European Journal of Clinical Nutrition (2008) 62, 365–372

0954-3007/08 $30.00
www.nature.com/ejcn

ORIGINAL ARTICLE

The coexistence of other micronutrient deficiencies in anaemic adolescent schoolgirls in rural Bangladesh

F Ahmed[1], MR Khan[2], CP Banu[2], MR Qazi[2] and M Akhtaruzzaman[2]

[1]*Department of Family Sciences, College for Women, Kuwait University, Safat, Kuwait and* [2]*Institute of Nutrition and Food Science, University of Dhaka, Dhaka, Bangladesh*

Objective: To investigate the prevalence of selected micronutrient deficiencies amongst anaemic adolescent schoolgirls in rural Bangladesh and to examine their relationship with haemoglobin (Hb) levels.
Design: A cross-sectional study.
Setting: Girls' high schools in rural areas of Dhaka District in Bangladesh.
Subjects and methods: Three hundred and ten anaemic adolescent girls aged 14–18 years from eight schools participated in the study. Information on personal characteristics and food habits were collected by interview. Parents were asked about their socio-economic conditions. Anthropometric data and blood samples were collected following the interview.
Results: Twenty-eight per cent of the girls had depleted iron stores (serum ferritin $<12.0\,\mu$g/l), 25% had folic acid deficiency (red blood cell folic acid <317 nmol/l), 89% had vitamin B_2 (erythrocyte glutathione reductase activity coefficient $\geqslant 1.4$) and 7% had vitamin B_{12} deficiencies (serum vitamin B_{12} <150 pmol/l). Although the prevalence of vitamins A and C deficiency was very low, a significant proportion had low vitamin A (serum retinol between 0.70 and $<1.05\,\mu$mol/l) and vitamin C status (plasma ascorbic acid between 11.4–23.0 μmol/l). Frequency of consumption of meat, serum ferritin and vitamin B_2 status were found to be strongly related to Hb by multiple regression analysis. For 1 μg/l change in serum ferritin, there was a 0.13 g/l change in Hb when adjusted for other factors.
Conclusions: There is coexistence of micronutrient deficiencies among anaemic adolescent girls in rural Bangladesh, although they do not suffer from energy deficiency. Of all micronutrients, only iron and vitamin B_2 concentrations were found to be related to the Hb concentration.
European Journal of Clinical Nutrition (2008) **62**, 365–372; doi:10.1038/sj.ejcn.1602697; published online 28 February 2007

Keywords: anaemia; iron deficiency; multiple micronutrient deficiency; adolescent girls; Bangladesh

Introduction

Anaemia is one of the most prevalent public health problems in developing countries, and has important health, social and economic consequences (INACG, 2002). Although iron deficiency is considered to be the main cause of anaemia, other identified non-genetic causes of anaemia are malaria (Stoltzfus *et al*., 1996; Dreyfuss *et al*., 2000), hookworm infestation (Stoltzfus *et al*., 1996; Bhargava *et al*. 2003), infection (Jansson *et al*., 1986) and deficiency of a number of micronutrients such as vitamins A, C, B_2, B_{12} and folic acid (Fishman *et al*., 2000).

In Bangladesh, the prevalence of anaemia among adolescent girls varies from 20 to 40% depending on the level of urbanization (HKI/IPHN, 1999a; Ahmed, 2000; BBS/UNICEF, 2004), and a significant proportion of this anaemia problem is found to be related to iron deficiency (Ahmed *et al*., 1996, 2000). However, dietary surveys indicate that a large proportion of the adolescent girls do not meet the daily requirements for various micronutrients, including iron,

Correspondence: Dr F Ahmed, Department of Family Sciences, College for Women, Kuwait University, PO Box: 5969, Safat 13060, Kuwait.
E-mail: F.Ahmed@cfw.kuniv.edu
Guarantor: F Ahmed.
Contributors: FA took the lead in study planning and design, conducted data analysis and wrote the manuscript with a substantial contribution from MRK. MRK also supervised blood collection and laboratory analysis. CPB and MRQ were responsible for blood collection, laboratory analysis and contributed to the writing of the manuscript. MA coordinated the field data collection, and contributed to the writing of the manuscript. There were no conflicts of interest for the authors.
Received 24 April 2006; revised 18 January 2007; accepted 22 January 2007; published online 28 February 2007

vitamin A, riboflavin (B_2) and vitamin C (Jahan and Hossain, 1998; Ahmed *et al*., 1998). The findings suggest that a substantial proportion of the girls may be suffering from sub-clinical deficiencies of micronutrients other than iron. However, confirmation of this by studies on physiological levels of the micronutrients are lacking, except for vitamin A (Ahmed *et al*., 1997; HKI/IPHN, 1999b).

