Original Article

Dietary and blood folate status of Malaysian women of childbearing age

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The protective role of folic acid taken during the periconceptual period in reducing the occurrence of neural tube defects (NTD) has been well documented by epidemiological evidence, randomized controlled trials and intervention studies. Much of the evidence is derived from western populations while similar data on Asian subjects is relatively nascent. Baseline data on folate status of Malaysian women is lacking, while NTD prevalence is estimated as 10 per 10,000 births. This study was conducted with the objective of determining the dietary and blood folate status of Malaysian women of childbearing age. A total of 399 women comprising 140 Malay, 131 Chinese and 128 Indian subjects were recruited from universities and worksites in the suburbs of Kuala Lumpur. Inclusion criteria were that the subjects were not pregnant or breastfeeding, not taking folic acid supplements, not habitual drinkers or smokers. Based on a 24-hour recall, the median intake level for folate was 202.4µg (59.4-491.8 µg), which amounts to 50.6% of the Malaysian Recommended Nutrient Intakes level. The median (5-95th percentiles) values for plasma and red cell folate (RBC) concentrations were 11 (4-33) nmol/L and 633 (303-1209) nmol/L respectively. Overall, nearly 15.1% showed plasma folate deficiency (< 6.8 nmol/L), with Indian subjects having the highest prevalence (21.5%). Overall prevalence of RBC folate deficiency (< 363 nmol/L) was 9.3%, and an almost similar level prevailed for each ethnic group. Only 15.2% had RBC concentration exceeding 906 nmol/L, which is associated with a very low risk of NTD. The result of this study point to the need for intervention strategies to improve the blood folate status of women of childbearing age, so that they have adequate protection against the occurrence of NTD at birth.

Key Words: blood folate, neural tube defects, dietary intake, women, Malaysia

Introduction

Folic acid or pteroylglutamic acid was synthesized in 1945 at the Lederle Laboratories in United Sates two years after pteroyl-triglutamate was isolated in the same laboratory.[1] While folic acid is in the form of a monoglutamate, most naturally occurring folates including those in food are a mixture of mono- and polyglutamates. Good food sources of folate include legumes, green leafy vegetables, peanuts and broccoli. It is recognized that the bioavailability of food folate is less than that of folic acid when consumed as part of a mixed diet.[2]

Folate functions as a co-enzyme in several biochemical reactions and is essential for growth and reproduction. Deficiency of folate leads to inability to synthesize thymidine, resulting in decreased DNA synthesis and reduced production of rapidly dividing cells such as erythrocytes. A serious consequence of severe folate deficiency is breakdown in central nervous system maturation producing neural tube defects (NTDs).[3] NTDs are a group of birth defects presumed to have a common origin in failure of the neural tube to develop properly during the embryonic stage. Spina bifida is a form of NTD that has a defective closure of the bony encasement of the spinal cord. The neural tube closes by approximately the 28th day of gestation, and so any potential exposures suspected to have caused an NTD would have to have occurred within the first month of gestation. Since the 1980s, definitive evidence from randomized controlled trials and intervention studies have demonstrated that folic acid taken during the periconceptual period substantially reduces the risk of neural tube defects (NTD) at birth.[4]

In the latter study in United Kingdom, 1817 women who had a history of NTD pregnancy, consumed 4 mg folic acid daily during the periconceptual period resulting in over 70% reduction in the recurrence of NTDs. In China, consumption of 400 µg of folic acid daily reduced the risk of NTD-affected pregnancy by 79% in Hebei province, where the incidence of NTD was high at 50-60 per 10,000.[5]

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There are ethnic differences in risk for NTDs. In the United States, NTD prevalence is highest among Hispanics followed in descending order by non-Hispanics whites, African-Americans and Asians.[6] A study in Canada involving five ethnic groups, women of First Nations origin were at markedly increased risk of NTD compared with white as the referent.[7] Differences in ethnic rates may be due to genetic susceptibility to NTDs, diet or other factors.

Data on folate intake and blood folate status of Malaysian women are lacking. Prevalence of NTD is suggested to be approximately 10 per 10,000 according to hospital-based data on the prevalence of congenital malformation diagnosed in the first week of birth.[8] In light of important public health implications arising from folate deficiency, this study was conducted to assess folate intake and blood folate status of Malaysian women of childbearing age.

Subjects and Methods

This study is part of a multi-centre research project that included Indonesia. A common protocol was followed as far as feasible. In Malaysia, the three main ethnic groups namely, Malay, Chinese and Indians were included given their distinctive dietary and lifestyle practices. The inclusion criteria were:

- aged 18-40 years
- not pregnant or breastfeeding
- not taking folate supplements regularly
- not consuming alcohol habitually
- not a habitual smoker
- without a history of diabetes, hypertension, liver, heart or gastrointestinal problems

The study was undertaken in Jan-March, 2005 after ethical approval was obtained from the Ethics Committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia in Dec, 2004. As in the case of Indonesia, owing to the absence of local data on folate deficiency, this study also used the estimation of folate deficiency among women aged 35-64 years in Shanghai, China.[9] Applying 95% confidence level, expected range proportion of 10% and 19% allowance for incomplete data, the minimum sample size computed for each ethnic group was 125 women. Recruitment was carried out in universities and other work sites located in the suburbs of Kuala Lumpur. Brochures about the study were distributed in purposively chosen locations. Interested participants were first screened to ensure that they fulfilled the inclusion criteria. For those who qualified, they were given a two-page information sheet about the study. If they agreed to participate, they were asked to sign a consent form, which included their email or telephone contact for their blood result to be sent to them. A total of 10 ml blood (non-fasting) was taken from each subject by a doctor or qualified laboratory technologist.

