Original Article

Do Thai women of child bearing age need pre-conceptional supplementation of dietary folate?

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Recent studies in western countries have indicated that women with low serum folate before pregnancy have greater risk of giving birth to babies with neural tube defects, and preconceptional folate supplementation has been recommended to prevent such defects. To determine whether Thai women needed folate supplementation before pregnancy, we carried out a cross-sectional study from September 2001 to January 2002. The objectives were to determine serum folate levels among women of child-bearing age and their relationship to dietary folate intake. One hundred and sixty-five apparently healthy, volunteer women aged 15 – 45 years were recruited from the Family Planning Clinic, Mother and Child Hospital, Health Promotion Centre, Region I, Bangkok. Data on general characteristics, nutritional status and dietary folate intake were recorded while venous blood was drawn for serum folate analysis. Results showed that 65.5% of the study group had low dietary folate intake, that 18% had low serum folate, and that there was a significant correlation between dietary intake and serum level (r = 0.68, P<0.001). There were also significant correlations between serum level and body mass index, (r = 0.13, P<0.001). However, there were no significant associations between serum level and age, educational level, occupation, family income, or duration vegetables were stored in the refrigerator before consumption. In conclusion, there is preliminary evidence that some pregnant Thai women may have sufficiently low serum folate levels to put their babies at risk. We recommend further study on a larger scale to confirm whether folate supplementation is needed for Thai women at child bearing age. In the interim, it may be wise for obstetricians to measure serum folate in pregnant women to determine whether folate supplementation is required.

Key Words: serum folate, dietary folate intake, child-bearing age women, preconceptual, Thailand

Introduction

Good health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity.[1] It also requires that there be no congenital abnormality that decreases the quality of life. The World Health Organization (WHO) recognizes the human right including the child's right "to the enjoyment of the highest attainable standard of physical and mental health".[2] Therefore, important strategies to prevent diseases or congenital abnormalities in children should be considered. Neural tube defects (NTDs) are congenital malformations of the central nervous system caused by failure of the neural tube to close and they result in the absence of major portions of the brain and spinal cord. NTDs include anencephaly, spina bifida and encephalocele.[3] The prevalence of NTDs in the United States was 1.02 in 1,000 live births in 1993[4] while that for Thailand has not been reported. However, our review of statistics from the Medical Statistics Unit, Ramathibodi Hospital, Mahidol University from 1994 – 2000 showed the occurrence of NTDs was 1.16, 0.78, 0.66, 0.91, 0.84, 0.66 and 0.42 per 1,000 live births, respectively.[5] As has been recognized by most western countries, these NTDs resulted in high treatment costs and parental stress. Folate is a water-soluble B vitamin that acts as a coenzyme in several single carbon transfer reactions to synthesise components of DNA, RNA and proteins.[6] It is classically used to treat megaloblastic anaemia. However, during the past 30 years, new functions of folate have been discovered and recent studies have suggested that periconceptional folic acid supplementation can reduce the risk of NTDs,[7-16] cardiovascular disease[17,18] and cancer.[19, 20]

The recommended dietary allowance (RDA) of folate is 400μg per day for women of childbearing age but this should be increased to 600-800 μg per day in pregnant women.[21,22] The serum folate concentration decreases within 2 – 3 weeks after inadequate dietary folate intake and concentrations lower than 3 ng/ml before pregnancy have been associated with a higher risk of NTDs.[13]

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Good food sources of folate include liver, dark green leafy vegetables, legumes, nuts, seeds, and oranges.[22,23] However, preparation and cooking processes can destroy dietary folate.[24-28] For example, modern lifestyles have changed and many people eat ready-cooked foods or like to shop ahead, keeping food in the refrigerator for several days. These habits can affect folate intake. We questioned whether Thai women of reproductive age received adequate daily dietary folate intake and whether their serum folate levels were adequate for prevention of NTDs. Thus, we examined the serum folate levels in Thai women of child-bearing age and its relationship to dietary folate intake.

Materials and methods

A cross-sectional study was carried out at the Family planning clinic, Maternal and Child hospital, Health Promotion Center Region I, Bangkok from September, 2001 to January 2002

The sample size was calculated from the formula

$$n = \frac{Z^2 \alpha/2 \, \sigma^2}{d^2} = \frac{(1.96)^2 (5)^2}{(0.8)^2}$$

=150

An additional 10 % was added to compensate for any incomplete data. "Word of mouth" was the method used for subject recruitment. Those who came to the clinic during the study period and were interested in joining the study were screened according to the inclusion and exclusion criteria. Healthy, volunteer women aged 15 – 45 years, not pregnant and not lactating were recruited with the additional criteria that they did not smoke or engage in excessive intake of alcohol, since both may affect serum folate levels.[29-31] None of the subjects had a history in the preceding 6 months of taking anticonvulsants, methotrexate or oral contraceptive pills, since these also may interfere with folate metabolism.[32-34] In addition, none were taking folic acid or other multivitamin supplements and none had a history of hypertension, diabetes mellitus or obesity. The Mahidol University Institutional Review Board approved the study and informed consent was obtained from all the subjects. For each participant, baseline data were recorded on sociodemographic information, food consumption pattern and life style. The food frequency questionnaire (FFQ) used in this study was validated against 3-day food records as follows: 1) 3 day food records (2 week days and one weekend day) were collected from 30 women of child bearing age to get varieties of food items that were food sources of folate 2) trials of FFQs were applied to other groups of women and adjusted until a significant correlation between the two methods was achieved at $P<0.05$, r=0.51. Dietary folate intake from the FFQs were computed by a specific nutrient program.

