for which there are no data on the rates of NTD.

Vitamin B_{12} plays an essential role in folate metabolism and there is increasing evidence that poor maternal vitamin B_{12} status may increase the risk of adverse pregnancy outcomes such as NTD[9]. Most recently Ray *et al.* measured holo-transcobalamin (holoTC), a sensitive indicator of vitamin B_{12} status, at 15 to 20 weeks' gestation, in Canadian women with (n 89) and without (n 422) an NTD-affected pregnancy[10]. Even against a background of mandatory folic acid fortification, low holoTC concentration was associated with a threefold higher risk of NTD. Suboptimal vitamin B_{12} status is common in many parts of the world[10]. Data are needed to determine the requirement for supplementation programmes or fortification in Vietnam. Accordingly we measured RBC

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and plasma folate levels of a representative group of women (15–49 years) living in the urban city of Hanoi and the rural province of Hai Duong and estimated the NTD rate based on RBC folate concentration. We also assessed the vitamin B_{12} status of these Vietnamese women using plasma holoTC and B_{12} as indicators.

Methods

Non-pregnant women aged 15–49 years were eligible to participate. Women were excluded if they were breast-feeding or had breast-fed within the last 12 months, or had a serious or chronic illness. Women were recruited from Hanoi City and Hai Duong Province. In Hanoi City, a district (Hai Ba Trung) and then a ward (Qunyh Mai) from within that district were randomly selected. A list of all 15–49-year-old women in Qunyh Mai ward was created and then organized by family. From this list a family was randomly selected and all women in that family were invited to participate in the study. From the first family, using the 'random walking' method, other families were approached and subjects added by inviting all women in the family who met the selection criteria. The selection procedure continued until 245 subjects were stratified equally into seven age groups (thirty-five women per group) as follows: 15–19, 20–24, 25–29, 30–34, 35–39, 40–44 and 45–49 years. In Hai Duong Province, Kim Thanh district was selected and within this district Tuan Hung and Cong Hoa communes were randomly selected. Selection of women was as described for Hanoi City; 154 (twenty-two women per group) and ninety-one (thirteen women per group) participants were recruited in Tuan Hung and Cong Hoa communes, respectively. Approval to conduct the studies was provided by the Ethical Committee of Science of the National Institute of Nutrition of Vietnam and all participants gave informed consent.

The survey was conducted between October 2006 and January 2007. Demographic details of the women were collected using questionnaires. Energy and folate intakes over the previous three days (including one weekend day and two weekdays) were estimated using 24 h recalls administered by a trained enumerator. Blood samples were taken by venepuncture into tubes containing EDTA following an overnight fast. Blood was processed and stored in a 1 % sodium ascorbate solution to prevent folate oxidation. RBC and plasma folate concentrations were measured as described by O'Broin and Kelleher using a microtitre technique with chloramphenicol-resistant *Lactobacillus casei* as the test micro-organism[11]. Whole blood standard (National Institute for Biological Standards and Control, Potters Bar, UK), with an assigned folate concentration of 29.4 nmol/l, was used to generate a standard curve. RBC folate was calculated from whole blood folate by subtracting plasma folate and adjusting for haematocrit. Plasma vitamin B_{12} and holoTC were measured by immunoassay using the ADVIA® Centaur™ (Bayer Healthcare, Tarrytown, NY, USA) and AxSym (Abbott Laboratories, Abbott Park, IL, USA), respectively. CV for these assays were 7·6 % for plasma folate, 10·8 % for RBC folate, 5·6 % for plasma vitamin B_{12} and 10·7 % for holoTC.

A plasma folate concentration <6·8 nmol/l or an RBC folate concentration <317 nmol/l was used to indicate folate deficiency[12]. RBC folate ≥905 nmol/l was used to indicate optimal folate status for NTD prevention. Predicted NTD risk was estimated based on each woman's RBC folate concentration using the predictive equation of Daly *et al.*[7]: $\exp[1{\cdot}6463 - 1{\cdot}2193 \times \ln(\text{RBC folate})]$. Vitamin B_{12} status was defined as deficient based on plasma vitamin B_{12} concentration of <148 pmol/l[13]. There are no established cut-offs for holoTC but the manufacturer recommends a cut-off of 35 pmol/l for serum or heparinized plasma samples. In order to use this cut-off for our samples which were collected in EDTA, we measured holoTC in plasma samples collected in both heparin- and EDTA-containing evacuated tubes from twenty healthy volunteers and adjusted our values accordingly (holoTC−EDTA plasma $= 0{\cdot}9699 \times$ holoTC − heparinized plasma $+ 17{\cdot}963$; $R^2 = 0{\cdot}8022$). Energy and nutrient intakes were estimated using Vietnam and ASEAN food composition databases[14,15]. Energy and folate intakes were not normally distributed and are presented as median and interquartile range. Differences between Hai Duong and Hanoi were determined using the χ^2 test for categorical variables and Student's t test for continuous variables. All statistical analyses were performed using the STATA statistical software package version 10 (StataCorp, College Station, TX, USA).