Blood collection and preparation for determination of folate and ferritin concentrations

1. From each subject, 10 ml of blood was drawn, out of which 3 ml was placed into a 3ml EDTA vacutainer tube, while the remaining 7ml was placed into a plain 10ml vacutainer tube
2. The EDTA sample was mixed well, following which full blood count was undertaken immediately and the hematocrit value noted.
3. The plain sample was centrifuged at 3,000 rpm for 10 minutes to separate the serum.
4. The serum was then added into two 2ml plastic tubes with lid as follows:
   a. Tube 1: 0.5-1.0 ml serum was transferred for serum ferritin
   b. Tube 2: 1.0 ml serum was transferred for serum folate. Ascorbic acid was added immediately at a concentration of 5 mg/ml of serum. Lid was closed and the content mixed well.
5. Tube 3: 1.9 ml of freshly prepared 1% aqueous solution of ascorbic acid was added followed by 0.1 ml of the well-mixed whole blood from the EDTA sample. Lid was closed and mixed well.

All three tubes were immediately stored at minus 20 ^{0}C until air freighted to New Zealand. The frozen blood samples were air freighted to the University of Otago in Dunedin, New Zealand for the determinations of blood folate (plasma and red cell) and iron (ferritin and hematocrit). The microtiter technique as described by O'Broin and Kellecher[10] with chloramphenicol resistant Lactobacillus casei as the test microorganism was used to determine whole-blood and plasma folate concentrations. Hemoglobin concentrations were determined in Universiti Putra Malaysia.

Body weight of the women was taken using a digital weighing scale (SECA Alpha Model 770, Germany). Height was measured by means of the body meter (SECA Model 208, Germany). Body mass index was computed and categorized according to WHO.[11] All subjects were interviewed for demographic and socio-economic background. Dietary intake was assessed by the use of a 24-hour dietary recall. The interview was conducted by nutritionists or trained enumerators. Dietary data was analyzed using the program Nutritionist Pro, version 2.5 (First Databank, California,USA).

Descriptive and statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS Inc, Chicago, U.S.A.). Median and 5^{th}-95^{th} percentile values are included for variables that did not have a normal distribution.

Results

Demographic and socio-economic background

A total of 399 women comprising 140 Malay, 131 Chinese and 128 Indians who fulfilled the inclusion criteria gave consent to participate in the study. The median age of the subjects was 23 years with 82.2% in the 18-30 years age category (Table 1). Majority of them were single (75.7%). The married women had a median number of 2 children. Education attainment was quite high (16 years), owing to a high proportion of the subjects,

Table 1. Demographic and socio-economic description of subjects.

	Malay (*N* = 135)	Chinese (*N* = 130)	Indian (*N* = 124)	Total (*N* = 389)[1]
		N (%)		
Age (years)				
18-30	107 (79.3)	121 (93.1)	91 (74)	319 (82.2)
31-40	28 (20.7)	9 (6.9)	33 (26.0)	70 (17.8)
Median (years)	25	22	24	23
5-95th percentiles (years)	20-38	20-33	20-39	20-38
Marital status				
Single	83 (61.9)	124 (96.1)	86 (69.4)	293(75.7)
Married	48 (35.8)	5 (3.9)	36 (29.0)	89 (23.0)
Divorced/widowed	3 (2.2)	-	2 (1.6)	5 (1.3)
Number of children				
N	51	5	38	94
Median	2	2	3	2
5-95th percentiles	0-4	2-3	0-7	0.6
Education				
≤ 6yrs (primary)	3 (2.2)	-	7 (5.7)	10 (2.6)
7-11 yrs (secondary)	35 (25.9)	3 (2.3)	24 9 (9.5)	62 (16.0)
Form 6/matriculation	23 (17.0)	15 (11.6)	14 (11.4)	52 (13.4)
College/University	74 (54.8)	111 (86.0)	78 (63.4)	263 (68.0)
Median (yrs)	16	16	16	16
5-95th percentiles (yrs)	11-16	13-16	7-16	11-16
Occupation				
Working	77 (58.8)	12 (9.4)	37 (30.6)	126 (33.2)
Students	50 (38.2)	116 (90.6)	75 (62.0)	241 (63.4)
Housewives	4 (3.1)	-	9(7.4)	13 (3.4)
Monthly income				
N	77	12	*N*=37	*N*=126
Median (RM)	1500	1680	900	1400
5-95th percentiles (RM)	600-3,900	200-4,000	465-3,730	420-4,000

[1] 399 subjects gave blood samples but only 389 questionnaires were obtained; the number for some questions added up to slightly less than 389 due to incomplete information.

