### **Original Article**

# Microcytic anaemia predominates in adolescent school girls in the delta region of Myanmar

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Objective: Anaemia is one of major nutritional problems in Myanmar affecting all age groups. However, there is lack of recent information and a study was conducted to acquire information on the current status of anaemia among adolescent schoolgirls in Nyaung Done township, Ayeyarwady division where an intervention study was planned. Subjects and Methods: A cross-sectional survey was conducted on 1269 subjects to obtain complete blood count, anthropometry and socioeconomic characteristics were obtained by questionnaire. Using red cell indices, we applied Bessman's, and Green and King's index classification to differentiate the types of anaemia. Electrophoresis was also done on a subsample (n=228). Results: Stunting was 21.2% and wasting was 10.7% respectively. Prevalence of anaemia was 59.1% and was mainly microcytic. Green and King's index showed 35.8% were iron deficient. Electrophoresis revealed 36 cases of Hb E haemoglobinopathy in the subsample. Conclusion: Anaemia is still a major nutrition problem in Myanmar. The reasons for this high prevalence should be explored and properly addressed. The study highlights the need for a comprehensive and large scale survey for the anaemia control programme in Myanmar.

Key Words: anaemia, haemoglobinopathy, Myanmar, adolescent, schoolgirls

#### INTRODUCTION

Myanmar, also known as Burma, is a country situated in South East Asia, where rice, which is rich in phytate, is the staple food. The causes of anaemia are multi-factorial yet iron deficiency (ID) is assumed to be the main cause of anaemia in the region as well as in the country. Children under five and women of child-bearing age are vulnerable to ID and to anaemia due to their high physiological requirements for iron and as a result, the prevalence is high among these groups. Worm infestation, deficiencies of other micronutrients such as vitamins B6, B12, folic acid, vitamin A and the presence of tropical diseases such as malaria, further complicates the problem. Several inherited haemoglobinopathies are also common in this region and contribute to the problem.

An iron supplementation program was launched in Myanmar in 1972 for pregnant women as part of the anaemia control program from the National Nutrition Center but there has been little attempt at regular follow up.<sup>1</sup> However, the prevalence is believed to be high and the most recent data in 2002 showed that the prevalence of anaemia was 45% among non-pregnant women, 26% among the adolescent schoolgirls and 71% among pregnant women.<sup>1</sup> In the recent years, studies have high-lighted the preconceptional period as an appropriate win-

dow of opportunity to improve iron status and benefit pregnancy outcome.<sup>2,3</sup> Early marriage is common among the girls in the region and in Myanmar up to 22.3% of the girls are married before the age of 20 years, especially in the rural areas.<sup>4</sup> This implies that in order to break the intergenerational problem of malnutrition, it is imperative to improve the nutritional status of adolescents.

In 2002, the highest prevalence of anaemia was reported to exist in Ayeyarwady Division, in the delta region of the country.<sup>5</sup> In the study described below, we reported the current information on anaemia among the adolescent girls in this division and we have used the measurements of mean cell volume (MCV) and red cell distribution width (RDW) to indicate the numbers with iron deficiency anaemia and thalassaemia traits.<sup>6,7</sup>

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#### MATERIALS AND METHODS Subjects

Nyaung Done Township was selected as it was 3 hours drive (~40 km) away from the former capital city Yangon and made it feasible to send the blood samples to the laboratory of Department of Medical Research (Lower Myanmar) within 4 hours of the blood collection. The study was conducted in July 2010 on 1269 adolescent school girls (13-18 y) from 6 schools. Background information on the subjects was obtained and nutritional status was assessed using anthropometry and biochemical measurements on all the subjects. The study described here was the screening phase of an intervention study, registered at Clinical Trials. Gov (NCT01198574) which will be reported elsewhere. Ethical approval was obtained from The Faculty of Medicine, University of Indonesia (128/PT02.FK/ETIK/ 2010) and in Myanmar (18/Ethics 2010, DMR-Lower Myanmar).

Body weight was measured by flat electronic weighing scale (SECA 874) to the nearest 0.1 kg and height by measuring tape (Microtoise) to the nearest 0.1 cm. All measurements were performed by two trained persons throughout the study following standard protocol. Z-scores were calculated for height-for-age, weight-for-age and BMI-for-age using WHO Anthroplus software.<sup>8</sup> Cut-offs of -2 SD were used to identify subjects who were stunted and thin.

Phlebotomy was done by experienced nurses. Two ml venous blood was drawn into closed EDTA-vacuumtubes and stored immediately at 4°C. The samples were transported at 4°C and analysed within 4 hours after the blood collection by Coulter Counter (ABX Pentra 60-HORIBA). The machine was standardized using the manufacturer's standards. Red cell parameters were classified using the established cut-off values.<sup>9</sup> Following Bessman's classification, six groups of subjects were identified using MCV (<80, 80-100, >100) and RDW ( $\leq$ 15, >15)<sup>6</sup>. The Green and King Index (MCV<sup>2</sup> x RDW/RBC) was used to differentiate the two most common causes of microcytic anaemia, ID and thalassaemia.<sup>8</sup> The index was applied as follows: values above and below 73 indicated thalassaemia trait or ID respectively.

