

The influence of vitamin A status on iron-deficiency anaemia in anaemic adolescent schoolgirls in Myanmar

Min Kyaw Htet^{1,2,*}, Umi Fahmida¹, Drupadi Dillon^{1,3}, Arwin Akib³, Budi Utomo⁴ and David I Thurnham⁵

¹South East Asian Ministers of Education Organization, Regional Center for Food and Nutrition, University of Indonesia, Salemba Raya 6, Jakarta 10430, Indonesia: ²Department of Health, Ministry of Health, Nay Pyi Taw, Myanmar: ³Faculty of Medicine, University of Indonesia, Jakarta, Indonesia: ⁴Faculty of Public Health, University of Indonesia, Depok, Indonesia: ⁵Northern Ireland Centre for Food and Health, School of Biomedical Science, University of Ulster, Coleraine, UK

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Abstract

Objective: The present study was conducted to investigate reasons for the high prevalence of anaemia among adolescent schoolgirls and to elucidate the role of vitamin A in contributing to Fe-deficiency anaemia (IDA).

Design: Among 1269 schoolgirls who were previously screened for anaemia (Hb < 120 g/l), 391 anaemic girls were further assessed for Fe, vitamin A and subclinical inflammation status. Fe and vitamin A indicators were corrected for inflammation and were compared in the Fe-deficient and non-deficient groups as well as between those with and without inflammation. Logistic regression was done to determine whether vitamin A status and subclinical inflammation were risk factors for Fe deficiency. The differences in Fe status among tertiles of vitamin A concentrations were assessed using ANOVA.

Setting: Myanmar.

Subjects: Adolescent schoolgirls (*n* 391).

Results: One-third of the anaemia (30.4%) was IDA. Prevalence of low vitamin A status (serum retinol < 1.05 µmol/l) was 31.5%. Fe and vitamin A status were significantly different between the IDA and non-IDA groups and also based on their inflammation status. Logistic regression showed that low vitamin A status was a significant predictor for being Fe deficient (OR = 1.81; 95% CI 1.03, 3.19 and OR = 2.31; 1.31, 4.07 in the middle (1.056–1.298 µmol/l) and low (≤ 1.056 µmol/l) vitamin A tertiles, respectively). ANOVA showed that better Fe status was associated with a higher concentration of serum retinol but only in IDA.

Conclusions: Fe deficiency was not the main cause of anaemia in the present population. The role of vitamin A as well as other micronutrients should be taken into account in addressing the problem of anaemia.

Keywords
Anaemia
Iron deficiency
Adolescents
Myanmar

Adolescence is a period of vulnerability as well as opportunity. Myanmar, also known as Burma, is a country in the South-East Asia region where about 20% of the demographic is adolescent and will be the future economic force of the country⁽¹⁾. Anaemia is one of the major nutritional problems in the region as well as in the country and affects all age groups, especially children and women⁽²⁾. Studies have shown that preconceptional anaemia is a significant predictor of poor pregnancy outcomes^(3,4) and thus preconceptional anaemia and Fe status of young women is a focus of our recent research. However, health and nutritional status information is limited for this age group. In the recently conducted survey in the delta region of Myanmar among 1269 adolescent

schoolgirls, we showed that the prevalence of anaemia was 59.1%⁽⁵⁾. In the present paper we give further information on the Fe and vitamin A status among these anaemic girls.

Although Fe deficiency (ID) is a major cause of anaemia, other micronutrient deficiencies can also contribute, be equally important and coexist with ID^(6–8). Adolescence is a period of growth and development in which the growth is faster than at any other time in an individual's life, except the first year. This imposes a high demand for energy and nutrients. Through modulation of Fe metabolism, haematopoiesis and susceptibility to infection, vitamin A is believed to play a significant role in the development of anaemia⁽⁹⁾. Trials have shown that vitamin A supplements

have synergetic effects with Fe on anaemia and indicated that the supplements should be combined for greater effectiveness^(10,11). However, some studies could not find any benefit from vitamin A on Fe supplementation⁽¹²⁾. Nutritional status indicators of Fe and vitamin A are often misinterpreted in the presence of subclinical inflammation (SCI) and markers of inflammation should always be measured for correct interpretation^(13–15). Therefore, a closer look at the association of Fe and vitamin A in relation to anaemia is still necessary and because the causes of anaemia are multifactorial, the contribution of other micronutrients and SCI should be sought wherever possible.