(60.3%) being university students. The working women were employed in a variety of positions including clerks bank executives, factory operators, laboratory technologists and nurses. Their median monthly income was RM1, 400 with a wide range from RM420 to RM4,000 (5-95th percentile).

Nutritional status

Overall, the mean body mass index (BMI) was within the normal range (22.6±4.5 kg/m^2) (Table 2). The Malay subjects had a significantly lower height and heavier body weight than the Chinese and Indians. Hence, the Malays had on average a significantly higher BMI than the other ethnic groups. With a mean BMI that verges on the border of overweight (24.1±4.7 kg/m^2), the Malay subjects showed the highest prevalence of overweight and obesity (20.0% and 12.6% respectively). In contrast, the Chinese group had a relatively higher proportion of underweight (22.7%), and less overweight (4.7%) and obese subjects (3.9%). The Indian subjects had as high a prevalence of underweight (22.5%) as the Chinese, and also relatively high proportions of overweight and obesity (14.6% and 9.0% respectively).

Energy and nutrient intake

The mean intake levels for energy and nutrients of the subjects were below the Malaysian recommended intake levels (RNI)[12] (Table 3a). The median folate intake of 202.4µg (59.4-491.8µg) amounted to only 50.6% of the Malaysian RNI level. Median intake of iron also fared poorly in achieving 35.9% or 52%, depending upon dietary iron bioavailability, of the RNI. Other nutrients with median intake levels below 50% of their respective RNIs were calcium, vitamin C, and vitamin B_{12}. Overall, the energy and nutrient consumption of the subjects was unsatisfactory.

Table 2. Body mass index of subjects

	Malay (*N* =135)	Chinese (*N* =128)	Indian (*N* =115)	Total (*N* =368)
		Mean ± SD		
Weight (kg)	58.69± 12.4^a	53.9 ± 10.0^b	55.72± 11.2^b	56.16 ± 11.4
Height (cm)	156 ± 5.1^a	159 ± 5.5^b	159 ± 6.5^b	158 ± 5.8
BMI (kg/m^2)	24.1± 4.7^a	21.2 ± 3.5^b	22.3 ± 4.8^c	22.6 ± 4.5
		N (%)		
Underweight <18.5 (kg/m^2)	10 (7.4)	29 (22.7)	24 (22.5)	63 (17.1)
Normal: 18.5-24.9 (kg/m^2)	81 (60.0)	88 (22.7)	63 (53.9)	232 (63.1)
Overweight 25.0-29.9 (kg/m^2)	27 (20.0)	6 (4.7)	7 (14.6)	40 (10.9)
Obese ≥ 30.0 (kg/m^2)	17 (12.6)	5 (3.9)	11 (9.0)	33 (8.9)

ANOVA post hoc test (Duncan) median values with the same superscript are not significantly different at $P<0.05$ level.

The Chinese subjects had on average the lowest levels of consumption of energy and several nutrients (Table 3b). Their intake of energy, protein, iron and vitamin A were significantly lower than those of the Malays and Indians. This could explain in part the comparatively higher prevalence of underweight among the Chinese. It is also likely that the women in this study might have under-reported their consumption levels.

Use of dietary supplements

Although the subjects did not take folic acid supplement by itself, a perceptible proportion of them (18.6%) reported taking dietary supplements regularly. Among the supplement users, 41.9% were Chinese, 37.8% Malay and 20.3% Indian. Vitamins were popular supplements being taken by nearly half of the users (Table 4). Vitamin C by itself or combined with multivitamins were taken by 25.7%, while 10.8% consumed multivitamins only. Vitamin B complex by itself or with other supplements was taken by another 9.5% of the users. Evening primrose oil and spirulina were also popular, taken on their own or in combination with vitamins, minerals and other non-nutrients. Overall, a wide variety of supplements were consumed and some subjects reported taking multiple types of supplements on a regular basis.

Table 3a. Energy and nutrient intake of subjects (*N*=383)*

	Intake Median (5-95th percentiles)	RNI Malaysia **	% RNI Median (9-95th percentiles)
Energy (kcals)	1325.0 (711-2453)	2000	66.3 (35.5-122.7)
Protein (g)	55.0 (21-100)	55	100.0 (38.2-181.8)
Fat (g)	45.0 (10-94)	400	50.6 (14.9-123)
Folate (DFE)	202.4 (59.4-491.8)	29 (10% bioavailability)	35.9 (14-131.7)
Iron (mg)	10.4 (4.2-38.2)	20 (15% bioavailability)	52.0 (21-191)
Calcium (mg)	291.0 (88-939)	800	36.4 (11.0-117.4)
Vit A (RE)[b]	591.0 (120-2087)	500	118.0 (24-417)
Thiamin (mg)	0.6 (0.2-1.5)	1.1	54.5 (18.2-136.4)
Riboflavin (mg)	0.9 (0.3-2.6)	1.1	81.1 (27.3-236.4)
Niacin equiv (mg)	8.0 (2.5-19.3)	14	57.1 (17.9-137.9)
Vit B_6 (mg)	1.0 (0.4-2.6)	1.3[c]	76.9 (30.8-200.0)
Vit C (mg)	28.9 (2.9-187.4)	70	41.3 (4.1-267.7)
Vit B_{12} (μg)	2.0 (0.2-14.4)	2.4[c]	83.3 (8.3-600)
Dietary fiber (g)	8.1 (2.3-17.5)	20-30	-