The coexistence of multiple micronutrient deficiencies with iron deficiency may increase the risk of anaemia and limit haematological response (Allen *et al*., 2000). In a meta-analysis of efficacy of iron supplementation in developing countries, the authors speculated that the factors other than iron deficiency might be operating to limit haematological response and thus the control of anaemia (Beaton and McCabe, 1999). Iron deficiency in anaemic subjects in poor communities may be complicated by one or more additional micronutrient deficiencies (Ronnenberg *et al*., 2000; Dijkhuizen *et al*., 2001). It is therefore important to understand the significance of how deficiencies of other micronutrients may cause anaemia in communities where concurrent deficiency of several micronutrients is common. This study was carried out to assess the prevalence of selected micronutrient deficiencies, including iron, in anaemic rural adolescent schoolgirls in Bangladesh, and also to examine their effect on haemoglobin (Hb) levels.

Subjects and methods

Subjects

The study was conducted in 310 post-menarcheal non-pregnant adolescent girls aged 14–18 years, from eight girls' high schools located in rural areas of Dhaka District in Bangladesh. The study was carried out between March and May 2003 during screening for anaemia for an intervention trial that examined the efficacy of multiple micronutrient supplementation (Ahmed *et al*., 2005). After obtaining the girls' and their parents' written consent, 1800 eligible girls of grades VIII–X in the selected schools participated in the initial screening for anaemia; 570 of them were identified as anaemic (Hb) concentration <120 g/l) using a B-Haemoglobin Analyzer (HemoCue, Angelholm, Sweden) on a finger prick blood sample. They were then invited to give a venous blood sample on an appointed day; 480 subjects provided a venous blood sample and their Hb concentration was measured again with a cyanmethemoglobin method using a commercial kit (Roche Diagnostics, Mannheim, Germany). Only the girls who were found to be anaemic ($n=310$) by both tests were included in this study. The blood samples of rest of the girls ($n=170$) who were not found to be anaemic by the cyanmethamoglobin method were not further analysed. Girls with fever, or any sign of infection, organ malfunction or metabolic disorder were excluded from the study. The study protocol was approved by the Ethical Review Committees of the Bangladesh Medical Research Council, Dhaka, and University of Queensland, Brisbane, Australia.

Socioeconomic and food habit data collection

Questionnaires were developed for socio-economic, personal characteristics and food habit data, and were pre-tested in the field before finalization. Information about family size, monthly income, parents' academic qualifications and occupations were obtained from the parents. The families earning below 659 Takas per capita monthly were considered to be living below the poverty line (BBS, 2003). The subjects were interviewed for personal characteristics and morbidity on the day of blood collection. A 7-day food frequency questionnaire (FFQ) containing a list of non-cereal food items was used to collect data on food habits. Although it is known that cereal grains contain phytates that interfere with absorption of iron, these girls consume only one type of cereal grain, that is, rice or any product from it. Rice is a common food that rural girls usually consume three times per day, so that any inhibitory effect due to phytates from the cereal grains may be assumed to be the same for all the girls. Therefore, for the purpose of this study, cereal foods were excluded from the FFQ.

Anthropometric measurements

Bare feet weight and height were measured by methods that have already been validated (Ahmed *et al*., 1998). Anthropometric data could be collected from 299 subjects. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). The prevalence of underweight was estimated as BMI-for-age at less than the 5th percentile of the reference population (WHO, 1995). According to the recommendation made by an World Health Organization (WHO) Expert Committee, a cutoff of less than 3rd percentile for height-for-age was used to define stunted growth (WHO, 1995).

Faecal specimens collection

Samples of morning stool were taken from 272 girls for assessment of helminth infection and were transported to the laboratory in a cold box on the day of collection. Faecal specimens were examined by the Kato–Katz method (WHO, 1994). If any helminth was found $>10\,000$ eggs/g stool, the sample was diluted by Stoll's technique (Suzuki, 1981) and then re-examined. Subjects were classified according to their worm load as described by Stoltzfus *et al*. (1996).

Biochemical and haematological measures

Five millilitres of venous blood were drawn from each subject. Details of blood collection, processing and analytical procedures for serum ferritin, red blood cell (RBC) folic acid, erythrocyte glutathione reductase activity coefficient

(EGRAC, an indicator of riboflavin status), plasma vitamin C, serum vitamins A and B_{12} have been described elsewhere (Ahmed *et al*., 2005). The number of samples varied for each of the biochemical variables because some were lost during analysis. One sample had a very high value for RBC folic acid that was considered to be abnormal for this population and was therefore excluded from the analysis.