In South-East Asia, tropical diseases such as malaria, dengue and haemorrhagic fever, worm infestations and genetic Hb disorders are common and all contribute to anaemia^(16,17). In Myanmar, reports show that malaria and dengue fever are the leading causes of morbidity and mortality and worm infestations are also common in the country^(18,19). The present study was conducted to investigate the factors contributing to the high prevalence of anaemia among adolescent schoolgirls in Myanmar and particularly the interdependence of vitamin A and Fe status in contributing to anaemia. In the present paper we report on the biochemical assessment of Fe and vitamin A status and anthropometry among anaemic adolescent schoolgirls from Myanmar and investigate the influence of vitamin A on Fe-deficiency anaemia (IDA).

Participants and methods

The data described in the present study were collected at the baseline of an intervention study conducted in the delta region of Myanmar (ClinicalTrials.gov ID: NCT 01198574). The objective of the intervention study was to investigate the role of SCI on the Fe and vitamin A status of anaemic girls during Fe supplementation. The present paper describes the baseline Fe, vitamin A and inflammation status of the girls prior to the supplementation.

Study site

The study was conducted in Nyaung Done Township, a peri-urban area in Ayeyarwady Division in the delta region of Myanmar where the highest prevalence of anaemia was reported to exist. It is located about a 3 h drive away (~40 km distance) from the former capital city of Yangon.

Participants

The participants were recruited in July 2010. The sample size was based on the requirements for the intervention study. It was calculated in order to detect a mean difference in Hb of 5 g/l between two groups, with 95% power at 5% level of significance based on a previous study in Indonesia⁽²⁰⁾. The sample size was seventy-three for each group and allowed for a dropout rate of 20%. To take

account of inflammation, the sample was increased to 100 and a total of 402 participants for four intervention groups were recruited.

Before the recruitment for the baseline study, a screening for anaemia and haemoglobinopathies was conducted among the adolescent schoolgirls (*n* 1269) from six schools in the township⁽⁵⁾. Postmenarcheal schoolgirls with anaemia (Hb < 120 g/l), not suffering from any major illness or disease at the time of data collection, were invited to join the study. Those with severe anaemia (Hb < 70 g/l) or those who took multivitamin supplements regularly for past three months were excluded⁽²⁾. The girls with severe anaemia were treated with Fe and not included in the study. Deworming with a single dose of albendazole (400 mg) was done in these girls one week before the blood collection. This treatment has been shown previously to effectively remove most gut parasites^(21,22) and we did not follow up to determine the effectiveness.

Ethical approval for the study was granted 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). The parents or caregivers were informed of the objectives of the study and their informed consent was obtained. The girls were also informed and the objectives and procedures in the study were explained. Those who fulfilled the inclusion criteria and gave their consent were invited to join the study.

Biochemical assessment

Phlebotomy was done by experienced nurses and 3 ml of non-fasting venous blood was taken from each participant in the morning school session. The blood samples were collected into non-heparinised vacuettes as well as into EDTA tubes and transported in ice-cooled containers to the laboratory of the Department of Medical Research, Lower Myanmar, within 4 h. Haemoglobin was measured using the EDTA-treated venous blood by the cyanmethaemoglobin method at the Nutrition Research Division, Department of Medical Research, Lower Myanmar. The Hb assessment was counter-checked with three different levels of external control (high, medium, low) from the National Health Laboratory on a daily basis and the CV of the assessment was $\leq 5.0\%$. After separation, serum samples were frozen in polyethylene vials and stored at -20°C for 3 months before further analyses were done. During the transport, the serum samples were packed in dry ice and kept frozen throughout the shipment to the laboratory of the SEAMEO-Regional Centre for Food and Nutrition, University of Indonesia, Jakarta, Indonesia. HPLC was used to measure serum retinol⁽²³⁾. The international certified NIST standards (National Institute of Standards and Technology, Gaithersburg, MD, USA) were used for quantification and the CV for the assessment was 4.94%. An in-house sandwich ELISA was used to measure serum ferritin (SF), soluble transferrin receptor (sTfR), retinol-binding

protein (RBP), C-reactive protein (CRP) and α_1 -acid glycoprotein (AGP) in 200 μ l of serum⁽²⁴⁾. For the quality assurance, certified quality control samples from the Centers for Disease Control and Prevention (Atlanta, GA, USA) and Bio-Rad Liquicheck controls (Bio-Rad, Munich, Germany) were used. The CV for all measurements were $\leq 5.0\%$ (3.68%, 3.00%, 2.91%, 4.68% and 3.30% for SF, sTfR, RBP, CRP and AGP, respectively). Body Fe store was calculated using the method of Cook *et al.*⁽²⁵⁾ which has been applied across different age groups⁽²⁶⁾.