Results

The response rate for the present study was 100 %. Demographic characteristics as well as energy and folate intakes of the participants are shown in Table 1. All women were ethnically Vietnamese (Kinh) apart from one Muong and one Thai woman. Over 80 % of the woman had at least one child. The women in Hanoi had received more education than those living in Hai Duong Province. For example, 76 % of women in Hanoi had completed grade 12 or higher compared with 19 % in Hai Duong Province. The majority of women in Hai Duong Province reported their occupation as farmer (68 %), whereas women in Hanoi were about evenly split between office clerk (26 %), factory worker (21 %), housewife (22 %) and business owner (21 %). Less than 2 % of women reported folic acid supplement use and mean folate intakes were similar in both areas, 248 μg/d.

Biochemical indices for folate and vitamin B_{12} are given in Table 2. RBC folate concentrations were similar in women from Hai Duong Province and Hanoi; however, Hai Duong women had higher mean plasma folate

Table 1 Demographic and dietary characteristics of the study population: non-pregnant Vietnamese women from Hanoi City and Hai Duong Province, October 2006–January 2007

	All (*n* 489)		Hanoi – urban (*n* 244)		Hai Duong – rural (*n* 245)	
Characteristic	Mean	SE	Mean	SE	Mean	SE
Age (years)	33	0·45	32	0·64	33	0·65
BMI (kg/m^2)	20·2	0·1	21·1	0·2	19·2	0·1*
	n	%	*n*	%	*n*	%
Has children	396	81	190	78	206	84
Alcohol consumers	5	1	3	1	2	1
Occupation						
Office clerk	101	18	71	26	30	11*
Factory worker	71	13	58	21	13	5*
Housewife	65	12	59	22	6	2*
Business owner	72	13	57	21	15	5*
Farmer	191	34	0	0	191	68*
Retired	11	2	10	4	1	0
Other	43	8	22	8	21	8
Highest education attained						
Less than grade 9	35	7	3	1	32	13*
Completed grade 9	221	45	53	22	168	69*
Completed grade 12	156	32	125	51	31	13*
Completed university	77	16	63	25	14	6*
Folic acid supplement use	8	2	5	2	3	1
	Median	IQR	Median	IQR	Median	IQR
Dietary intake						
Energy (MJ/d)	7·2	6·0, 8·7	6·8	5·6, 8·7	7·4	6·5, 8·7
Folate (μg/d)	248	157, 364	245	150, 377	252	164, 354

IQR, interquartile range.
Mean values or percentages were significantly different from those of Hanoi (χ^2 test or Student's *t* test): **P*<0·05.

Table 2 Biochemical indices of folate and vitamin B_{12} status: non-pregnant Vietnamese women from Hanoi City and Hai Duong Province, October 2006–January 2007

	All (*n* 423 to 481)		Hanoi – urban (*n* 212 to 244)		Hai Duong – rural (*n* 211 to 237)	
	Mean	95 % CI	Mean	95 % CI	Mean	95% CI
RBC folate (nmol/l)	856	837, 876	854	826, 883	858	831, 886
<317 nmol/l (%)	0		0		0	
≥905 nmol/l (%)	37	33, 41	34	28, 40	39	33, 46
Plasma folate (nmol/l)	23·5	22·7, 24·2	22·0	21·0, 23·0	24·9**	23·8, 26·1
<6·8 nmol/l (%)	1	0, 1	0	0, 1	0	0, 1
Plasma vitamin B_{12} (pmol/l)	494	475, 513	461	436, 487	527**	499, 556
<148 pmol/l (%)	0		0		0	
Plasma holoTC (pmol/l)	78	74, 82	72	66, 77	84**	78, 90
<35 pmol/l (%)	4	2, 6	5	2, 8	2	0, 4

RBC, red blood cell; holoTC, holo-transcobalamin.
Mean values were significantly different from those of Hanoi (Student's *t* test): ***P*<0·001.