The deficiency for each biochemical index was defined as the prevalence of girls below an appropriate cutoff value. SF concentration $<12\,\mu$g/l was defined as iron depleted and an SF concentration between $12\,\mu$g/l and $<20\,\mu$g/l was defined as iron deficient (INACG, 1985; McDonnell and White, 1997). The RBC folate <317 nmol/l was defined as folic acid deficiency, and the RBC folate between 317 and 363 nmol/l was considered to be low in folic acid (Senti and Pilch, 1985; Gibson, 2005a). Serum vitamin B_{12} <150 pmol/l (Dong and Scott, 1982) and EGRAC $\geqslant 1.4$ (Powers *et al*., 1983) were defined as vitamin B_2 and vitamin B_{12} deficiency, respectively. Vitamin C deficiency was defined as a plasma ascorbic acid $<11.4\,\mu$mol/l, and low vitamin C status was when plasma ascorbic acid lay between 11.4 and 23.0 μmol/l (Jacob, 1999). Subclinical vitamin A deficiency was defined as serum retinol $<0.70\,\mu$mol/l (WHO, 1996) and suboptimal vitamin A status was defined by serum retinol between 0.70 and $<1.05\,\mu$mol/l (Ballew *et al*., 2001).

Statistical analysis

Univariate analysis consisted of the simple frequency distribution of selected variables. The normality of distribution was checked using the Kolmogorov–Smirnov goodness-of-fit test. Hb, serum vitamin B_{12} and serum ferritin (SF) concentrations were not normally distributed. The serum vitamin B_{12} data were log-transformed and the SF data were square root-transformed for statistical analyses. For presentation, the values were transformed back to the original units. The data are summarized as mean$\pm$s.d. when normally distributed and median (25–75 percentile) where not normally distributed. Otherwise data are presented as mean with a 95% confidence interval.

To investigate the relationship between the indicators of micronutrient status and the degree of severity of anaemia, the Hb concentrations were classified into two groups: mild anaemia (Hb between 110 and <120 g/l) and moderate to severe anaemia (Hb <110 g/l) levels. In populations exposed to frequent infection, it is often difficult to interpret SF and vitamin A data, as it is an acute phase reactant (Thompson *et al*., 1992). Serum C-reactive protein (CRP) concentration was measured so as to identify subjects who were in an acute phase of an infection at the time of study. A cutoff of >5.0 mg/l for CRP was used for screening (Pepys, 1981). The data were analysed using analysis of variance (ANOVA) after excluding those who had elevated serum CRP levels. Furthermore, inflammation has often been found to be associated with anaemia (Abshire and Reeves, 1983; Jansson *et al*., 1986), therefore, Hb levels were compared between girls with and without high CRP levels using ANOVA.

Pearson's correlation test was used to assess the association of Hb with various socio-economic, dietary and biochemical variables, and with worm infestation load. The same test was also used to examine the association among other micronutrients. Finally, backward-stepwise multiple-regression analysis was done to determine the independent relationship of Hb with various social, dietary, health and biochemical indices. The data were analysed using SPSS for Windows (version 12; SPSS Inc, Chicago).

Results

The characteristics of the study participants are presented in Table 1. Most of their parents had achieved good educational levels. Only 10% of the fathers and 12% of the mothers were illiterate. Nearly two-thirds of the fathers and half of the mothers had completed at least secondary school education. The largest proportion (43.9%) of their fathers ran businesses, 29.5% worked in farming, 22.3% had office jobs and only 4.3% were manual workers. Ninety-six per cent of the mothers were housewives (data not shown). The majority of the subjects (63.1%) came from medium size families consisting of five or six members. Nearly half of the families had per capita monthly income below rural poverty line income level.

Table 1 Characteristics of the study participants[a]

Variable	*Percent distribution*
Education of father	
Illiterate	10.0
Primary	25.1
Secondary	45.2
Higher secondary and above	19.7
Education of mother	
Illiterate	12.2
Primary	35.1
Secondary	48.0
Higher secondary and above	4.7
Occupation of father	
Manual work	4.3
Farming	29.5
Service (office)	22.3
Business	43.9
Family size	
Small, $\leqslant 4$ members	13.6
Medium, 5–6 members	63.1
Large, $\geqslant 7$ members	23.3
Per capita monthly income	
Below poverty line[b]	49.8
Above poverty line	50.2

[a]$n=310$.
[b]Per capita monthly income $<$Taka 659/month (BBS, 2003).