* number of completed questionnaires; ** Recommended Nutrient Intakes for Malaysia for ages 30-50 years[13a,b]; RE = Retinol equivalent;
[c] based on the US dietary Reference Intakes (DRI) (IOM)[25]

Table 3b. Energy and nutrient intake by ethnicity (Mean ± SD)

	Malay (*N*=138)	Chinese (*N*=126)	Indian (*N*=119)	Total (*N*=383)
Energy (kcals)	1443 ± 525[a]	1251 ± 421[b]	1505 ± 563[a]	1402 ± 518
Protein (g)	56.8 ± 24.6[a]	50.0 ± 20.6[b]	57.4 ± 27.4[a]	54.8 ± 24.5
Fat (g)	46.2 ± 22.4[a]	41.6 ± 33.6[a]	46.3 ± 26.8[a]	45.0 ± 28.0
Folate (DFE)	227.9 ± 160.0[a]	219.2 ± 135.1[a]	196.3 ± 92.5[a]	227.2 ± 142.6
Calcium (mg)	372 ± 265[a]	338 ± 283[a]	453 ± 403[b]	386 ± 322
Iron (mg)	15.8 ± 11.0[a]	11.3 ± 8.3[b]	16.0 ± 11.4[a]	14.4 ± 10.6
Vit A (RE)	1021 ± 1396[a]	614 ± 553[b]	860 ± 625[a]	838 ± 970
Vit C (mg)	60.3 ± 73.5[a]	52.7 ± 61.7[a]	55.7 ± 59.6[a]	56.4 ± 65.4
Thiamin (mg)	0.7 ± 0.3[a]	0.6 ± 0.4[a]	0.9 ± 0.6[b]	0.7 ± 0.5
Riboflavin (mg)	1.0 ± 0.6[a]	1.1 ± 0.7[a]	1.3 ± 1.1[b]	1.1 ± 0.8
Niacin equiv (mg)	9.2 ± 4.5[ab]	8.1 ± 5.0[a]	9.7 ± 6.3[b]	9.0 ± 5.3
Vit B_6 (mg)	1.4 ± 1.0[a]	1.1 ± 0.6[b]	0.9 ± 0.5[c]	1.2 ± 0.7
Vit B_{12} (μg)	8.2 ± 26.9[a]	15.5 ± 95.4[a]	7.1 ± 52.8[a]	10.2 ± 64.2
Dietary fiber (g)	7.9 ± 4.4[a]	8.2 ± 5.1[a]	8.3 ± 5.0[a]	8.1 ± 4.8

ANOVA post hoc test (Duncan): mean values with the same superscript are not significantly different at $P< 0.05$ level

Table 4. Types of dietary supplements consumed according to ethnicity.

Dietary supplements	Malay (*N* =28)	Chinese (*N* = 31)	Indian (*N* =15)	Total (*N* =74)
Vitamin C only	4	9	1	14 (18.9)
Vitamin C + multivitamins	2	3	0	5 (6.8)
Multivitamins only	4	2	2	8 (10.8)
Vitamin B complex only or with other vitamins/mineral	0	5	2	7 (9.5)
Evening primrose oil only or with other dietary supplements[a]	9	4	2	15 (20.3)
Spirulina only or with other dietary supplements[b]	7	1	4	12 (16.2)
Others[c]	2	7	4	13 (17.6)
Total *N* (%)	28 (37.8)	31 (41.9)	15 (20.3)	74 (100)

[a] includes vitamins, minerals, fish oil;[b] includes vitamins, cod liver oil
[c]includes wheat grass, garlic, lecithin,slimming tea,ling zhi (ganoderma)

Blood folate status

The median (5-95th percentiles) values for plasma folate and red blood cell (RBC) folate of the subjects were 11 (4-33) nmol/L and 633 (303-1209) nmol/L respectively (Table 5). On the basis of mean ± SD values, the Chinese subjects had a statistically higher plasma folate than the Malays and Indians. The Malays also had a statistically lower mean RBC folate than either the Chinese or Indians. Thus, on average, the blood folate status of the Malay subjects was the lowest among the ethnic groups. It is more appropriate to make comparisons using RBC folate than plasma folate concentrations, because the latter fluctuates according to recent intake and thus does not reflect body stores. In contrast, RBC folate concentrations reflect tissue content of folate throughout the body, and a low level is explicit evidence of folate deficiency (a consequence of a reduced supply of folate occurring over several months). In this respect, the mean RBC folate concentration of Malaysian women (673.8±302.4 nmo/L) was about 200 nmol/L lower than that of the Indonesian study counterparts (876.7±219.4 nmol/ L).[13] Nonetheless, the median folate intake for the Indonesian subjects at 131.5 (36.9-316.1) μg/day is lower than that of the Malaysian subjects at 202.4 (59.4-491.8) μg/day. In Indonesia, wheat flour is fortified with folic acid at 2 mg/kg and flour is a constituent of many commonly consumed food items including noodles, local cakes and snacks. It is plausible that the folate intake levels of Malaysians may have been over estimated. In terms of folate deficiency, 15.1% of the Malaysian subjects showed plasma folate deficiency (<6.8 nmol/L), with Indian subjects having the highest prevalence (21.5%). The overall prevalence of RBC folate deficiency (<363 nmol/L) was 9.3%, and a similar level prevailed in each of the ethnic groups. In contrast, none in the Indonesian study showed plasma folate or RBC folate deficiency.[13] Table 5 also shows that only 15.2% of the subjects had RBC folate concentration that exceeded 906 nmol/L, a level that is associated with very low risk of NTD.[14]

