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Folate and vitamin B$_{12}$ status and dietary intake of anaemic adolescent schoolgirls in the delta region of Myanmar

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Abstract

The aim of the present study was to assess the prevalence of deficiency of folate and vitamin B$_{12}$ and, simultaneously, the nutrient intake adequacy of folate, vitamin B$_{12}$, iron, vitamin A, vitamin C, vitamin B$_6$ and calcium in 391 adolescent anaemic (Hb < 120 g/l) schoolgirls living in the delta region of Myanmar (Burma). Dietary intakes were assessed using a 3 d estimated food record. The distribution of observed intakes calculated from the food records were adjusted for usual intakes, and the prevalence of inadequacy was estimated using the estimated average requirement cut-point method. Median (first, third quartile) serum folate and vitamin B$_{12}$ concentrations were 6·5 (4·6, 8·5) nmol/l and 612·8 (443·2, 795·2) pmol/l, respectively. The prevalence of folate deficiency defined as <6·8 nmol/l was 54 %; however, vitamin B$_{12}$ deficiency defined as <148 pmol/l was negligible (<1 %). The prevalence of inadequate intake of folate was high (100 %) as was the prevalence of inadequate intakes of vitamin A, vitamin C, vitamin B$_6$ and calcium, ranging from 60 to 100 %. Red meat or poultry was rarely consumed, but fish was consumed on a daily basis. Green leafy vegetables were also consumed frequently but consumption of dairy products was uncommon. Folate deficiency was high, and the prevalence of inadequate intake of folate among other key micronutrients was relatively common in this sample of anaemic adolescent schoolgirls. Appropriate strategies such as food fortification and dietary diversification are needed to improve the micronutrient status of these young women to ensure optimal health and future reproductive success.

Key words: Serum folate: Dietary folate intake: Vitamin B$_{12}$: Adolescent girls: Myanmar

Folate and vitamin B$_{12}$ are essential for optimal growth, development and maintenance of health throughout all life stages. Folate is involved in the synthesis and methylation of DNA, and vitamin B$_{12}$ is an important cofactor in folate metabolism[1]. Dietary inadequacies of these nutrients can lead to impaired DNA synthesis and megaloblastic anaemia. During pregnancy, deficiencies of folate and vitamin B$_{12}$ are associated with a number of negative health outcomes[1–4], including the well-established link between inadequate folate intake during the periconceptional period and increased risk of congenital neural tube defects.

Folate-rich food sources include leafy vegetables, legumes as well as nuts, fruits and berries. With the exception of liver, meat is not a good source of folate. In contrast, vitamin B$_{12}$ is found almost exclusively in animal source foods including red meat, poultry, fish and dairy products. In developing countries, meeting the recommended dietary requirement of folate and vitamin B$_{12}$ may be difficult to achieve by food consumption alone due to the high cost and limited availability of folate-rich and/or vitamin B$_{12}$-rich foods. Daily iron and folic acid supplementation for pregnant women is recommended as part of antenatal care[5]; however, these programmes reach women later in pregnancy and compliance is often poor. Thus, additional cost-effective interventions such as food fortification may be needed to prevent the prevalence of inadequate intakes.

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Abbreviation: EAR, estimated average requirement.

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The World Health Organization[6] recommends the collection of food and nutrient intake data along with information on nutritional status of the target population before planning and implementing any fortification programme. In Myanmar, a country in South East Asia, food fortification is non-existent with the exception of universal salt iodisation. Earlier evidence suggested that folate status of the population may be poor[7], but little work has been carried out recently to assess the nutrient intake of the population with no data on the adequacy of folate and vitamin B_{12} intakes in women of reproductive age. Recently, we reported a high prevalence of anaemia (59 %, Hb < 120 g/l) and coexistence of iron deficiency and suboptimal vitamin A status in a group of adolescent schoolgirls in the delta region of Myanmar[8,9]. In this study, we provide additional information on the folate and vitamin B_{12} status of the adolescent girls who were anaemic at the time. We also investigated the prevalence of inadequate intake of folate, vitamin B_{12} and other micronutrients including vitamin A, vitamin C, vitamin B_6 and calcium in the same girls.