The mean (s.d.) height was 150.5 (4.7) cm, weight was 40.9 (5.6) kg and BMI was 18.1 (2.3) kg/m^2 (Table 2). The prevalence of stunting (<3rd percentile of WHO reference value) was 33.4% and only 8.4% were underweight (<5th percentile WHO reference value). The distribution of Hb concentrations was skewed towards the lower values and median value was 110.2 g/l (Table 2). Nearly 36% of the subjects had moderate anaemia (Hb between 80 g/l and <110 g/l) and only 1.3% had severe anaemia (Hb <80 g/dl). The distributions of both SF and serum vitamin B_{12} concentrations were skewed towards higher values. The median (25–75 percentiles) values of SF and serum vitamin B_{12} were 20.5 μg/l (10.2–31.7 μg/l) and 306.6 pmol/l (194.8–377.4 pmol/l), respectively. Table 3 shows that 28% of the girls had depleted iron stores (SF <12.0 μg/l) and another 20% had iron deficiency (SF between 12.0 and <20.0 μg/l). About 7% of the girls had vitamin B_{12} deficiency (serum vitamin B_{12} <150 pmol/l). The mean (s.d.) RBC folate concentration was 427.6 (160.5) nmol/l, and it was normally distributed. Nearly a quarter of the girls had folic acid deficiency (RBC folic acid <317 nmol/l) and another 12% had low folic acid status (RBC folic acid between 317 and <363 nmol/l). Mean (s.d.) EGRAC was 1.84 (0.4). Eighty-nine per cent of the girls had EGRAC $\geqslant$1.4, indicating vitamin B_2 deficiency (Table 3). Mean (s.d.) serum retinol concentration was 1.18 (0.29) μmol/l. Nearly 4% of the girls had subclinical vitamin A deficiency (serum retinol <0.70 μmol/l) and another 31% had low vitamin A status (serum retinol between 0.70 and <1.05 μmol/l). Mean (s.d.) plasma ascorbic acid concentration was 46.1 (20.0) μmol/l. About 10% of the girls had low vitamin C status (plasma ascorbic acid between 11.4 and 23.0 μmol/l) with only 2% vitamin C deficient (plasma ascorbic acid <11.4 μmol/l, Table 3).

A little over half (52.5%) of the girls were infected with at least one form of intestinal helminths. However, only six girls (2.2%) were infected with hookworm. One hookworm-infected girl was infected with all three types of worm; the remaining hookworm infected subjects were also infected with another worm. The prevalence of infection with *Ascaris lumbricoides* and *Trichuris trichura* was 44.6% and 26.6%, respectively. Overall, 18.4% were infected with both *A. lumbricoides* and *T. trichura*. The incidence of hookworm was light (1–1999 eggs/g). Only 1.1% of the subjects were heavily infected with *T. trichura,* whereas none were heavily infected with *A. lumbricoides*.

A substantial proportion of the girls did not consume meat (31.7%), eggs (35.3%) and milk (35.6%) in the week

Table 3 Prevalence of micronutrient deficiency in anaemic adolescent schoolgirls in rural Bangladesh

Micronutrient	*Percentage*
Depleted iron stores[a]	28.0
Iron deficiency[b]	20.0
Folic acid deficiency[c]	24.8
Low folic acid status[d]	12.3
Vitamin B_{12} deficiency[e]	6.8
Vitamin B_2 deficiency[f]	89.0
Subclinical vitamin A deficiency[g]	4.1
Low-serum vitamin A status[h]	31.0
Vitamin C deficiency[i]	2.0
Low vitamin C status[j]	9.6

[a]Serum ferritin <12.0 μg/l.
[b]Serum ferritin 12.0 and <20.0 μg/l.
[c]RBC folic acid <317 nmol/l.
[d]RBC folic acid between 317 and <363 nmol/l.
[e]Serum vitamin B_{12} <150 pmol/l.
[f]Erythrocyte glutathione reductase activity coefficient (EGRAC), a dimensionless number inversely proportional to the vitamin B_2 (riboflavin) status. $\geqslant$1.4.
[g]Serum retinol <0.70 μg/l.
[h]Serum retinol between 0.70 and <1.05 μmol/l.
[i]Plasma ascorbic acid <11.4 μg/l.
[j]Plasma ascorbic acid between 11.4–23.0 μg/l.