Leishman-stained blood films were examined under light microscopy by an experienced pathologist for all the results with Hb <120 g/L, and samples provisionally diagnosed with haemoglobinopathy (n=228) were submitted for electrophoresis (Jookoo ECP-10, Japan).

Data were checked for normality using the Kolmogorov-Smironov test. Summary statistics was calculated as means ( $\pm$ SD), medians (quartiles) and proportions (%). Chi square test was used to analyze associations between nutritional status among groups of anaemia with or without haemoglobinopathy.

#### RESULTS

Of the 1269 subjects recruited, samples of 30 subjects were excluded from analysis due to mechanical errors and five subjects were older than 19 years and thus the characteristics of total 1,234 subjects were presented (Table 1). Mean age was 14.0 $\pm$ 1.3 years. Stunting (<-2 height-forage z-scores) was 21.2%, and thinness (<-2 BMI-for-age z-scores) was 10.7%. Subjects with Hb E haemoglobinopathy tend to be thinner than those without haemoglobinopathy but it was not statistically significant (*p*=0.09).

The prevalence of anaemia among the girls in the study area was 59.1% (n=729) and mean Hb concentration was

**Table 1.** Characteristics of the whole study population and the anaemic sub-groups<sup>†</sup>

	Total	Anaemic subjects	Haemoglobinopathy <sup>‡</sup>
	(n=1234)	(n=729)	(n=36)
Age (y)	$14\pm1.30$	$14\pm1.28$	$14 \pm 1.04$
Attending class (%)			
Middle school	39.5	35.9	30.6
High school	60.5	64.1	69.4
Religion (%)			
Buddhists	94.3	94.9	100
Christians	3.6	3.4	0
Hindu	0.2	0.3	0
Muslim	2.0	1.4	0
Ethnicity (%)			
Burmese	56.4	58.9	83.3
Mon	0.1	0.1	0
Kayin	24.1	22.9	5.6
Indian	2.0	1.6	0
Mixed	17.5	16.4	11.1
Anthropometry			
Weight (kg)	$41.5 \pm 6.44$	$41.4 \pm 6.06$	$40.8 \pm 6.70$
Height (m)	$1.51 \pm 0.05$	$1.51 \pm 0.05$	$1.51 \pm 0.05$
Body mass index (BMI)	$18.3 \pm 2.40$	$18.2 \pm 2.25$	$17.9 \pm 2.65$
Height-for-age z score	$-1.41 \pm 0.79$	$-1.43 \pm 0.75$	$-1.44 \pm 0.80$
BMI-for-age z score	$-0.78 \pm 0.99$	$-0.82 \pm 0.95$	$-1.01 \pm 0.06$
Stunting <sup>‡</sup> (%)	21.2	21.5	30.6
Thinness <sup>‡</sup> (%)	10.7	10.3	19.4

<sup>†</sup> values are presented as means  $\pm$  SD or %

<sup>‡</sup>Total examined by electrophoresis was n=228

<sup>§</sup>Stunting and thinness were defined by height-for-age z score (<-2 SD) and BMI-for-age z score (<-2 SD)

Variables	Median (1 <sup>st</sup> , 3 <sup>rd</sup> quartiles)	Cut-off	Abnormal status (%)
Haemoglobin (g/L)	117 (111, 123)	<120	59.1
HCT (%)	29.9 (25.2, 36.0)	<36	74.7
MCV (fL)	62.0 (53.0, 76.0)	<80	82.0
MCH (pg)	24.6 (23.1, 26.4)	<27	82.0
MCHC (g/dL)	38.7 (32.0, 45.0)	<32	24.7
RDW <sup>‡</sup> (%)	17.6 (16.3, 18.8)	>15	88.0

**Table 2.** Red cells parameters of the adolescent school girls from screening  $(n=1234)^{T}$ 

HCT: Haematocrit, MCV: Mean cell volume, MCH: Mean cell haemoglobin, MCHC: Mean cell haemoglobin concentration, RDW: Red cell distribution width

<sup>†</sup>Cut-off values from Gibson's Principles of Nutritional Assessment<sup>9</sup> and Bessman's study<sup>6</sup>

<sup>‡</sup> RDW is the Standard Deviation of the MCV expressed as a percentage of the mean MCV.

**Table 3.** Classification of subjects (%) based on mean corpuscular volume (MCV) and red cell distribution width  $(RDW)^{\dagger}$ 

	MCV <80 fL (Microcytosis)	MCV=80~100 fL (Normocytosis)	MCV >100 fL (Macrocytosis)
	(n=1012)	(n=210)	(n=12)
RDW ≤15 (n=148)	7.4	3.7	0.9
RDW >15 (n=1086)	74.6	13.3	0.1

<sup>†</sup> classification based on Bessman's study<sup>6</sup>

117 g/L (Table 2). The data showed that mean cell volumes (MCV) and haemoglobin (MCH) were low in more than 80% of the girls and the low red cell volumes contributed to a wider distribution of red cell sizes (red cell distribution width, RDW) in 88% of the girls.