Methods

This study used data collected at baseline of an interventional study that investigated the role of inflammation on iron and vitamin A levels during iron and vitamin A supplementation in anaemic adolescent females (ClinicalTrials.gov ID: NCT 01198574). Details of data collection on socio-demographics, growth and baseline iron and vitamin A levels were described earlier[9]. In brief, participants aged between 13 and 19 years were recruited in July 2010 from Nyaung Done Township, a peri-urban area in Ayeyarwady division, located in the delta region of Myanmar. Post-menarcheal schoolgirls, with anaemia (Hb < 120 g/l), not suffering from any major illness or disease at the time of data collection were recruited to the study. Those with severe anaemia (Hb < 70 g/l) were excluded and treated with iron supplements. A total of 402 participants were recruited, and data were complete for 391 participants. A socio-demographic questionnaire was administered to the mother or caregiver of each schoolgirl, and anthropometric measurements including weight and height were taken using standardised techniques with the participant wearing light clothing and no shoes[10].

Ethical approval of the study protocol was obtained from the Faculty of Medicine, University of Indonesia (128/PT 02.FK/ETIK/2010), and from the Department of Medical Research, Lower Myanmar (18/Ethics 2010 DMR-Lower Myanmar). Written informed consent was obtained from the parents or guardians of the adolescent girls who participated in the study.

Dietary assessment

Dietary intakes were calculated from estimated food records. Participants were trained on how to record dietary intake for a 3 d non-consecutive period. They were provided with pictures to assist them with estimating portion size in household measure units, which were then converted into gram intakes. Nutrisurvey software (www.nutrisurvey.de) was used to calculate the usual energy and nutrient intakes from the food using nutrient values derived from an existing Myanmar food composition table supplemented, where necessary, with data from Indonesia food composition tables[11–13]. The missing values for folate and B_{12} data were imputed from United States Department of Agriculture food composition table, except for manufactured foods[14].

Goldberg's method was used to check under-reporting, and under-reporters were not included in the results[15]. Software of Intake Distribution Estimation (PC-SIDE, Iowa State University) was used to calculate the proportion of subjects with inadequate intake using the estimated average requirement (EAR) cut-point approach. EAR for nutrients were calculated from the FAO/WHO requirement estimates using appropriate recommended conversion factors[6]. For the intake of iron, the probability approach was used as the EAR approach was not appropriate for menstruating girls[6].

Dietary patterns of the girls were also assessed by interview using a fifty-item FFQ designed to assess usual intake of iron and vitamin A. The FFQ was administered during the midline (sixth week) of the study. For vitamin A from β-caroetene, a conversion factor of 1/6 was used, whereas for other carotenoids a conversion factor of 1/12 was used. The FFQ used in this study was developed in order to include major food sources of iron and vitamin A, which was aligned with the objective of the main intervention study. The foods included in the FFQ were obtained from the list of foods reported in 24 h dietary recalls of the same subjects at baseline. Standard portions were assigned for each food item and respondents reported their usual portion as a proportion or multiple of their standard portion.

Biochemical assessment

Serum folate concentrations were measured using microbiological assays according to the methods of O'Broin and Kelleher[16] and Molloy and Scott[17] in a ninety-six-well plate (Costar; Corning Inc.) using the chloramphenicol-resistant *Lactobacillus rhamnosus* (ATCC 27773). The National Institute of Standards and Technology Standard Reference Material 1955 (three levels) was included for each plate run (*n* 8), with analysis yielding a folate content for level 1 of 5·5 (SD 0·37) nmol/l (inter-assay CV of 6·9 %) (certified value 5·6 (SD 1·2) nmol/l), for level 2 of 14·3 (SD 1·3) nmol/l (CV of 9·1 %) (certified value 14 (SD 3) nmol/l) and for level 3 of 44·4 (SD 4·5) nmol/l (CV of 4·5 %) (certified value 44 (SD 11) nmol/l). Cut-off values for the assessment of folate status were defined using values proposed by the WHO, with deficiency defined as serum folate concentrations <6·8 nmol/l, marginal as 6·8–13·4 nmol/l and normal folate status >13·4 nmol/l[18].

