Original Article

Red cell folate and predicted neural tube defect rate in three Asian cities

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Background: Periconceptional folic acid reduces neural tube defect (NTD) risk. Red blood cell folate concentration is inversely associated with NTD risk. In many countries there is a lack of information on NTD rates. Red cell folate status in women of childbearing age may be a surrogate for NTD rates and may be helpful in identifying countries or regions most likely to benefit from improved folate status.

Objective: To predict NTD rates using red cell folate concentrations in women of childbearing age living in three Asian cities

Design: Cross-sectional convenience samples of non-pregnant women living in Beijing (n=220), Kuala Lumpur (n=389), and Jakarta (n=129).

Results: Red cell folate concentrations were highest (p<0.001) in women from Jakarta at 872 nmol/L (95% CI; 833, 910) followed Kuala Lumpur at 674 nmol/L (95% CI: 644, 704) and lowest in Beijing at 563 nmol/L (95% CI: 524, 601). Accordingly, predicted NTD rates were highest in Beijing at 30/10000 (95% CI: 27, 33), followed by Kuala Lumpur at 24/10000 (95% CI: 22, 25), and lowest in Jakarta at 15/10000 (95% CI: 14,15).

Conclusion: Our red blood cell folate data suggests that of the three cities improving the folate status of women in Beijing would have the greatest impact on NTD rates.

Key Words: red cell folate, NTD, fortification, Asia, women

Introduction

High folate status in women of childbearing age reduces the risk of neural tube defects (NTDs). In a large cohort of Irish women the risk of having an NTD-affected pregnancy was lowest in women with red cell folate concentrations above 905 nmol/L.[1] Taking folic acid during the periconceptional period reduces individual risk and population incidence of NTD.[2-4] Any programme that increases folate status is likely to have the greatest impact on NTD rates in populations with low folate status. For example, in a southern region of China, the background NTD rate was approximately 10 per 10000 births and mean red cell folate concentration in women aged 35 - 44 y was 911 nmol/L, whereas, in a southern region where red cell folate status was lower (508nmol/L), the background NTD rate was much higher, 50 - 60 per 10000 births.[5] Furthermore, the NTD rate in these regions following a public health campaign that encouraged women to take a daily supplement containing 400 µg folic acid per day was 6-7/10000 in compliant women.[6] Thus, despite large pre-supplementation differences in NTD rates between the northern and southern regions, NTD rates post-supplementation were similar. This is suggestive of a folate-responsive NTD threshold, possibly around a rate of 5-6/10000 below which the prevalence of NTD cannot be reduced further with folic acid. This is consistent with NTD rates in North America which have not fallen below 5/10000 since mandatory fortification.[2-4]

In many countries, information on birth defects is lacking. In the absence of this information, it is possible that red cell folate status in women of childbearing age may be a surrogate for NTD rates and helpful in identifying countries or regions most likely to benefit from improved folate status. Wald et al used this approach to compare predicted and actual decline in NTD rates in North America.[7] We have used a similar approach to predict NTD rates using red cell folate concentrations in women of childbearing age living in three Asian cities, Kuala Lumpur, Jakarta, and Beijing.

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Methods

Subjects

Non-pregnant women 18-40 y were recruited using convenience sampling from Beijing, Kuala Lumpur, and Jakarta. Recruitment was via mail, the posting of flyers, the Internet, and word of mouth. Women were excluded if they were breastfeeding or had breastfed within 12 months, or had a serious or chronic illness. In Beijing, 220 women 20-35 y were recruited between February and June 2002. A total of 389 women 18-40 y comprising approximately equal proportions of Malay, Chinese and Indian ethnicities were recruited from worksites and universities in Kuala Lumpur and it's suburbs between January and March 2005. In Jakarta, 129 women 18-40 y were recruited in late 2004. Demographic details of the women were collected using questionnaires. Approval to conduct the studies was provided by Ethics Committees within each of the study regions: The Peking Union Medical College Hospital Ethics Committee, Beijing, China; the Ethics Committee of the Faculty of Medicine and Health Sciences, Universiti Putra, Malaysia; Ethical Committee Faculty of Medicine, University of Indonesia, Jakarta.

Blood collection and laboratory analysis

Blood samples were taken by venipuncture into tubes containing EDTA following an overnight fast. Blood was processed and red cell folate concentrations measured as described by O'Broin and Kelleher using a microtiter technique with chloramphenicol-resistant *Lactobacillus casei* as the test microorganism.[8] Whole blood standard (National Institute for Biological Standards and Control, UK) with an assigned folate concentration of 29.4 nmol/L was used to generate a standard curve. The intra-assay coefficient of variation was 10.4% for whole blood based upon repeated measurements of pooled samples.

Analysis

Predicted NTD risk was estimated based on each woman's red cell folate concentration using the predictive equation of Daly et al [1.643-1.2193 x ln(red cell folate)].[1] ANOVA was used to test for differences between cities in red cell folate and predicted NTD rates, with a Bonferroni correction for multiple comparisons.