Table 2 Anthropometric and biochemical measures of the participants

Variable	n	*Mean± s.d.*	*Median*	*25–75 percentile*
Anthropometry				
Height (cm)	299	150.5±4.7		
Weight (kg)	299	40.9±5.6		
BMI (kg/m^2)	299	18.1±2.3		
Biochemistry				
Haemoglobin (g/l)	310		110.2	106.4–116.1
Serum ferritin (μg/l)[a]	288		20.5	10.2–31.7
Erythrocyte folic acid (nmol/l)	309	427.6±160.5		
Serum vitamin B_{12} (pmol/l)	310		306.6	194.8–377.4
EGRAC[b]	306	1.84±0.4		
Serum retinol (vitamin A)(μmol/l)[a]	287	1.18±0.29		
Plasma vitamin C (μmol/l)	307	46.1±20.0		

Abbreviations: BMI, body mass index; EGRAC, erythrocyte glutathione reductase activity coefficient.
[a]Samples with C-reactive protein (CRP) >5.0 mg/l were excluded from the analysis.
[b]Erythrocyte glutathione reductase activity coefficient, a dimensionless number inversely proportional to the vitamin B_2 (riboflavin) status.

preceding the interview; while 21.2% ate meat, 20.3% ate eggs and 29.4% consumed milk at least four times a week. The intake patterns of large and small fish (species weighing under 10 g) were similar. Only a few (0.7%) did not eat any fish at all, whereas more than a third ate fish at least four times. However, 25% did not eat any small fish. Pumpkin was not consumed by 51.6% and dark green leafy vegetables (DGLVs) were eaten by 17% of the girls. Fruit was highly popular among the girls, being consumed more than three times by 76.5% of them.

Table 4 shows the relationship of Hb concentration with the selected micronutrient status. The mean SF concentration in the girls with moderate to severe anaemia (Hb < 110 g/l) was significantly ($P = 0.001$) lower than in the girls with mild anaemia (Hb between 110 and < 120 g/l). Serum vitamin B_{12} concentrations were higher in the girls with higher Hb levels ($P = 0.09$). No significant differences were observed for EGRAC, plasma ascorbic acid, serum vitamin A and RBC folate concentrations by the Hb groups.

Table 5 shows the association between various biochemical variables. Hb concentrations were found to be correlated with SF ($r = 0.264$, $P = 0.001$), serum vitamin B_{12} ($r = 0.106$, $P = 0.07$) and serum vitamin A ($r = 0.11$, $P = 0.07$). Serum ferritin was significantly associated with serum vitamin A. RBC folic acid was also positively correlated with serum vitamin B_{12}, serum retinol and plasma ascorbic acid. Serum vitamin A was found to be associated with plasma ascorbic acid. EGRAC (an indicator of vitamin B_2) was negatively associated with RBC folic acid, serum vitamins A and B_{12}. Furthermore, Hb concentrations were also positively correlated with the frequency of intakes of meat ($r = 0.13$; $P = 0.03$) and eggs ($r = 0.12$; $P = 0.04$). Serum vitamin A was associated with meat ($r = 0.18$; $P = 0.002$) and egg ($r = 0.11$; $P = 0.07$) intakes. Serum vitamin B_{12} was also associated with egg ($r = 0.19$; $P = 0.001$) intake. Among the socio-economic variables, only the size of the family was significantly positively correlated with Hb ($r = 0.126$; $P = 0.03$). Of all helminths, the load of *A. lumbricoides* was negatively correlated with Hb levels ($r = -013$; $P = 0.04$).

The factors influencing Hb concentrations of the girls were examined using a backward stepwise multiple regression analysis (Table 6). In the analysis, age, family size, frequency

Table 4 Mean concentrations of selected micronutrients by haemolgobin concentration

Indices	*Haemoglobin < 110 g/l*		*Haemoglobin ⩾ 110 g/l*		P-*value*
	n	*Mean (95% CI)*	n	*Mean (95% CI)*	
Serum ferritin (μg/l)[a]	106	18.5 (15.3–21.6)	182	24.9 (22.5–27.3)	0.001[b]
RBC folic acid (nmol/l)	113	428.3 (397.9–458.7)	196	427.2 (404.7–449.6)	0.95
Serum vitamin B_{12} (pmol/l)	114	286.4 (261.3–311.5)	196	318.3 (294.5–342.1)	0.09[c]
EGRAC	113	1.9 (1.8–2.0)	193	1.8 (1.7–1.9)	0.20
Serum vitamin A (μmol/l)[a]	106	1.16 (1.11–1.21)	181	1.20 (1.15–1.24)	0.34
Plasma vitamin C (μmol/l)	114	45.7 (42.2–49.3)	193	46.3 (43.4–49.3)	0.79

Abbreviations: CI, confidence interval; EGRAC, Erythrocyte glutathione reductase activity coefficient, a dimensionless number inversely proportional to the vitamin B_2 (riboflavin) status; RBC, red blood cell.
[a]Samples with serum CRP > 5.0 mg/l were excluded from the analysis. Differences between groups were compared by ANOVA.
[b]Based on square root transformed value.
[c]Based on log transformed value.