Serum vitamin B_{12} concentrations were assayed using the Elecsys® 2010 (Roche Diagnostics) automated electrochemiluminescence immunoassay. The control samples provided by the manufacturer were within the recommended range, and the inter-assay CV based on pooled serum was 3·2 % (*n* 9). Vitamin B_{12} status was defined as normal (serum vitamin B_{12} > 221 pmol/l), marginal (148–221 pmol/l) or deficient (<148 pmol/l)[19].

Statistical analysis

Statistical analyses were performed using statistical software package SPSS (SPSS) version 15.0 for windows. Normality of distribution of the variables was checked using the Kolmogorov–Smirnov test. Differences between this study group and the overall group from screening stage, in mean socio-demographics and anthropometric measurements, were examined using an independent sample *t* test. Dietary intakes of the participants were expressed as median (first, third quartile) for consistency because of non-normal distributions of some nutrients. A *P* value <0·05 indicated statistical significance.

Results

Serum folate and vitamin B_{12} concentrations were available for 389 and 391 adolescent schoolgirls, respectively. For dietary intake analyses, data were available for 391 participants.

The mean age of the girls was 15·9 (SD 1·2) years, with age at menarche of 13·2 (SD 0·9) years. Overall, 31 % of the girls were stunted (HAZ < –2) and 16 % were classified as thin (BAZ <–2). There were no significant differences between the anaemic adolescent females in this study and the overall group for socio-demographic status and mean anthropometric measurements[8].

The proportion of females with normal, marginal and deficient folate and vitamin B12 status is shown in Table 1. Overall, 153 (39·3 %) participants had folate deficiency with 211 (54·2 %) having marginal folate status. In marked contrast, vitamin B_{12} concentrations were high among the adolescent schoolgirls, with only one participant (0·3 %) classified as B_{12} deficient.

Table 1. Biochemical indicators of folate and B_{12} status of participating anaemic adolescent girls*
(numbers and percentages)

	n	%
Serum folate (nmol/l)	389	
Normal (>13·5 nmol/l, %)	25	6·4
Marginal (6·8–13·4 nmol/l, %)	211	54·2
Deficient (<6·8 nmol, %)	153	39·3
Serum vitamin B_{12} (pmol/l)	391	
Normal (>221)	370	94·6
Marginal (148–221)	20	5·1
Deficient (<148)	1	0·3

* To convert serum folate to ng/l, divide by 2·677.

Table 2 shows the median (first, third quartiles) energy and nutrient intakes of the adolescent schoolgirls per day, along with the FAO/WHO EAR. The proportion of girls with intakes less than the EAR are also reported. The median estimated energy intakes of these adolescent schoolgirls 7154·6 kJ/d (1710 kcal/d) were slightly lower than the energy requirement for median physical activity level 7811·5 kJ/d (1867 kcal/d)[20]. Only 1·5 % of schoolgirls (*n* 6) were identified as under-reporters of energy intake using the Goldberg method. Median intake of folate was well below the EAR, with all the participants having an intake less than the EAR. Likewise, the prevalence of inadequate intakes for vitamin B_6 (97·7 %) and calcium (99·7 %) was very high. Dietary intake of vitamin A and vitamin C was slightly better among participants, yet remained a concern with the percentage of participants with intakes less than the EAR of 59 and 61 %, respectively.

Weekly consumption of relevant food groups and sub-groups based on the qualitative food frequency assessment is shown in Table 3. Red meat and organ meat were rarely consumed among the girls. Fish consumption was common, with half of the adolescent girls consuming fish on a daily basis. Nearly all the participants consumed vitamin A-rich green leafy vegetables and vitamin C-rich fruits on alternate days in a week, but dairy product consumption was uncommon. Table 4 is based on the food frequency assessment and depicts the food items most frequently consumed, their average portion sizes and folate content (μg/100 g). Water spinach, duck egg and rice were the major contributors of folate to the diet of the adolescent girls based on their weekly consumption. Similarly, Table 5 shows the main sources of B_{12} from the diets of these adolescent girls.