To measure serum folate, about 5ml of venous blood was taken from non-fasting participants. After centrifugation at 3000 × g for 15 min, serum was transferred to 5ml vials and stored at -20°C. Serum folate was assessed using a commercial radioimmunoassay (Dual – count solid phase no boil assay for folic acid, Diagnostic Products Corporation, Los Angeles). The haematocrit values were measured by a micro-method using calibrated heparinized microhaematocrit tubes. After filling the capillaries with blood, they were centrifuged at 14,000g in an IEC MB microhaematocrit centrifuge and haematocrit values were measured by using microhaematocrit reader. For statistical analysis, the computer software package SPSS for Windows version 9.0 (Statistic Package for the Social Science/windows) was used. Specific measures included percentages, means, standard deviation and tests included Pearson correlations and ANOVA.

Results

For the 165 participants, ages ranged from 17 to 43 years with a mean age of 27.8 years (Table 1). Thirty percent were unemployed while the remaining 70% worked in the government, government enterprises or business or were self-employed. The average family income was 12,643 baht per month (45 baht is approximately equal to US $1). With respect to nutritional status (Table 2), 80% of the participants had a normal body mass index (BMI) (18.5-24.9 kg/m^2) and 20% were found to be undernourished (BMI <18.5 kg/m^2).[35]

Table 1. General characteristics of the study group

Characteristic	Number	%
Total number of participants	165	100.0
Age (years)		
< 20	23	13.9
20-35	123	74.6
> 35	19	11.5
$\bar{x} \pm SD$ (min-max) *27.89 ± 6.28 (17-43)*		
Occupation		
Not working/unemployed	50	30.3
Housewives	32	19.4
Students	18	10.9
Working /employed	115	69.7
Government/Gov. Enterprise	17	10.3
Business	30	18.2
Private employee	30	18.2
Business owner	26	15.7
Other	12	7.3
Education		
Primary school	31	18.8
Junior high school	35	21.2
Senior high school	38	23.0
Diploma	32	19.4
Bachelor degree or higher	29	17.6
Family Income/ month (baht)		
≤ 5000	16	9.7
5000-10000	57	34.5
10001-15000	35	21.2
15001-20000	24	14.5
20000-25000	5	3.0
25001-30000	17	10.3
> 30000	11	6.7
Median± QD 12642.8 ± 6000		

Serum folate and haematocrit status

Serum folate levels (Table 2) were low and considered marginal for folate deficiency for 18% of the participants while they were normal (6.1 – 24.0 ng/ml) for 82%. Only 3% were anaemic (haematocrit level <36%).

Table 2. The nutritional status, haematocrit and serum folate of the study group

Parameters	*N* = 165	%
BMI (kg/m^2)		
< 18.5	34	20.6
18.5-24.9	131	79.4
$\bar{x}$ ± SD (min-max) 20.4± 2.4; 15.4-24.6		
Haematocrit (%)		
<36	7	3.2
≥36	158	96.8
$\bar{x}$ ± SD (min-max) 40.8±2.9; 32-51		
Serum folate (ng/ml)		
3.0-6.0	30	18.2
6.1-24.0	135	81.8

Dietary folate intake

In the whole study group, 60% of the foods consumed were ready to eat foods while the majority of the remainder were self prepared foods (Table 3). The methods used for self-preparation included stir-frying and boiling and some vegetables were eaten uncooked (fresh). Table 4 shows that the most frequent dietary folate food source from animal protein was egg 2.8 times/week followed by drinking yoghurt and soymilk twice a week. Regarding vegetables, fresh yard long beans were consumed more than other vegetables at around 2-3 times a week. The most common fruit consumed was orange 1-3 times a week, followed by guava and pineapple. Cooked rice, the staple food of Thai people, was consumed 2.15 times per day. It was found (Table 5) that the mean dietary folate intake in the study group was 172μg/d which was considered inadequate (RDA= 400μg/d[21,22] and a total of 65.5% of the participants in the study had low dietary folate intakes.

There was a statistically significant correlation between dietary folate intake, BMI, and serum folate status at $P<0.001$ (r = 0.68, 0.13) (Table 6). However, there was no significant correlation between age, haematocrit status and serum folate. Also, the ANOVA test did not show significant association between serum folate status and educational level, occupation, family income or duration of vegetable storage in the refrigerator before consumption.