(24·9 *v.* 22·0 nmol/l; *P*<0·001). Based on RBC folate <317 nmol/l or plasma folate <6·8 nmol/l, there was no evidence of folate deficiency. Thirty-seven per cent of women had an RBC folate concentration ≥905 nmol/l; RBC folate above this concentration is associated with low risk of NTD. Using the equation of Daly *et al.*[7] we predict the NTD rate (95 % CI) for women living in these regions of Vietnam to be 14·7 (14·2, 15·1) per 10 000 pregnancies. Mean plasma B_{12} and holoTC were higher in women in Hai Duong Province than Hanoi. No women had a plasma B_{12} concentration indicative of deficiency (<148 pmol/l). Only 4 % of women had a low holoTC (<35 pmol/l).

The percentages of women falling into various categories of RBC folate concentration are shown in Fig. 1. The categories are the same as those used by Daly *et al.*[7] in developing the equation we used to predict NTD risk. Most of the women had an RBC folate concentration in the upper three quintiles, i.e. >453 nmol/l, with 41 % of women falling between 680 and 905 nmol/l.

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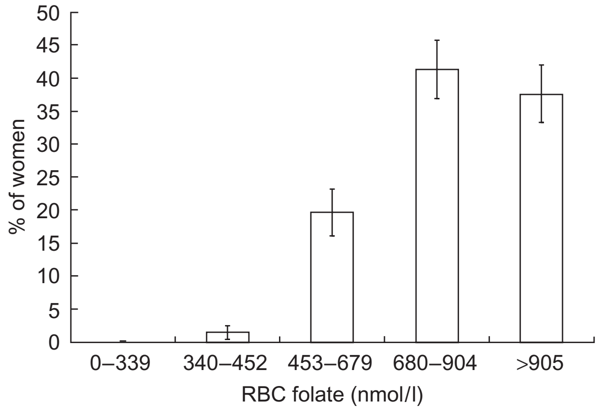

Fig. 1 Red blood cell (RBC) folate concentration according to category of neural tube defect risk based on Daly *et al.*[7], among 15–49-year-old, non-pregnant Vietnamese women from Hanoi City and Hai Duong Province, October 2006–January 2007. Values are means with their 95 % confidence intervals represented by vertical bars

Discussion

To our knowledge, the present study is the first one to examine the folate and vitamin B_{12} status of women of reproductive age in Vietnam. Our results indicate that the prevalence of vitamin B_{12} and folate deficiency is low among women in a rural and an urban area of Vietnam. Indeed, based on biochemical indicators used in the study, the folate and vitamin B_{12} status of these women is good. Our mean RBC folate concentration of 856 nmol/l is substantially higher than what we reported for women of childbearing age living in Kuala Lumpur (674 nmol/l) or Beijing (563 nmol/) and very similar to that of women living in Jakarta (872 nmol/l), where there is mandatory folic acid fortification of wheat flour at 200 μg/100 g[16].

The mean folate intake of 240 μg/d is much greater than the mean intake of 84 μg/d that we reported for women in Kuala-Lumpur[16]. Hao *et al.* attribute the large difference in RBC folate between people (35–64 years) in northern *v.* southern China (520 *v.* 864 nmol/l) to a greater availability of fresh vegetables throughout the year in south China[8]. The mean intake of vegetable leaves in the Vietnam General Nutrition Survey 2000 in the Red River Delta Region, which includes Hai Duong Province and Hanoi, was high at 160 g/d[17]. The high blood folate concentrations of women in our study are suggestive of a folate intake higher than 240 μg/d. In young New Zealand women consuming a similar amount of folate (232 μg/d), mean RBC folate concentrations were some 100 nmol/l lower[18]. One explanation for this discrepancy is that the food composition database used in the present study is incomplete and may be unreliable, particularly for wild vegetables which are good sources of folate; thus we may be under-reporting the folate intake of these Vietnamese women[19]. Unfortunately most folate values in food composition databases are derived from assay procedures that are now known to underestimate folate content[20].