Discussion

The dietary folate intakes of the anaemic adolescent girls of this study were very poor, which was consistent with the high prevalence of biochemical folate deficiency. Adequate folate status among reproductive age women is critical, given the important biological role of folate in gene expression, cell division and reproduction. Moreover, this finding is a concern given our earlier reports of iron deficiency and low vitamin A

Table 2. Nutrient intake of participants (*n* 391) assessed using a 3 d food record
(Median and 25th–75th percentile)

	Intake			
	Median	25th–75th percentile	EAR	Proportion of girls with an inadequate intake
Energy (kcal)	1767	1540–2039		
Energy (kJ/d)	7393·1	6443·4–8531·2		
Protein (g)	53	45–63		
Vitamin A (μg RE)	344	208–571	428·6	62·3
Folate (μg)	125	90–184	320·0	94·3
Vitamin B_{12} (μg)	2·4	1·5–3·7	2·0	36·7
Vitamin B_6 (mg)	0·5	0·4–0·6	1·0	97·0
Vitamin C (mg)	40	22–61	54·2	68·5
Calcium (mg)	326	233–436	833·3	98·7
Iron (mg)*	11	8–15		66·8

EAR, estimated average requirement; μg RE, μg retinol equivalent.
* EAR should not be used for menstruating girls due to the high variability and the skewed nature of the distribution of the requirement for iron[6].

status among these reproductive age study participants[9]. Anaemia is a significant public health problem in Myanmar[21]. However, little or no attempt has been made to investigate the underlying causes for the high prevalence of anaemia in the country. Folate is one of the key nutrients for haemopoiesis, and the poor folate status among the population may contribute to the high prevalence of anaemia in the country among other factors including infection, genetic Hb disorders and deficiencies of iron, vitamin A, zinc and riboflavin[8,9,22–24]. In addition, both folate deficiency and iron depletion have shown to be associated with the severity of anaemia[25,26], suggesting that the suboptimal intake of both nutrients needs to be addressed concomitantly.

To our knowledge, our study is the first report on the folate status of adolescent females in Myanmar. Published data on folate status of any life-stage group in Myanmar are lacking, with the exception of an earlier report in 1976 showing a fairly low prevalence of folate deficiency (13 %) among a group of pregnant women[7]. Serum folate concentrations of the girls in the present study were much lower than those of reproductive age women from neighbouring countries such as Thailand, Bangladesh and Malaysia[27–29]. Low folate status is likely to be attributable to a predominant rice-based diet (which is low in folate) and a low intake of legumes and fruits (which are high in folate). In addition, Burmese usually consume well-cooked vegetables, and substantial amounts of folate may be lost during food processing, preparation and cooking[26,30–32].

Fortification of food with folic acid is considered to be one of the most cost-effective strategies to prevent folate deficiency with proven success in both developed and less-developed countries, including neighbouring South East Asian countries[33,34]. For example, the government of Indonesia mandated folic acid fortification of wheat flour nearly 15 years ago in an effort to mitigate the prevalence of folate deficiency[33]. A post-fortification report of red blood folate status of reproductive age women in Jakarta demonstrated the success of the programme, showing relatively high blood folate levels with no indication of deficiency[33,35].

Rice is a staple food in the delta region of Myanmar[36], accounting for approximately 45 % of the energy consumed in

Table 3. Weekly frequency of consumption of relevant food groups and sub-groups
(Median values and 10th, 90th percentiles)

	Frequency/week	
Food group/sub-group	Median	10th, 90th percentiles
Grains and grain products		
Rice	17	14, 21
Meat, fish, poultry		
Red meats	0·25	0, 2
Fish	7	1·8, 14
Poultry	0·5	0, 4
Organ meat	0	0, 1
Eggs	2	0, 6
Vegetables		
Vitamin A-rich dark green leafy vegetables	2	0, 2·7
Fruits		
Vitamin A-rich fruits	0	0, 1
Vitamin C-rich fruits	0	0, 2·7
Dairy products	0	0, 1

Table 4. Top contributing food sources of dietary folate and estimated daily intake among participating adolescent schoolgirls, determined using an FFQ