Thus, by this criterion, it is deduced that the majority of the subjects did not have the threshold level of RBC folate for full protection against a NTD risk. In comparison, the Indonesian study found 60.6% of its subjects

Table 5. Blood folate and iron status of subjects

	Malay	Chinese	Indian	Total
Plasma folate (nmol/L)	*N* = 137	*N* = 127	*N* = 121	*N* = 385
Mean ± SD	12.0±8.7[a]	17.1±10.7[b]	13.0±9.0[a]	14.0±9.7
Median (5-95th percentile)	10 (4-23)	14 (5-37)	11 (3-30)	11 (4-33)
N (%) < 6.8 nmol/L[1]	23 (16.8)	9 (7.1)	26 (21.5)	58 (15.1)
Red cell folate (nmol/L)	*N* = 140	*N* = 126	*N* = 123	*N* = 389
Mean ± SD	622.9±228.4[a]	705.2± 319.5[b]	706.5±351.4[b]	673.8±302.4
Median (5-95th percentile)	599 (304-1093)	657 (294-1330)	632 (294-1410)	633 (303–1209)
% < 363 nmol/L[2]	13 (9.3)	12 (9.5)	11 (8.9)	36 (9.3)
% > 906 nmol/L[3]	16 (11.4)	21 (16.7)	22 (17.9)	59 (15.2)
Hematocrit (%)	*N* =140	*N* = 131	*N* = 124	*N* = 395
Mean ± SD	35.1± 2.9[a]	34.4±3.0[a]	34.7±3.9[a]	34.8±3.3
Median (5-95th percentile)	35.3 (30.2-39.7)	34.4 (29.1–39.0)	35.0 (27.8-40.2)	35.0 (29.2-39.5)
% < 36.0%[4]	87 (62.1)	91 (69.5)	80 (64.5)	258 (65.3)
Hemoglobin (g/dl)	*N* = 140	*N* = 131	*N* = 117	*N* = 388
Mean ± SD	12.7± 1.8[a]	12.8±1.2[a]	12.4±1.6[b]	12.7±1.6
Median (5-95th percentile)	13.0 (10.6-14.6)	12.8 (10.6-14.6)	12.6 (9.2-14.7)	12.8 (10.2-14.7)
% < 12.0 g/dl[5]	23 (16.4)	27 (20.6)	31 (26.5)	81 (20.9)
Ferritin (μg/L)	*N* = 125	*N* = 115	*N* = 113	*N* = 353
Mean ± SD	35.0±31.9[a]	32.4±27.7[a]	20.1±20.2[b]	29.3±27.8
Median (5-95th percentile)	27.5 (4.5-101.2)	24.7 (3.3-82.5)	13.0 (2.4-65.8)	21.5 (2.9-85.1)
% < 15 μg/L[6]	23 (18.4)	27 (23.5)	31 (27.4)	81 (23.0)

[1,2] Folate deficiency (Choumenkovitch *et al*., 2001)[17]; [3] associated with very low risk of neural tube defect (Daly *et al*., 1995)[14]; [4,5] presence of anemia; and[6] depleted iron stores (WHO, 2001)[26]; ANOVA post hoc test (Duncan): mean values with the same superscript are not significantly different at $P<0.05$ level

Table 6. Comparison of blood folate and iron status between users and non-users of dietary supplements

	Users				Non-users			
	Malay	Chinese	Indian	Total	Malay	Chinese	Indian	Total
Folate								
Plasma								
N	29	30	13	72	103	93	105	301
Mean±SD	14.4±9.3[a]	19.6±14.2[b]	19.9±14.2[b]	17.6±12.6	10.5±5.2[a]	16.4±9.3[a]	12.0±7.7[a]	12.8±7.9*
RBC								
N	29	30	14	73	106	93	106	305
Mean±SD	694±275[a]	743±383[a]	854±428[a]	745±354	605±206[a]	691±303[a]	691±341[a]	661±290**
Iron								
Hct								
N	29	31	14	74	106	96	107	309
Mean±SD	34.9±3.0[a]	34.6±2.3[a]	1436.1±2.7[a]	35.0±2.7	35.2±2.9[a]	34.4±3.1[a]	34.5±4.0[a]	34.7±3.4
Hb								
N	29	31	13	73	106	96	101	303
Mean±SD	12.5±2.6[a]	12.7±0.9[a]	12.7±0.9[a]	12.7±1.8	12.8±1.5[a]	12.8±1.3[a]	12.3±1.7[a]	12.7±1.5
Ferritin								
N	29	26	12	64	95	85	98	278
Mean±SD	32.6±19.6[a]	34.4±19.8[a]	16.9±11.8[b]	30.4±19.4	36.4±34.9[a]	32.5±30.0[a]	20.9±21.2[b]	29.8±29.8