There was little evidence of vitamin B_{12} deficiency in these Vietnamese women. The mean plasma vitamin B_{12} and holoTC concentrations were more than twice the cut-offs of 148 and 35 pmol/l, respectively[13]. Our findings are in contrast to reports of widespread vitamin B_{12} insufficiency in other developing countries, particularly India, Mexico, Central and South America, as well as parts of Africa[21–24]. However, there is scant information on the vitamin B_{12} status of Asian women outside India[23]. In a recent study in China, the mean plasma vitamin B_{12} concentrations of women (35–64 years) was 333 pmol/l in the south and 233 pmol/l in the north, both lower than our mean of 494 pmol/l[25]. Vitamin B_{12} absorption tends to decrease with age, which may explain some of the difference between the Chinese and younger Vietnamese women in our survey[26]. Because the Vietnamese food composition database does not include values for vitamin B_{12} we are unable to examine dietary explanations. However, meat and fish consumption was common in these areas in the Vietnam General Nutrition Survey 2000, with mean values of 55 and 33 g/d, respectively[17]. Also fish sauce, which contains 0.5 μg vitamin B_{12}/18 g (1 tablespoon), is commonly consumed in Vietnam.

Blood folate concentrations are variable among laboratories; the variability is largely method-dependent with better agreement found among laboratories using the same method[27,28]. We used a microbiological assay according to the method described by O'Broin and Kelleher[11]; the same method as that used by Daly *et al.*[7] in their observational study in which the data showed an inverse association between NTD risk and blood folate concentrations. Using that relationship, we would predict the NTD rate (95 % CI) in these areas of Vietnam to be 14·7 (14·2, 15·1) per 10 000 pregnancies. This rate is lower than we predicted for Beijing (30/10 000) and Kuala Lumpur (24/10 000), and similar to that for Jakarta (15/10 000)[16]. We recognize that the predictive equation for calculating NTD rates is based on a single observational study from Ireland, where, at the time of the study, NTD rates were high. Maternal blood samples were collected prospectively (i.e. before birth) and the association between NTD rate and RBC folate concentration was determined. We used the equation to predict the absolute rate of NTD and acknowledge this is speculative. Nevertheless, the close agreement between the actual and predicted decline in NTD rates in countries with mandatory folic acid fortification suggests the equation is valid[29] for predicting change in NTD rate with a change in population folate status. Furthermore, the finding of lower blood folate concentrations and higher NTD rates in northern *v.* southern China suggests that this relationship applies in an Asian setting[8].

Although our study population appears to have adequate vitamin B_{12} status we do not know the optimal

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plasma B_{12} or holoTC concentration for pregnancy. For example, in the case–control study by Ray *et al.*[10], holoTC concentration <55·3 pmol/l was associated with a tripling of NTD risk. Using this cut-off over a third of our women would be at increased risk of suboptimal B_{12} status. Further study is needed to clarify the association between adverse pregnancy outcomes and vitamin B_{12} status, including determining optimal levels of plasma B_{12} and holoTC for pregnancy.

Although our sample is representative of Hanoi and Hai Duong Province we cannot extrapolate our findings to the rest of Vietnam. Vietnam is a country that is geographically, climatically and ethnically diverse, with up to fifty ethnic minority groups. Clearly these differences could affect the food supply, dietary practices, and consequently folate and vitamin B_{12} intakes. For example, the mean folate intake of ethnic Vietnamese (Kinh) women (*n* 44) aged 19–60 years living in the Central Highlands was estimated as 407 μg/d using an FFQ[30]. Among Pa-Ko (*n* 29) women living in the same area folate intake was >800 μg/d, indicating that the folate status of these women may be better than that of our study subjects. Folate intakes may be seasonally affected in Hanoi and Hai Duong Province dependent upon the supply of fresh vegetables. In China, RBC folate, plasma folate and plasma B_{12} were lower in autumn than in spring even in the south[8,25]. Our survey was conducted following summer when there might have been a greater supply of folate-rich foods. Further study is needed to examine the folate status of women of different ethnicities in other provinces of Vietnam.

Conclusion

Our results indicate a low level of folate and vitamin B_{12} deficiency in Hanoi City and Hai Duong Province. Based on biochemical indices one might predict that these Vietnamese women were relatively well protected against folate-deficient NTD. Nevertheless, an improvement in folate status is likely to lower NTD risk. Given the serious nature of NTD and the ease with which increased protection can be afforded, Vietnamese women planning a pregnancy should take a supplement or consume fortified foods that provide at least 400 μg folic acid/d.

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