Food item	Average times consumed/week	Average portion size (g)	Folate content (μg/100 g)	Estimated folate intake (μg/d)
Cooked rice	16·6	250	2	11·8
Duck egg	1·9	50	80	10·8
Water spinach	2·0	50	73	10·4
Banana	1·4	113	20	4·6
Condensed fish paste	5·8	10	48	4·0
Guava	2·3	80	14	3·7
Cucumber	2·4	50	19	3·2
Fish	6·4	30	8	2·2
Apple	1·4	200	3	1·2
Fish sauce	1·7	10	29	0·7

Table 5. Top contributing food sources of vitamin B_{12} and estimated daily intake among participating adolescent schoolgirls, determined using an FFQ

Food item	Average times consumed/week	Average portion size (g)	B_{12} content (μg/100 g)	Estimated B_{12} intake (μg/d)
Duck egg	1·8	50	5·3	0·68
Fish	5·0	30	1	0·21
Prawn	1·2	35	1·3	0·08
Milk	0·4	200	0·4	0·05
Chicken egg	0·5	40	1·1	0·03
Fish sauce	1·5	10	1·4	0·03
Pork	0·6	30	1	0·03
Beef	0·3	30	1·8	0·02
Fish paste	3·7	10	0·4	0·02
Liver	0·3	30	0·9	0·01

our study participants (data not shown). Although fortification of rice or other increasingly popular wheat-based foods such as instant noodles can contribute to improving the intake of key problem nutrients[37], the decision to fortify a widely consumed food staple with folic acid is influenced by a number of factors including the concern of adverse effects due to low vitamin B_{12} status[38,39]. For example, the Pune Indian study revealed that high folate intakes in vitamin B_{12}-deficient mothers may contribute to increased adiposity and insulin resistance in the offspring[38,40]. Remarkably, vitamin B_{12} deficiency in our population of adolescent females was rare, occurring in only one woman. According to the dietary data, frequent consumption of eggs and fish are likely to have contributed to the very low prevalence of biochemical vitamin B_{12} deficiency (Table 5). Unlike western diets, milk is not commonly consumed among the Burmese population, but fish was a significant contributor. Fish, however, is not a good source of iron, and red meat was not commonly consumed in this predominantly Buddhist group of participants.

In addition to previous reports of poor iron intake in this sample group[9], the present study revealed a widespread risk of co-existing micronutrient dietary inadequacies in this sample population of adolescent anaemic females. Food consumption data indicated that a significant proportion of the girls did not consume foods rich in iron, vitamin A and calcium such as liver, milk and eggs. Although consumption of green leafy vegetables was common – such as water spinach, which is available all year-round – the amounts consumed were likely to be insufficient to fulfil the recommended nutrient requirements for folate. Fruits and dairy products were also not consumed often, which is consistent with previous reports[41]. Additional work is needed to determine whether it is possible to formulate a balanced diet using nutrient-dense locally available and culturally acceptable foods in this age group. This can be achieved using linear programming, a mathematical approach designed to optimise the diet[42]. In doing so, linear programming takes into account the existing food selection pattern, nutritional goals and cost constraints. If local foods are unable to provide the desired key nutrient levels, linear programming can be used to formulate new food products based on local commodities and determine the need and potential impact of food fortification or supplementation strategies.

This study had several limitations. First, this study was conducted in one of the townships in the delta region of the country, and thus cannot be generalised to the whole country. However, the dietary patterns observed in our study participants are typical of the dietary habits throughout rural Myanmar, although they do not reflect seasonal variability in intakes. Second, nutrient composition of food items was acquired from different food composition tables, which may not accurately reflect the nutrient composition of the food. It is appreciated that there is variability in the composition of foods between countries, likely due to differences in season, cultivar or variety.

In summary, our study highlights the poor folate status and the coexistence of multiple micronutrient inadequacies in the diet of these anaemic adolescent schoolgirls, with the most common deficits being folate, calcium and vitamin B_6. Despite the very low intake of meat and poultry, the prevalence of vitamin B_{12} deficiency was very low, demonstrating the valuable contribution of fish in the diet with regard to dietary vitamin B_{12} intake in this population group. Many of these young women will be married and pregnant within the next few years. Given the high demands for folate and iron during pregnancy, urgent survey work is needed to inform recommendations to improve the micronutrient health and the reproductive success of young Burmese women.

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