ANOVA post hoc test (Duncan) among ethnic groups within Users and Non-users: mean values with the same superscript are not significantly different at $P < 0.05$ level; T-test (2-tailed) between "Users Total" and "Non-users Total" mean values: * significant difference at $P < 0.05$; ** at $P < 0.01$

had RBC folate level <906 nmol/L.[13] It appears that while the flour fortification program has raised the blood folate concentrations of Indonesian women in general, the benefit has yet to reach all segments of the women population. As for blood iron status, the median values for hemoglobin (Hb) and hematocrit concentrations were 12.8 g/dl and 35% respectively (Table 5). Anemia (Hb < 12.0 g/dl) was present in 20.9% of the subjects overall, with the highest prevalence among the Indians (26.5%). The Indian subjects also showed a significantly higher prevalence (27.4%) with depleted iron stores (ferritin <15 μg/L) than the Malays and Chinese. Poor iron status among Indian women is likely a consequence of poor iron intake, especially if they are vegetarians. In examining the potential influence of dietary supplements on blood folate and iron status, Table 6 shows that users of dietary supplements had a significantly higher average plasma folate and RBC folate concentrations than non-users. Among the users, their mean plasma folate and RBC folate concentrations were 17.6±12.6 nmol/L and 745 ± 354 nmol/L respectively, compared with the corresponding values of 12.8±7.9 and 661±290 nmol/L among the non-users. It is likely that some of the dietary supplements used contained some folic acid. Users of dietary supplements may also be more health conscious and practice better food preparation and eating habits than the non-users.

Discussion

Epidemiological studies have demonstrated the inverse relationships between folate nutritional status and the risk of cardiovascular disease, and the occurrence of neural tube defect at birth. In light of important public health implications arising from folate deficiency, many countries including Malaysia have recommended that women of childbearing age consume 400 μg folic acid/day towards decreasing the risk of having an NTD-affected pregnancy. Achieving this amount could be challenging however. In this study, total folate intake among the subjects was found to meet only 16.5% of the Malaysian RNI. Notwithstanding the limitation of a single 24-hour method used to assess dietary intake, and the likelihood of under-reporting by the subjects, the median and 5-95th percentiles values were low.

When comparing among the ethnic groups, the Chinese subjects were shown to have significantly lower intake of folate. However, their blood folate concentrations were significantly higher than those of the Malays and Indians. This may be due to more Chinese consuming various types of dietary supplements, which might have contributed to their higher blood folate concentrations. Ethnic-specific methods of food preparation may also be a factor here. Folate is easily lost in pro-longed cooking. Chinese usually stir-fry over a short duration leafy green vegetables and legumes, which are good folate sources. Malays and Indians on the other hand, generally prefer their vegetables to be well-cooked. Also the bioavailability of dietary folate, especially in relations to the overall diet composition, might contribute to the different blood folate concentrations of the ethnic groups. However, the influence of folate bioavailability to blood folate status remains unclear presently.[15]

The mean RBC folate concentration of the Malaysian subjects at 673.8 ± 302.4 nmol/L is lower than that of women reported in other studies. Women aged 18-49 years in New Zealand had baseline RBC folate concentrations in a supplementation trial that ranged from 837 ± 291 to 944 ± 364 nmol/L.[16]

As pointed out previously, the Indonesian subjects appear to have benefited from the wheat flour fortification program, as their RBC folate concentration was about 200 nmol/L higher than the Malaysian counterparts. In the United States too, there has been improvement in RBC folate concentrations of the women in general, since

cereal-grain products were fortified with folic acid in early 1998. The amount of folic acid added to different cereal-grain products ranges form 95 to 309 μg/100 g of product. At this range of fortification, it was projected that folic acid intake in the general population attributable to fortification would be approximately 100 μg/day.[17] Caudill *et al.*,[18] reported that Californian women of childbearing age from socio-economically-advantaged background had a mean RBC folate concentration of 1307 nmol/L, four times higher than the level deemed acceptable (≥362 nmol/L). Asian women in this study had RBC folate level of 1269 ± 274 nmol/L and the Hispanics 1586±417 nmol/L. These folate concentrations were 2-3 times higher than pre-fortification red cell levels. The NHANES III data collected in 1988-91 reported mean RBC folate concentration of 515.9 nmol/L for whites, 455.7 nmol/L in Mexican Americans, and 415.4 nmol/L among African Americans.[19]

It appears that the subjects in this study had RBC folate concentrations at about the pre-fortification levels of US women or slightly higher. More importantly, is the finding that the majority of the Malaysian subjects had RBC folate concentration below the concentration of 906 nmol/L, which is deemed to confer maximum protection against the occurrence of NTD at birth. There is therefore a need to improve the blood folate status of Malaysian women of childbearing age.