Results

Demographic characteristics of the participants are shown in Table 1. In Beijing, around half of the women were single, whereas in Kuala Lumpur and Jakarta over two thirds of the women were single. The majority of women had received secondary education or higher. Mean red cell folate concentrations and predicted NTD rates are shown in Table 2. Red cell folate concentrations were higher ($p<0.001$) in women from Jakarta than from Beijing or Kuala Lumpur by 198 (95%CI; 140 to 255) and

Table 1. Demographic and dietary characteristics

Descriptive	Beijing	Kuala Lumpur	Jakarta
Number of women	220	389	129
Ethnicity	Chinese	Malay (27%) Chinese (31%) Indian (32%)	
Age (y)	28 ± 8.1	25 ± 5.5	30 ± 6.8
BMI (kg/m^2)	20.9 ± 3.4	22.6 ± 4.5	22.5 ± 4.3
Smokers n (%)	2 (1%)	5 (1%)	9 (7%)
Oral contraceptive users	94 (43%)	NA	NA
Ever been pregnant	94 (43%)	NA	90 (69.8%)
Alcohol consumers	11 (5%)	20 (5%)	NA
Multivitamin or B-complex supplement use	NA	20 (5%)	20 (16%)
Marital status			
Single	113 (51%)	293 (76%)	96 (74%)
Married	104 (47%)	89 (23%)	30 (23%)
Widowed/divorced	1 (<1%)	5 (1%)	3 (2%)
Highest Education			
Primary	2 (1%)	10 (2.6%)	28 (21.7%)
Secondary	23 (11%)	62 (16%)	20 (15.5%)
Matriculation	71 (32%)	52 (13%)	60 (46.5%)
Polytechnic/University	122 (55%)	263 (68%)	21 (16.3%)

Table 2 Red cell folate concentrations[1] and predicted NTD rate

	Beijing	Kuala Lumpur	Jakarta
Red cell folate (nmol/L)	563 (524 to 601)[a]	674 (644 to 704)[b]	872 (833 to 910)[c]
Predicted NTD rate (/10000)	30 (27 to 33)[a]	24 (22 to 25)[b]	15 (14 to 15)[c]

[1]n = 220, 389, and 129 women in Beijing, Kuala Lumpur and Jakarta, respectively; values are means (95%CI). Numbers not sharing a common superscript are significantly different, P<0.001.

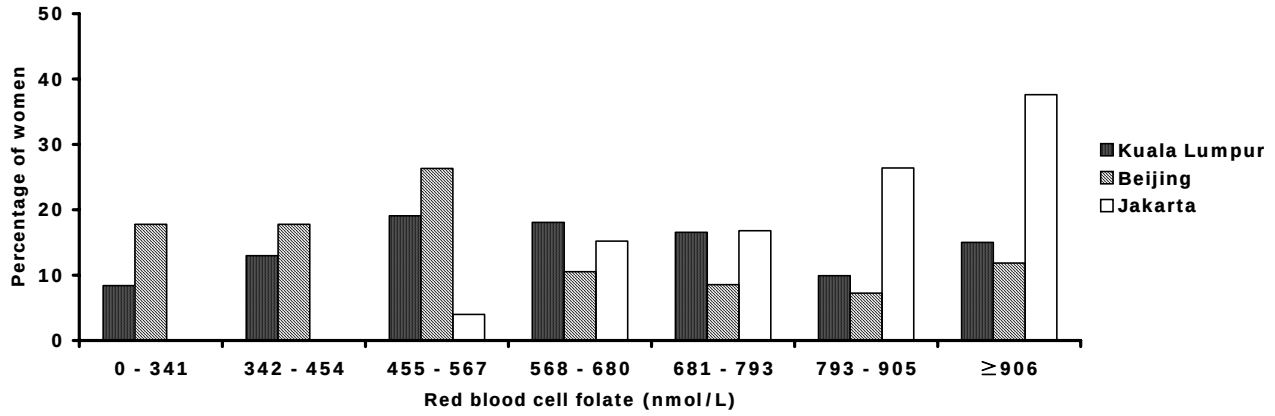

Figure 1. Distribution of red blood cell folate concentrations among women in Kuala Lumpar (n=389), Beijing (n=220) and Jakarta (n=129).