Discussion

Dietary folate intake

The estimates of dietary folate intake in this study were obtained from FFQ and included 69 food items as potential folate sources. Since it was impossible to include every food type in the survey, it is certain that some sources of folate were unintentionally overlooked in preparing the questionnaire. Under-reporting or over-reporting of portion sizes by the participants and failure to remember all foods eaten would also affect the estimate. Given that the calculated mean dietary intake of 172 μg/d for the whole group was lower than that recommended, while the serum levels were normal for most, it is likely that some significant dietary sources were underestimated or overlooked in this study. Thus, the calculated mean dietary folate intake must be regarded with some caution, and the tested serum levels should be considered more scientifically reliable.

Hougton *et al.*[36] reported similar findings in a study of Canadian female adolescents (14-19 years) for whom the mean daily folate intake was 242 ± 2.8 μg/d. In addition, Subar *et al.*[37] found that the intake of folate in the US population between ages 19 and 74 years was 207 micrograms for females. Orange juice, white breads, dried beans, green salad, and ready-to-eat breakfast cereals were the major food sources of folate in that study. All these intake values are considerably lower than the recommended RDA of 400 μg/d.

Serum folate

Although serum folate level reflects recent dietary folate intake rather than habitual dietary folate intake, our study

Table 3. Storage times, types of food and cooking methods used

Parameters	Number	%
Storage time		
1 day	40	24.2
2-3 days	99	60.0
> 3 days	26	15.8
Types of food		
Self-cooked food	61	36.9
Ready-to-eat food	97	58.8
Frozen food	5	3.0
Instant food	2	1.2
Cooking methods		
Uncooked (fresh vegetables)	35	21.2
Stir-frying	67	40.6
Boiling	60	36.4
Steaming	2	1.2
Frying	1	0.6

Table 4. Overview of the top ten most frequently consumed food items

Food items	Folate (μg/100m)	Average frequency score (times/week/person)
Rice, cooked	2.9	15.1
Egg	36.9	2.8
Orange	12.2	2.8
Rice noodle	7.2	2.7
Bread, white, slice	12.2	2.7
Guava	9.5	2.5
Soy milk	1.95	2.5
Drinking yoghurt	2.2	2.5
Yard long beans, fresh	105.0	2.2
Kale Chinese, cooked	80.2	2.1

Table 5. Number and percentage of women of child-bearing age listed by dietary folate intake

Dietary folate intake (μg/d)	*N*	%
Folate intake (μg/d)		
< 100	29	17.6
100-199	79	47.9
200-299	53	32.1
300-399	4	2.4
$\bar{x}$ *±SD (min- max) 172 ± 58 (47-376)*		

Table 6. Correlation coefficients for associations between dietary folate intake (D-folate), haematocrit , BMI, age and serum folate (S-folate)

Parameters	Haematocrit	S-folate	BMI	Age
D- folate	0.093	0.680**	0.060	-0.065
Haematocrit		0.043	-0.093	0.025
S- folate			0.134*	0.068
BMI				0.025

*Significant at P<0.05; ** Significant at P<0.01

demonstrated that there was a statistically significant correlation between estimated dietary folate intake and serum folate status (P<0.001, r=0.68). Indeed, 18% of the participants had low serum folate levels and would thus be considered at risk of folate deficiency when pregnant. This, in turn, might increase the chance of NTDs in their babies.[15]

Brussaard *et al.*[38] assessed the adequacy of folate intake and folate status among 20–49 year old women in the Netherlands and found that the mean folate intake was 270μg/d. The prevalence of intakes below the lower limit of the recommended range (200μg/d) was 20%. Potatoes, vegetables and fruits provided 36% of the folate in the diet while other important sources were bread (18%) and dairy products (16%). Mean serum folate levels in younger and older women were 12.2 and 14.2 nmol/l, respectively, and were clearly influenced by recent folate intake.

Relationships of folate status and demographic characteristics.

Ford and Bowman[39] found that age and educational level were weakly related to serum folate concentration, and they anticipated that higher educational attainment resulted in better health knowledge, purchasing power, and access to high quality consumer goods. We found a statistically significant correlation between BMI, dietary folate intake and serum folate status. We believe that patterns of food consumption such as use of ready to eat foods that are not freshly prepared, high temperature and long cooking time and long vegetable storage time in the refrigerator may be significant reasons for loss of folate, leading to low folate intake. Folate is temperature and storage sensitive and cooking can cause a significant fall in its concentration in food.[24-28] However, Gami and Chen[40] proposed that storage in plastic bags at low temperature helped preserve the vitamin and visual qualities of fresh vegetables. In this study, there was no association between vegetable storage time and serum folate, possibly because the majority of the study group consumed ready to eat foods.

Conclusion

This pilot study on serum folate status and dietary folate intake among Thai women yielded preliminary information suggesting that a significant number of pregnant women may have sufficiently low serum folate levels to put their babies at risk. Further study on a larger population should be conducted to confirm whether preconceptional folate supplementation might be needed to reduce risk of NTDs for Thai women at child bearing age.

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