One strategy is fortifying selected foods or food ingredients with folic acid as practiced in countries like Indonesia, United States and Canada. In United States, the birth prevalence of NTDs is estimated to have decreased from pre-fortification level of 37.8 per 100,000 live births to 30.5 per 100,000 conceived after mandatory folic fortification.[20] A similar fortification program in Canada begun in late 1998 has also led to a decline (38%) in the prevalence of NTD in Ontario.[21] A study in New Zealand in which women of childbearing age were given milk fortified with 375μg folic acid daily over 12 weeks had folate status raised by 51% compared to baseline. The mean RBC folate of the fortified group was 1262 nmol/L, which is well above 906 nmol/L associated with the greatest reduction of NTD.[22]

Another strategy is to encourage all women capable of becoming pregnant to take folic acid supplements. The daily use of 400 μg of folic acid remains the most effective practice to prevent NTD defects. Norsworthy *et al.*,[23] demonstrated that, while once-a-week 2800μg of folic acid supplement resulted in 50% of the women achieving a RBC folate concentration exceeding 905nmol/L, this was significantly lower that the daily 400μg supplement group, where 74% exceeded 905 nmol/L. The authors also highlighted their finding of only 23% of women taking the daily folic acid supplement showed a RBC folate concentration that exceeded 905nmol/L after 6 weeks. This is noteworthy because many countries recommend women to take folic acid for only 4 weeks before pregnancy. Thus, the authors recommended a longer period to achieve blood folate concentration associated with the lowest risk of NTD risk.

This study also found the persistence of poor iron status among the women particularly among the Indians. Iron-folate supplementation is a feasible strategy to improve ferritin-Hb status of Malaysian adolescents.[24] Health promotion on good nutritional practice and less reliance on costly dietary supplements is desirable among young female adults at work sites and the universities. There was also a noticeable prevalence of overweight and obese subjects in this sample, ranging from 8.8% in the Chinese to 32.6% among the Malays. A study in China found that being too thin (BMI <21 kg/m^2) increases the odds of blood folate deficiency two folds, and being overweight (BMI ≥27 kg/m^2) also has a 1.3 times higher chance of having folate deficiency.[9] The relationship between nutritional status and folate deficiency needs further investigation. In Malaysia, the leading cause of mortality of adults is cardiovascular disease, and this is often attributed to lifestyle-related conditions such as diabetes, hypercholesterolemia and obesity. The implications of low folate and high homocysteine as cardiovascular risk in Malaysians should be studied.

In conclusion, there is a definite need to improve the consumption of folate and blood folate status of Malaysian women of childbearing age. The public health authority has to weigh the costs and benefits of various strategic interventions. Research is also needed to generate data on (a) the folate content of Malaysian foods, (b) the bioavailability of folate in commonly consumed foods, (c) dietary intake of folate and other B vitamins of adolescents and adults, and (d) dietary intake and blood folate of pregnant women and birth outcomes.

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References

1. Rosenberg IH. Virtual folate: virtual success? Am J Clin Nutr 1999; 70:177-8.
2. Gregory JF. Dietary folate in a changing environment: bioavailability, fortification and requirements. F Food Sci 2004; 69: SNQ59-SNQ60.
3. Czeizel AE, Dudas I. Prevention of the first occurrence of neural tube defects by periconceptional vitamin supplementation. N Engl J Med 1992; 327: 1832-5.
4. MRC Vitamin Study Research Group. Prevention of NTDs: results of the Medical Research Council Vitamin Study. Lancet 1991; 338:131-137.
5. Berry RJ, Li Z, Erikson JD, Li S, Moore CA, Wang H, Mulinare J, Zhao P, Wong L-Y C, Gindler J, Hong SX, Correa A. Prevention of neural-tube defects with folic acid in China. N Engl J Med 1999; 341:1485-90.
6. Centre for Disease Control (CDC). Spina bifida incidence at birth – United States 1983-1990. MMMR Morb Mortal Wkly Rep1992; 19:399-404.
7. Ray JG, Vermeulen MJ, Meier C, Cole DEC, Wyatt PR. Maternal ethnicity and risk of neural tube defects: a population-based study. Canadian Med Assoc J 2004; 171:343-5.
8. Ho J. Folic acid in the prevention of neural tube defect – a programme for Malaysia? Med J Malaysia 2004; 59:94-9.
9. Hao L, Ma J, Stanpfer MJ, Ren A, Tian Y, Tang Y, Willet WC, Li Z. Geographical, seasonal and gender differences in folate status among Chinese adults. J Nutr 2003; 133: 3630-35.