Table 5 Correlation between various biochemical measures including haemoglobin

Variable	*Haemoglobin*	*Serum ferritin*	*Serum vitamin* B_{12}	*RBC folic acid*	*EGRAC*	*Serum vitamin A*	*Serum ascorbic acid*
Haemoglobin		0.264 $P = 0.001$	0.106 $P = 0.07$	NS	NS	0.11 $P = 0.07$	NS
Serum ferritin			NS	0.107 $P = 0.07$	NS	0.168 $P = 0.004$	NS
Serum vitamin B_{12}				0.201 $P = 0.001$	−0.174 $P = 0.003$	0.101 $P = 0.09$	NS
RBC folic acid					−0.300 $P = 0.001$	0.352 $P = 0.001$	0.187 $P = 0.002$
EGRAC (vitamin B_2)						−0.198 $P = 0.001$	NS
Serum vitamin A							0.200 $P = 0.001$

Abbreviations: EGRAC, Erythrocyte glutathione reductase activity coefficient; NS, not significant or *P* value more than 0.10; RBC, red blood cell.
Erythrocyte glutathione reductase activity coefficient, a dimensionless number inversely proportional to the vitamin B_2 (riboflavin) status

Table 6 Multiple regression analysis for haemoglobin[a]

Variable	B	*s.e.* B	*Beta*	P *value*
Serum ferritin (μg/l)	0.131	0.03	0.266	0.0001
EGRAC	−2.631	1.20	−0.135	0.03
Meat intake (frequency/week)	1.327	0.68	0.123	0.05
Ascaris lumbricoides infection	−0.702	0.364	−0.121	0.06
Family size	0.364	0.201	0.112	0.07

Abbreviations: *B*, unstandardized coefficient; Beta, standardized coefficient; s.e. *B*, standard Error; EGRAC, Erythrocyte glutathione reductase activity coefficient.
Multiple $r=0.353$; $r^2=0.124$; Adjusted $r^2=0.105$; F-ratio = 6.6 (d.f. 5); $P=0.001$.
[a]Samples with serum CRP > 5.0 mg/l were excluded from the analysis.
Erythrocyte glutathione reductase activity coefficient, a dimensionless number inversely proportional to the vitamin B_2 (riboflavin) status.

of consumption of meat and eggs, SF, RBC folic acid, EGRAC, plasma vitamin C, serum vitamins A and B_{12}, and infection with *A. lumbricoides* were included. Using a $P>0.10$ for exclusion, only frequency consumption of meat, SF and EGRAC (vitamin B_2 status) were found to be statistically significantly related to Hb. The overall F ratio was 6.6 (d.f. 5) and was statistically significant ($P=0.001$). The adjusted r^2 was 0.105 (multiple $r=0.353$).

Discussion

This study reports the magnitude of selected micronutrient deficiencies that have important roles in haematopoiesis, in anaemic rural adolescent schoolgirls in Bangladesh. Earlier studies reported the relationship of Hb with iron and vitamin A levels in adolescent girls in Bangladesh (Ahmed *et al*., 1996, 2000). In this study, we explored the relationship between Hb and various micronutrients levels in anaemic adolescent girls only. Nearly half of the girls came from families with per-capita monthly income below the rural poverty line income. This proportion closely corresponds with the prevalence of rural poverty in Bangladesh. The sample may therefore be reasonably considered to be representative of a rural population group. In the present study, one third of the girls were short, suggesting an inadequate availability of nutrients in their younger days. On the other hand, most achieved a better body proportion with only 8.4% being thin. This suggests that energy intake was adequate for most of the girls but they were still anaemic, probably due to the low intake of micronutrient rich foods. In the present study, we collected information on the intake of micronutrient rich foods over a period of one week, and the data revealed that a significant proportion of the girls do not take food rich in iron, vitamins A, B_2 and B_{12} such as meat, liver, milk and eggs. Among the foods from animal sources, fish were consumed by almost all the girls, although 25% did not eat any small fish. Small fish are usually eaten whole and thus may contribute significant amounts of vitamins and minerals, particularly vitamin A, iron, and calcium (Roos *et al*., 2003). This study was carried out during the months of March, April and May. Tropical dark green leafy and other vegetables are abundant in this season. In addition to several vitamins, DGLVs contain iron. As the girls were rural residents, it might be expected that they had the good habit of consuming vegetables regularly. However, only a little over half of the girls ate DGLVs more than three times in a week and 17% did not consume any item from this food group in the given period.