Definition of Fe deficiency, Fe-deficiency anaemia, vitamin A deficiency, low vitamin A store and subclinical inflammation

Anaemia was defined when Hb was <120 g/l⁽²⁷⁾, ID when SF was <15 μ g/l and/or sTfR was >8.5 mg/l, and IDA by the occurrence of ID and anaemia^(28,29). A high risk of vitamin A deficiency was defined when serum retinol or RBP concentration was <0.7 μ mol/l and low vitamin A status was defined when retinol or RBP concentration was <1.05 μ mol/l⁽³⁰⁾. SCI was defined when serum CRP concentration was >5 mg/l and/or AGP concentration was >1 g/l^(13,14). Participants were grouped into four categories according to their acute-phase proteins CRP and AGP: (i) healthy (both CRP and AGP normal); (ii) incubation (CRP high and AGP normal); (iii) acute convalescence (both CRP and AGP high); and (iv) late convalescence (CRP normal and AGP high). The values of serum retinol and SF were corrected using meta-analysis correction factors obtained from Thurnham *et al.*^(13,14,31).

Dietary assessment

Three non-consecutive-day estimated food records were recorded by the participants after explanation on how to complete the form by the Principal Investigator (M.K.H.). Participants were given pictures to help them choose the closest portion size (in household measure units) which were later converted into gram intakes. Dietary intake data were entered into Nutrisurvey software (www.nutrisurvey.de) to calculate usual dietary Fe intake, i.e. the average of the three days. The nutrient composition database from the Myanmar food composition table was used, supplemented with data from the Indonesian food composition table.

Anthropometry assessment

Anthropometric assessments were carried out by trained persons. Body weight was measured using a flat electronic weighing scale (SECA 874, Hamburg, Germany) to the nearest 0.1 kg and height measured by a measuring tape (Microtoise SECA 206) to the nearest 0.1 cm. Nutritional status was assessed using Z-scores and the WHO Anthro-Plus software was used for the analysis. Stunting was defined when height-for-age Z-score was <-2 and thinness was defined as BMI-for-age Z-score of <-2 ⁽³²⁾. BMI was weight divided by the square of the height (kg/m²).

Statistical analysis

Descriptive data were presented as means or geometric means with their standard deviations for continuous variables and as proportions for categorical data. Normality was checked with the Kolmogorov–Smirnov test and data were transformed if not normally distributed. Hb, body Fe store, serum retinol and RBP were normally distributed and SF and sTfR concentrations were transformed using logarithms. Analysis of the difference in plasma concentrations of Fe and vitamin A status indicators between groups was done using ANOVA or the independent-sample *t* test in both log-transformed and arithmetic data. *Post hoc* analysis using least significant difference was applied when ANOVA was statistically significant. Multivariate logistic regression was used for predicting the risk of IDA. Participant age, school cluster, dietary Fe intake and BMI category were included in the model as independent variables. Results were presented as odds ratios with 95% confidence intervals. A two-sided *P* value of 0.05 was considered significant. Statistical analyses were performed using the statistical software package SPSS version 15.0 for Windows.

Results

In the original sample of 1269 adolescent girls, there were 729 with anaemia of whom 414 were eligible according to the inclusion criteria. Of the 414 eligible girls, twelve did not attend school on the blood collection days and data were incomplete for eleven. Therefore data from 391 girls were analysed, and Table 1 shows their general characteristics and nutritional status. Most girls had Burmese ethnicity and were Buddhists. The prevalence of stunting and thinness was 31.1 and 15.7%, respectively. Mean usual Fe intake was 11.7 (SD 4.8) mg/d. Table 2 shows the percentage of girls with abnormal Fe, vitamin A and inflammation status indicators. It also shows that correction of Fe and vitamin A data for inflammation using published correction factors had only a very small effect on the prevalence of low Fe and vitamin A status. Both serum retinol and RBP were measured in the present study and there was a significant correlation between the two indicators ($r=0.63$, $P<0.001$). For the remainder of the current analyses serum retinol concentration is used as the primary indicator of vitamin A status; however, data for RBP are presented in the text and the online supplementary material.