309 (254 to 365) nmol/L, respectively. Folate concentrations in Kuala Lumpur were 112 (58 to 166) nmol/L higher than in Beijing. More details on the Malaysian dietary and blood folate study will be published in the Asia Pacific Journal of Clinical Nutrition (in press). Accordingly, predicted NTD rates were highest in Beijing, followed by Kuala Lumpur and lowest in Jakarta. The percentages of women falling into various categories of red cell folate concentrations are shown in Figure 1. The categories are the same as those used by Daly et al.[1] in developing the equation we used to predict NTD risk. Women from Jakarta are distributed into the higher red cell folate categories, whereas women from Beijing and Kuala Lumpur are represented across a wider range of the categories. Eighteen percent of the women in Beijing, 8% in Kuala Lumpur and no women in Jakarta had red cell folate concentrations below 305 nmol/L, a cut-off value indicative of folate deficiency.[9]

Discussion

Our results have shown that red cell folate concentrations in women living in three major Asian cities are markedly different, with the highest concentrations in Jakarta and lowest in Beijing. Indeed, eighteen percent of women in Beijing had red cell folate concentrations that would place them at increased risk of folate deficiency anaemia, major health consequences of which would be fatigue and suboptimal cognitive performance.[10] Moreover, for women who become pregnant, low maternal folate status increases the risk of low birth weight[11] and birth defects.[6, 12]

The most likely explanation for the higher folate status in women in Jakarta is the implementation of mandatory folic acid fortification in 2001 of domestic and imported wheat, at a dose of 200 µg per 100 g;[13] neither China nor Malaysia have a mandatory fortification scheme. Mean red cell folate concentrations in Jakarta (872 nmol/L) are slightly higher than those reported in the United States post-fortification (787 nmol/L), which suggests a higher intake of total folate (naturally occurring and added) by women in Jakarta.[14, 15] If consumption of wheat flour is similar between the two countries, the slightly higher red cell folate concentrations in Jakarta are consistent with the higher level of fortification in Jakarta than in the United States (140 µg/100 g). In countries without mandatory fortification, such as Malaysia and China, the main determinant of red cell folate is dietary folate intake.[16] Although we did not assess folate intake, the differences in red cell folate concentrations between Kuala Lumpur and Beijing will reflect differences in the folate consumption of women in the cities.

We cannot generalize the results to all women living in China, Indonesia, and Malaysia because convenience samples of women living in the three urban centres were recruited that may not be representative of the population. Whether these women have higher or lower folate status compared with other urban-dwelling or rural women is not known. Nevertheless, our red cell folate concentrations in women living in Beijing are consistent with those reported by Hao et al, where a representative sample of Beijing women (n=205) living in urban or rural settings had a mean red cell folate of 508 nmol/L; a difference of 55 nmol/L compared with women in our study.[5]

In many countries NTD incidence rates are unknown, may not include pregnancy terminations, or may not be based on complete ascertainment of all pregnancy outcomes. In the absence of NTD incidence data, it is possible to predict NTD rates using results from a case-control study conducted in Ireland which quantified the relation between red cell folate and the risk of NTDs.[1] Though applying the formula to predict NTD rates in other populations has limitations, because there are factors other than folate status that determine NTD rates, this approach was used by COMA[17] when considering mandatory fortification in the United Kingdom. Furthermore, Wald et al.[7] used this model to compare predicted and actual changes in NTD rates from large randomized trials and mandatory fortification in the United States. Applying the equation of Daly et al.[1] to our red cell folate data suggests that the NTD rate in Beijing is twice that of Jakarta. The applicability of NTD risk estimates found in Irish women has not been confirmed in an Asian setting; however, the totality of evidence is unequivocal that implementing folic acid fortification in areas with low folate status reduces NTD rates.[3, 5, 6, 18]

Our results indicate low folate status amongst women living in Beijing and suggest that of the three cities improving the folate status of women in Beijing would have the greatest impact on NTD rates.

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三大亚洲城市的红血球叶酸及神经管畸形的预测发生率

背景：妊娠期间的叶酸摄取有效降低神经管畸形的风险。红血球的叶酸水平及神经管畸形的风险是互为相比的。现今的很多国家都缺乏神经管畸形发生率的相关资料。育龄妇女的红血球叶酸水平也许是可代替神经管畸形发生率的另一种指标，并且可用于确认那一些从提高叶酸水平计划当中最可能受惠的国家或区域。

目的：使用三大亚洲城市的育龄妇女之红血球的叶酸水平以预测神经管畸形的发生。

方法：采用断面式方法以录取居于北京(人数：220)，吉隆坡(人数：389)及雅加达(人数：129)的未孕妇女参与此项调查。

結果：雅加达的妇女拥有最高的(p<0.001)红血球叶酸水平，872 nmol/L (95% CI; 833, 910)，其次是吉隆坡妇女，叶酸水平高达674 nmol/L (95% CI: 644, 704)。北京妇女的叶酸水平为之最低，只达563 nmol/L (95% CI: 524, 601)。有鉴于此，预测北京的神经管畸形的发生率将是最高，达至30/10000 (95% CI: 27, 33)，接着是吉隆坡，24/10000 (95% CI: 22, 25)，而雅加达的发生率将是最低，只是15/10000 (95% CI: 14,15)。

結論：根据我们的红血球叶酸研究结果显示，在这三大城市中，北京将是在提高叶酸水平以降低神经管畸形的发生率的计划中最为受惠的城市，并拥有最显着的效果。

關鍵詞：红血球叶酸，神经管畸形，食品强化，亚洲，妇女。