Data on various biochemical indices reveal a widespread deficiency of several micronutrients in this population. If a cutoff of SF of $<20\,\mu$g/l is used, iron deficiency could account for anaemia in 48% of the girls. This suggests that iron deficiency alone may not adequately explain anaemia in more than half of the subjects in this study population. It is noteworthy that iron deficiency is established as the single important cause of anaemia in girls who suffers from severe and moderate anaemia. At higher Hb concentrations, the influence of other nutritional factors on Hb concentration became more apparent. In the present study, nearly two-thirds of the girls had mild (Hb between 110 g/l and <120.0 g/l) anaemia. Furthermore, 25% of the girls had folic acid deficiency (RBC folate <317 nmol/l), another 12% were at risk of folate deficiency (RBC folate between 317 and 363 nmol/l) and 7% of were found to be B_{12} deficient. These two vitamins are involved in DNA synthesis and their deficiency may cause megaloblastic anaemia (Fishman *et al*., 2000; Gibson, 2005a). In addition, both folate and vitamin B_{12} deficiencies have been found to be associated with depression and dementia (Bottiglieri, 1996). Also, poor vitamin B_2 status, judged by EGRAC $\geqslant 1.4$, in this population was as high as 89%. This prevalence is very similar (90%) to that reported in a study of low-income urban school children in Hyderabad, India (Prasad *et al*., 1987). Although prevalence of vitamin C and subclinical vitamin A deficiencies were insignificant in this population, one in 10 had low vitamin C status and one in three girls had inadequate vitamin A status (serum retinol $<1.05\,\mu$mol/l).

To explore the relationship between the severity of anaemia and various indices of micronutrient status, the girls were divided into two groups (mild versus moderate-to-severe anaemia). The result of bivariate analysis (ANOVA) showed that of all micronutrients tested, only SF was significantly ($P=0.001$) lower in the girls with moderate to severe anaemia (Hb <110 g/l) than the girls with mild anaemia (Hb between 110 g/l and <120 g/l). It is well known that there may be physiological interaction between nutrients after absorption, including a sparing effect of one nutrient on other. These interactions may underlie the relationship between the indicators of various micronutrients and Hb levels. In addition, we have observed an association between Hb levels and various socio-economic, dietary and non-dietary factors. Therefore, the independent relationship between various biochemical measures and Hb concentrations was further explored along with important

socio-economic, dietary and health related factors using multiple regression analysis. The findings revealed that consumption of meat, iron (judged by SF) and vitamin B_2 (assessed by EGRAC) status were independently significantly positively related to Hb concentration in these anaemic girls. Iron deficiency impairs erythropoiesis in the bone marrow and thus leads to microcytic anaemia (Gibson, 2005b). Vitamin B_2 (riboflavin) is known to mobilize stored iron from the liver and thus its deficiency may lead to anaemia (Yu and Cho, 1990). Folic acid deficiency impairs DNA synthesis and leads to ineffective erythropoiesis and, as a result, macrocytic anaemia develops (Fishman *et al*., 2000). However, the contribution of folic acid deficiency to anaemia in this study population could not be ascertained, because no peripheral blood cell morphology examinations or functional tests for folic acid deficiency were carried out. Vitamin A exerts an influence on the metabolic availability of iron, and hence Hb formation (Mejia, 1992). Vitamin C in the diet may increase the bioavailability of non-haem iron (Lonnerdal, 1989) and enable iron mobilization from the storage (Fishman *et al*., 2000). Although in the present study we did not find any significant relationship between Hb and vitamins A and C levels, there was significant association between serum ferritin and serum vitamin A ($r=0.168$; $P=0.001$).

Among the non-nutritional factors worm infestation, particularly hookworm infection, is considered to be an important cause of anaemia (Stoltzfus *et al*., 1996). We found no significant relation between worm infection and Hb levels. In the present study, the overall burden of helminth infection was low to medium. Furthermore, hookworm infection was found in only six cases. In addition, acute inflammation has often been found to be associated with anaemia (Abshire and Reeves, 1983; Jansson *et al*., 1986). Although the precise mechanism has not yet been established, shortened RBC survival, cytokine inhibition of erythropoiesis and abnormalities in iron mobilization and delivery have all been implicated in development of anaemia in inflammatory diseases (Duffy, 2004). Therefore, in the present study we also explored the impact of inflammation by comparing the Hb levels between those girls without any inflammation and those with acute inflammation using a cutoff of >5.0 mg/l for CRP. We did not find any significant differences in Hb levels between the two groups. One explanation for this finding could be due to small number of girls with inflammation.