The mean Hb concentration of the girls was 88.9 (SD 11.8) g/l and 30.4% (n 119) of anaemia was IDA (Table 3). Although the prevalence of vitamin A deficiency (serum retinol <0.70 μ mol/l) among the girls was low (1.8%), the prevalence of low vitamin A status (serum retinol <1.05 μ mol/l) was relatively high (31.5%). Furthermore, there were slightly more girls with low vitamin A status in those with IDA (37.0%) than in those without IDA (29.0%, $P=0.125$). SCI, indicated by raised serum CRP

Table 1 Characteristics of the adolescent schoolgirls (*n* 391), delta region of Myanmar, 2010

Variable	<i>n</i>	%	Mean	SD
Age (years)			15.9	1.2
Age at menarche (years)			13.2	0.9
Religion (%)				
Buddhist	378	96.7		
Christian	8	2.0		
Hindu	1	0.3		
Muslim	4	1.0		
Ethnicity (%)				
Burmese	223	57.0		
Kayin	95	24.3		
Indian	4	1.0		
Mixed	69	17.7		
Father's education status*				
Illiterate	22	5.6		
Primary school	176	45.0		
Middle school	108	27.6		
High school	63	16.1		
Graduate	12	3.1		
Mother's education status*				
Illiterate	23	5.0		
Primary school	222	56.8		
Middle school	85	21.7		
High school	44	11.3		
Graduate	12	3.1		
Father's occupation status (%)				
Farmer	210	53.7		
Free labourer	62	15.9		
Vendor	41	10.5		
Unemployed	38	9.7		
Government employee	30	7.7		
Mother's occupation status (%)				
Farmer	141	36.1		
Housewife	129	33.0		
Vendor	82	20.0		
Free labourer	22	5.6		
Government employee	12	3.1		
Nutritional status				
Weight (kg)			41.8	6.2
Height (m)			1.5	0.1
BMI (kg/m ²)			18.3	2.3
BMI classification by Asian standard				
Undernutrition (<18.5 kg/m ²)	221	56.5		
Normal (18.5–22.9 kg/m ²)	160	40.9		
Overweight (23.0–27.4 kg/m ²)	7	1.8		
Obese (≥27.5 kg/m ²)	3	0.8		
Height-for-age Z-score			-1.70	0.8
BMI-for-age Z-score			-1.19	0.9
Prevalence of stunting (%)†	122	31.1		
Prevalence of thinness (%)†	61	15.7		
Dietary Fe intake (mg/d)			11.7	4.8

*Ten fathers and five mothers had passed away.

†Stunting and thinness defined as height-for-age Z-score < -2 and BMI-for-age Z-score < -2, respectively.

and/or AGP concentrations, was found in only 6.6% of the girls. Fe, vitamin A and SCI status of the girls were compared between non-IDA and IDA groups; Hb, Fe and vitamin A status indicators were significantly different between the two groups while inflammation status was not.

The effect of SCI on Fe and vitamin A status was investigated by comparing Fe and vitamin A status indicators of the girls in the presence and absence of inflammation (data not shown). Results showed that apart from Hb and sTfR concentrations, other indicators were significantly

different depending on inflammation status. However, since the prevalence of girls with SCI was low, SF and vitamin A data were corrected for the influence of SCI for the remainder of the analyses^(13,14) (Table 4, Table 5 and Supplemental Table 2).

Logistic regression was done adjusting for age, clustering by school, Fe intake and BMI category. The results showed that among these anaemic adolescent girls, poor vitamin A status was a significant predictor for IDA. In the two lower vitamin A tertiles (serum retinol ≤1.056 μmol/l and 1.056–1.298 μmol/l), the risk of IDA was two times higher than in the upper vitamin A tertile (serum retinol >1.298 μmol/l). IDA was not significantly associated with other risk factors studied such as age, BMI, clustering by school, Fe intake and inflammation (Table 4). When the analysis was run using RBP (tertiles for RBP were ≤1.232, 1.232–1.465 and >1.465 μmol/l), the same pattern was observed even though it failed to reach statistical significance and be a significant predictor for IDA (Supplemental Table 1).