Workers have suggested that the type of anaemia can be classified using MCV and RDW measurements.<sup>6</sup> On that basis, there were 1,012 girls (82%) with microcytosis (MCV <80 fL) and 1086 (88%) with red cell sizes outside the normal range (RDW >15) (Table 3). Only 210 subjects (17%,) were within the normal MCV range (80-100 fL) but even for these subjects RDW was high in the majority.

We applied Green and King's index to our data for those with anaemia (Hb <120 g/L, n=729). According to the index, ID and  $\beta$ -thalassaemia trait (BTT) were 35.8% (n=261) and 64.2% (n=468) respectively.

Among the subjects submitted to electrophoresis (n=228), the results revealed that 36 subjects suffered from haemoglobinopathies, 5 with homozygous Hb E, and 31 with Hb EA.

#### DISCUSSION

The subjects in the study were adolescent school girls from the delta region and more than half of the subjects were of Burmese ethnicity. The findings suggested moderate prevalence of stunting but high prevalence of thinness among the girls. Almost 60% of the girls were anaemic (Hb <120 g/L) which indicated the existence of a severe public health problem among the girls in the study area .<sup>10</sup> The Bessman classification showed 82% had small red cells (MCV <80 fL) and almost 90% had an abnormal cell size (RDW >15). Most red cells were small indicating ID or  $\beta$  Thalassemia trait (BTT) but the large RDW does not rule out the possibility that some cells were larger than average due to folate or B-12 deficiency. Our dietary data on a sub-sample of the anaemic girls (n=391) also suggested that the proportion of inadequate intakes of folate was higher than that of iron amongst these subjects, which highlights that folate might also be a problem nutrient. This dietary data will be reported separately. In addition, iron deficiency without anaemia may also be present in those subjects with a high RDW.<sup>11</sup>

There is a high prevalence of haemoglobinopathy in the region wherein Hb E haemoglobinopathy is the most common type, followed by  $\alpha$  Thalassemia.<sup>12</sup> In the present study, the sub-sample examined for haemoglobinopathies found only 36 cases, but the Hb E trait was consistently found to be common especially among people of Burmese ethnicity.<sup>13</sup>

The low MCV and high RDW strongly suggest that ID is an important cause of the anaemia <sup>7</sup> in the Myanmar girls, and according to the Green and King's index, the estimated prevalence of iron deficiency was 35.8%. Anaemia is major nutritional problem in the region and it is generally assumed that iron deficiency is the common cause. The findings from our study highlighted that iron deficiency is an important cause for this high prevalence of anaemia and was consistent with studies conducted among neighbouring countries which showed that approximately 30% of anaemia among adolescent girls were due to iron deficiency.<sup>14-16</sup>

A more comprehensive and large scale study should be conducted to determine the causes of anaemia in the country. In the recently conducted survey among Cambodian children, haemoglobinopathy EE was identified as an important risk factor for anaemia together with other risk factors such as low vitamin A status, storage iron depletion and sub-clinical inflammation. This study demonstrates the complex nature of anaemia, and that comprehensive applications of biochemical and haematological indicators are needed to identify the cause.<sup>17</sup> We believe further research is needed to determine the true contributions of the factors causing anaemia among the vulnerable age groups in Myanmar which will probably have implications for the anaemia control programme in Myanmar.

In conclusion, the study showed that there was a high prevalence of anaemia in these Myanmar adolescent girls in the delta region and that the anaemia was predominantly microcytic. The reasons for this high prevalence should be explored and properly addressed. This study highlights the magnitude of anaemia in the study area and a large scale survey should be conducted for better programme implementation.

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#### AUTHOR DISCLOSURES

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## Original Article

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# 小球性貧血在緬甸三角洲地區青春期的女學生中佔多數

目的:貧血是緬甸所有年齡層群眾的一個主要營養問題,然而缺少近期的相關資料。有一個介入型研究計劃將在伊洛瓦底省的央東鄉進行,因此先做一橫斷性研究,以得到緬甸青春期女學生貧血現況的資料。受試者與方法:針對 1269 名受試者做橫斷面調查,進行完整血液檢查與體位測量,社會經濟特性則由問卷而得。使用 Bessman's 和 Green and King's 紅血球指數分類法來區分不同類型的貧血,其中 228 個血液樣本有進行電泳分析。結果:發育遲緩和過度消瘦者分別占 21.2%與 10.7%。貧血的盛行率是 59.1%,其中以小球性為主;從 Green and King's 指數來看,有 35.8%是由於鐵缺乏。電泳分析的樣本中顯示有 36 名是屬於HbE 血紅蛋白病。結論:在緬甸,貧血仍然是一個主要的營養問題,其高盛行率的原因應被繼續探討與提出。本研究凸顯在緬甸貧血控制計畫中進行大規模且全面調查的必需性。

### 關鍵字:貧血、血紅蛋白病、緬甸、青少年、女學生