10. O'Broin S, Kellecher B. Microbiological assay on micro-titre plates of foalte in serum and red cells. J Clin Pathol 1992; 45:344-7.
11. WHO Obesity. Preventing and Managing the Global Epidemic. Report of a WHO Consultation on Obesity. Geneva, WHO; 1998.
12. National Coordinating Committee on Food and Nutrition (NCCFN), Ministry of Health Malaysia. Recommended Nutrient Intakes for Malaysia. Putrajaya, Ministry of Health Malaysia; 2005.
13. Muslimatun S, Augustina R, Wiradnyani RAR. Folate status among selected child bearing-age and pregnant women in Jakarta, Indonesia. Unpublished Research Report. SEAMEO-TROPMED Regional Centre for Community Nutrition, Jakarta; 2005.
14. Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM. Folate levels and neural tube defects: implications for prevention. J Am Med Assoc 1995; 274: 1698-1702.
15. Gregory JF. Case study: folate bioavailability. J Nutr 2001; 131:1376S-82S
16. Venn BJ, Green TJ, Moser R, Mckenzie JE, Skeaff CM, Mann J. Increases in blood folate indices are similar in women of childbearing age supplemented with (6S)-5-methytetrahydrofolate and folic acid. J Nutr 2002; 132: 3353-3355.
17. Choumenkovitch SF, Jacques PF, Nadeau MR, Wilson PWF, Rosenberh IH, Selhub J. Folic acid fortification increases red blood cell folate concentrations in the Framingham Study. J Nutr 2001; 131:3277-80.
18. Caudill MA, Le T, Moonie SA, Esfani ST, Cogger EA. Folate status in women of childbearing age residing in Southern California after folic acid fortification. J Am Coll Nutr 2001; 20:129-34.
19. Ford ES, Bowman BA. Serum and red blood cell folate concentrations, race, and education: findings from the third National Health and Nutrition Examination Survey. Am J Clin Nutr 1999; 69:476-81.
20. Choumenkovitch SF, Selhub J, Wilson PWF, Rader JI, Rosenberg IH, Jacques PF. Folic acid intake from fortification in United States exceeds predictions. J Nutr 2002; 132: 2792-98.
21. Ray JG, Meier C, Vermeulen MJ, Boss S, Wyatt PR, Cole DEC. Association of neural tube defects and folic acid fortification. Lancet 2002; 360: 2047-48.
22. Green TJ, Skeaff CM, Rockell JEP, Venn BJ. Folic acid fortified milk increases blood folate and lowers homo-cysteine concentration in women of childbearing age. Asia Pac J Clin Nutr 2005; 14:173-8.
23. Norsworthy B, Skeaff CM, Adank C, TJ Green. Effects of once-a-week or daily folic acid supplementation on red blood cell folate concentrations in women. Eur J Clin Nutr 2004; 58:548-54.
24. Tee ES, Kandiah M. Awin N, Chong SM, Satgunasingam N, Kamarudin I, Milano S, Dugdale AE, Viteri FE. School-administered weekly iron-folate supplements improve hemoglobin and ferritin concentrations in Malaysian adolescent girls. Am J Clin Nutr 1999; 69: 1249-56.
25. Institute of Medicine, National Research Council. Dietary Reference Intakes for Thiamine, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline. Washington DC: National Academy Press, 2000.
26. WHO. Iron Deficiency Anaemia. Assessment, Prevention and Control. A guide for programme managers. Geneva, WHO; 2001

Original Article

Dietary and blood folate status of Malaysian women of childbearing age

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马来群岛育龄妇女的饮食和体内叶酸水平的研究

流行病学证据、随机对照试验以及干预性研究都证明妇女在怀孕期间服用叶酸对胎儿具有保护作用，能够降低胎儿神经管缺乏（NTD）发生率的作用。这些证据主要来自西方人群，而在亚洲类似的研究相对来说还是比较缺少的。在马来群岛，每 10000 个新生婴儿中就有 10 个患有 NTD 疾病，然而却缺少关于其育龄妇女的叶酸水平的基线数据。本项研究的目的是测定马来群岛育龄妇女的饮食和体内叶酸水平。共有 399 个妇女参加本次研究，其中包括 140 个马来人，131 个华人以及 128 个印第安人，这些人来自吉隆坡的大学和工厂。选择志愿者的标准是这些妇女没有怀孕或者未处于哺乳期，不服用叶酸，没有酗酒和吸烟的习惯。根据这些志愿者 24 小时的饮食回忆，叶酸摄入中值量为 66μg (15.7-207.8 μg)，占了马来群岛人推荐营养素摄入量的 16.5%。血浆和红细胞叶酸（RBC）中值（5-95 百分点）分别为 11 (4-33) nmol/L and 633 (303-1209) nmol/L。总的来讲，将近 15.1%的被调查人员的叶酸摄入量缺乏(< 6.8 nmol/L)，而叶酸缺乏人数最高的是印第安人为 21.5%。总的红细胞叶酸缺乏是 9.3 %，一个对每个同种同文化之民族最相似的流行水平。只有 15.2%的人的红细胞叶酸浓度大于 906 nmol/L，这个浓度与 NTD 低发生率相关。本项研究结果说明处于育龄期妇女应采取一定的干扰措施来改善她们体内叶酸缺乏的状况，以便她们防御孩子出生时患 NTD 的几率。

关键词：血液叶酸浓度、饮食摄入、妇女。