Fe status indicators of the girls were compared across tertiles of vitamin A status. For all girls, results showed that all the Fe status indicators except Hb were significantly different among the tertiles of serum retinol concentrations. *P* values from ANOVA were 0.251, 0.003, 0.02 and 0.001 for Hb, SF, sTfR and body Fe store, respectively (results not shown).

When the girls were stratified by their Fe status (IDA and non-IDA), their vitamin A status did not influence Hb and sTfR for both the IDA and the non-IDA group. However, in the IDA group, poor vitamin A status was significantly associated with low SF concentration and low body Fe stores. The sTfR concentration showed a similar trend but it failed to reach statistical significance. No relationships between the Fe status indicators and vitamin A status were observed in the non-IDA group (Table 5). When the analysis was done using RBP, similar findings were obtained in the IDA group for SF (9.4, 17.2, 16.7 μg/l; *P* = 0.04) except that differences between body Fe stores (-1.8, 0.22, 0.41 mg/kg, *P* = 0.07) failed to reach statistical significance (Supplemental Table 2).

Discussion

In the present study, we have found that 30% of anaemia cases were due to ID. More importantly, not only low retinol concentration (≤1.056 μmol/l) but also moderate retinol concentration was associated with significantly increased risk of IDA. Among these anaemic adolescent girls, the association between vitamin A and Fe status indicators (SF and body Fe stores) was found to be significant only in those with IDA. These findings were consistent when RBP was used instead of serum retinol.

The results from the study suggest that a third of the anaemic adolescent girls have poor Fe and/or

Table 2 Abnormal iron, vitamin A and inflammation status biomarkers among adolescent schoolgirls (*n* 391), delta region of Myanmar, 2010

	Median	Range (1st–3rd quartile)	Cut-off	Deficient or abnormal (%)
Hb (g/l)	89.7	81.1–96.7	<120	99.5
SF (μg/l)	37.5	20.6–56.5	<15	19.9
MA-corrected SF (μg/l)*	36.9	20.2–54.2	<15	19.9
Serum sTfR (mg/l)	6.4	5.4–8.0	>8.5	16.6
Body Fe store (mg/kg)	4.9	2.1–6.7	≤0	16.4
Serum RBP (μmol/l)	1.3	1.2–1.6	<0.70	1.0
MA-corrected RBP (μmol/l)*	1.4	1.2–1.6	<1.05	13.0
Serum retinol (μmol/l)	1.2	1.0–1.4	<0.70	1.8
MA-corrected serum retinol (μmol/l)*	1.2	1.0–1.4	<1.05	30.4
CRP (mg/l)	0.2	0.1–0.3	>5	2.6
AGP (g/l)	0.7	0.6–0.8	>1	6.6

SF, serum ferritin; MA, meta-analysis; sTfR, soluble transferrin receptor; RBP, retinol binding protein; CRP, C-reactive protein; AGP, α₁-acid glycoprotein.
*Correction factors obtained from Thurnham *et al.*^(13,14,31).

Table 3 Iron, vitamin A and inflammation status according to IDA status* among adolescent schoolgirls (*n* 391), delta region of Myanmar, 2010

	Non-IDA (<i>n</i> 272)		IDA (<i>n</i> 119)		<i>P</i> value†
	Mean	SD	Mean	SD	
Hb (g/l)	90.9	10.9	84.3	12.5	<0.001
SF (μg/l)	44.1	1.67	13.5	3.51	<0.001
Serum sTfR (mg/l)	5.75	1.22	10.34	1.6	<0.001
Body Fe store (mg/kg)	5.9	2.1	−0.5	4.9	<0.001
Serum RBP (μmol/l)	1.39	0.32	1.31	0.28	0.018
Serum retinol (μmol/l)	1.24	0.33	1.15	0.27	0.013
Vitamin A deficiency (%)‡	0.7		1.7		0.588
Low vitamin A (%)§	29.0		37.0		0.125
CRP (mg/l)	0.21	4.18	0.17	3.85	0.263
AGP (g/l)	0.73	1.24	0.72	1.26	0.503
SCI (%)	7.4		5.0		0.510

IDA, Fe-deficiency anaemia; SF, serum ferritin; sTfR, soluble transferrin receptor; RBP, retinol binding protein; CRP, C-reactive protein; AGP, α₁-acid glycoprotein; SCI, subclinical inflammation.

*Geometric means and standard deviations for SF, serum sTfR, CRP and AGP.

†Independent *t* test (for continuous variables) or χ^2 test (for categorical variables) was used for comparing the Fe and vitamin A status indicators between the groups.

‡Serum retinol <0.70 μmol/l.

§Serum retinol <1.05 μmol/l.

||SCI defined as CRP >5 mg/l and/or AGP >1 g/l.

Table 4 Risk factors for IDA among adolescent schoolgirls (*n* 391), delta region of Myanmar, 2010

	OR	95% CI OR	<i>P</i> value*
Serum retinol			
Highest tertile (>1.298 μmol/l), ref.	1.000	–	0.013
Lowest tertile (≤1.056 μmol/l)	2.314	1.314, 4.073	0.004
Middle tertile (1.056–1.298 μmol/l)	1.814	1.031, 3.191	0.039
SCI†	1.592	0.595, 4.263	0.355

IDA, Fe-deficiency anaemia; ref., reference category; SCI, subclinical inflammation; C-reactive protein; AGP, α₁-acid glycoprotein.

*Logistic regression adjusted for age, school cluster, dietary Fe intake and BMI category, significant at *P* < 0.05.

†SCI defined as CRP >5 mg/l and/or AGP >1 g/l.

vitamin A status. Generally, nutritional status of the girls did not differ much compared with the data reported by the National Nutrition Center⁽³³⁾ but a 31.1% prevalence of stunting is relatively high and represents the existence of chronic nutritional problems among the population. More than half (56.5%) of the girls were underweight

according to their BMI. In previous surveys in Myanmar, the Fe status of the population was not comprehensively studied and Hb only was used as a proxy indicator of ID. The prevalence of anaemia among adolescents was reported to be 26.0% according to data from the National Nutrition Center⁽³³⁾. ID was assumed to be the main cause

Table 5 Comparison of mean Hb and iron status indicators across different serum retinol tertiles stratified by IDA status*† among adolescent schoolgirls (*n* 391), delta region of Myanmar, 2010

	Non-IDA (<i>n</i> 272)							IDA (<i>n</i> 119)						
	Tertile of serum retinol						<i>P</i> value‡	Tertile of serum retinol						<i>P</i> value‡
	Low (<i>n</i> 79)		Moderate (<i>n</i> 91)		High (<i>n</i> 102)			Low (<i>n</i> 48)		Moderate (<i>n</i> 44)		High (<i>n</i> 27)		
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Hb	91.9	11.4	89.3	9.9	91.5	11.4	0.237	83.3	13.6	84.7	12.9	85.4	10.2	0.753
SF	45.08	1.60	39.09	1.60	44.69	1.60	0.09	9.24 ^a	3.60	15.90 ^b	3.20	18.01 ^b	3.09	0.033
sTfR	5.68	1.25	5.85	1.20	5.73	1.22	0.59	11.23	1.61	9.92	1.66	9.55	1.47	0.277
Body Fe store	6.10	2.16	5.47	2.00	6.03	2.10	0.09	-2.10 ^a	5.37	0.27 ^b	4.02	0.99 ^b	4.51	0.011

IDA, Fe-deficiency anaemia; SF, serum ferritin; sTfR, soluble transferrin receptor.

^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).

*Vitamin A status classified by serum retinol tertile (lowest tertile $\leq 1.056 \mu\text{mol/l}$, middle tertile $1.056\text{--}1.298 \mu\text{mol/l}$ and highest tertile $> 1.298 \mu\text{mol/l}$).

†Mean and standard deviation for Hb and body Fe store, geometric mean and standard derivation for SF and sTfR.

‡One-way ANOVA, with *post hoc* analysis done using least significant difference.

of anaemia in the region and in the country at large. The findings from our study suggest that poor vitamin A may also be an important contributor to anaemia in Myanmar. The prevalence of ID among the anaemic adolescent girls in our study is comparable to that in other countries in South-East Asia. In Bangladesh, Thailand and Indonesia, the prevalence of ID was reported to be 33.0%, 21.5% and 27.3%, respectively^(7,20,34–36). In Vietnam, the prevalence of ID among women of reproductive age was reported to be as low as 8.9% while it contributed as high as 42% among adolescent Kenyan girls with anaemia^(12,37) suggesting that it is an important determinant. However, there is evidence for the coexistence of multiple micronutrient deficiencies in the Asian countries^(6,7) and therefore it is important that the status of other micronutrients, such as vitamin B₁₂ and folate, be properly examined in attempts to control anaemia.

Inflammation was not very prevalent among our study population and only 6.7% displayed evidence of SCI. Inflammation is the body's physiological response to infections and injuries and the purpose is to minimise damage to body tissues, although it can have negative implications for nutritional assessment^(38,39). However the low prevalence of SCI meant that inflammation had little impact on nutritional biomarkers in the present study (Table 2). The results also suggest that sTfR concentrations were not affected by SCI although other workers have reported different results^(40–42). The prevalence of SCI will depend on the intensity of infections and the age of the individuals. For example, a recent study from Cambodia showed SCI (i.e. elevated AGP) was a significant risk factor for anaemia among young children⁽¹⁶⁾. Similarly, another study from Papua New Guinea highlighted that SCI (i.e. elevated CRP and AGP) among children under 5 years old was significantly associated with the high prevalence of anaemia⁽⁴³⁾. However, in a study conducted among pre-school children from the Marshall Islands, it was reported that both Fe and vitamin A deficiencies, but not inflammation, were independent risk factors for anaemia⁽⁴⁴⁾. Our study in Myanmar suggests that

poor vitamin A status was the significant predictor of ID whereas inflammation was not.

Logistic regression showed a twofold higher risk of IDA when the concentration of serum retinol was in the middle and lower tertiles (Table 4). This finding suggests that good vitamin A status may be necessary to prevent ID. The interdependence of vitamin A and Fe has been reported in previous studies. Blackfan and Wolbach reported that anaemia as well as haemosiderosis of the spleen and liver could be reversed by repletion with vitamin A in vitamin A-deficient patients⁽⁴⁵⁾. Anaemia refractory to Fe supplementation but responsive to vitamin A was reported among vitamin A-deficient, healthy volunteers by Hodges *et al.*⁽⁴⁶⁾. A study from Guatemala indicated that vitamin A was probably important in mobilising Fe stores where a vitamin A-fortified sugar programme was implemented among pre-school children⁽⁴⁷⁾. A randomised controlled trial from Indonesia showed an increase in SF concentrations by 14.9 $\mu\text{g/l}$ when vitamin A-deficient children were treated with vitamin A supplements⁽⁴⁸⁾. These studies suggest that vitamin A may directly enhance erythropoiesis as well as the mobilisation of Fe from storage. Similarly, other studies have confirmed the beneficial effect of additional vitamin A to Fe supplementation^(10,11,49,50) while others found no benefit^(12,51,52).

Further analysis of Fe status in the vitamin A tertiles showed that Fe status was better in the two groups with the higher vitamin A status (i.e. serum retinol $> 1.056 \mu\text{mol/l}$). Similar findings were reported before, where higher Hb concentrations were associated with better vitamin A status^(44,46). However, when we stratified the anaemic girls by their Fe status, the association did not exist in the non-IDA group whereas it was maintained in the IDA group (Table 5). This finding is interesting since it might explain the inconsistent findings where Fe-deficient and non-Fe-deficient anaemic subjects are not considered separately. Many studies have shown the positive effect of vitamin A in reducing the prevalence of anaemia but not all^(12,51,52), and

this inconsistency might be better explained if the association of Fe and vitamin A status is taken into account.

Our findings showed that poor Fe and vitamin A status contributed to anaemia among adolescent schoolgirls in Myanmar but it could not explain the full extent of the high prevalence of anaemia among the girls. The girls in the study were apparently healthy and anaemia due to infections or inflammations was unlikely given the low prevalence of inflammatory biomarkers among the girls. In the region, multiple micronutrient deficiencies frequently coexist and the role of other micronutrients such as vitamin B₁₂ and folate should also be carefully considered and explored further^(6,8,53). It would appear that improvements in vitamin A status may be required to reduce IDA and should be considered as part of programme implementation even though clinical vitamin A deficiency does not appear to be a public health problem in the region studied.

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Supplementary material

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