In conclusion, this study shown that anaemic adolescent girls in rural Bangladesh face substantial risk with regard to the multiple micronutrient deficiencies, although they are not at risk of energy deficiency. Of all micronutrients examined, only iron and vitamin B_2 concentrations were found to be independently related to Hb concentrations. Given the other benefits of micronutrients for good health, the findings of this study highlight the importance of planning for micronutrient intervention in this population group.

Acknowledgements

We thank the Nestle Foundation, Lausanne, Switzerland for the financial support to conduct this study. We acknowledge the technical support of the Biomedical Research Group of BIRDEM, Dhaka, for helping with RBC folate and vitamin B_{12} assays. The authors also express their sincere thanks to the participants of this study.

References

Abshire TC, Reeves JD (1983). Anemia of acute inflammation in children. *J Pediatr* **113**, 868–871.

Ahmed F, Hasan N, Kabir Y (1997). Vitamin A deficiency among adolescent female garment factory workers in Bangladesh. *Eur J Clin Nutr* **51**, 698–702.

Ahmed F, Khan MR, Akhtaruzzaman M, Karim R, Marks GC, Banu CP *et al*. (2005). Efficacy of twice-weekly multiple micronutrient supplementation for improving the hemoglobin and micronutrient status among anemic adolescent schoolgirls in Bangladesh. *Am J Clin Nutr* **82**, 829–835.

Ahmed F, Khan MR, Islam M, Kabir I, Fuch G (2000). Anaemia and iron deficiency among adolescent schoolgirls in peri-urban Bangladesh. *Eur J Clin Nutr* **54**, 678–683.

Ahmed F, Khan MR, Karim R, Taj S, Hyderi T, Faruque MO *et al*. (1996). Serum retinol and biochemical measures of iron status in adolescent schoolgirls in urban Bangladesh. *Eur J Clin Nutr* **50**, 346–351.

Ahmed F, Zareen M, Khan MR, Banu CP, Haq MN, Jackson AA (1998). Dietary pattern, nutrient intake and growth of adolescent school girls in urban Bangladesh. *Pub Health Nutr* **1**, 83–92.

Ahmed F (2000). Anaemia in Bangladesh: a review of prevalence and aetiology. *Pub Health Nutr* **3**, 385–393.

Allen LH, Rosado JL, Casterline JE, Lopez P, Garcia OP, Martinez H (2000). Lack of hemoglobin response to iron supplementation in anemic Mexican preschoolers with multiple micronutrient deficiencies. *Am J Clin Nutr* **71**, 1485–1494.

Ballew C, Bowman BA, Sowell AL, Gillespie C (2001). Serum retinol distribution in residents of the United States: third National Health and Nutrition Examination Survey, 1988–1994. Bureau of Statistics, Statistics Division, Ministry of Planning, Government of the Peoples Republic of Bangladesh, Dhaka.

BBS (2003). *Report of the household income and expenditure survey 2000*. Bangladesh Bureau of Statistics: Dhaka, Bangladesh.

BBS/UNICEF (2004). Anaemia prevalence survey of urban Bangladesh and rural Chittagong Hill Tracts 2003. Bangladesh Bureau of Statistics, Statistics Division, Ministry of Planning, Government of the Peoples Republic of Bangladesh and UNICEF, Dhaka, Bangladesh.

Beaton GH, McCabe GP (1999). *Efficacy of intermittent iron supplementation in the control of iron deficiency anaemia in developing countries: an analysis of experience*. The Micronutrient Initiative: Toronto.

Bhargava A, Jukes M, Lambo J, Kihamia CM, Lorri W, Nokes C *et al*. (2003). Antihelminth treatment improves hemoglobin and serum feffitin concentrations of Tanzanian schoolchildren. *Food Nutr Bull* **24**, 332–342.

Bottiglieri T (1996). Folate, vitamin B_{12} and neuropsychiatric disorders. *Nutr Rev* **54**, 382–390.

Dijkhuizen MA, Wieringa FT, West CE, Muherdiyantiningsih, Muhilal (2001). Concurrent micronutrient deficiencies in lactating mothers and their infants in Indonesia. *Am J Clin Nutr* **73**, 786–791.

Dong A, Scott SC (1982). Serum vitamin B12 and blood cell values in vegetarians. *Annals Nutr Metab* **26**, 209–216.

Dreyfuss ML, Stoltzfus RJ, Shrestha JB, Pradhan EK, LeClerq SC, Khatry SK *et al*. (2000). Hookworms, Malaria and vitamin A deficiency contribute to anemia and iron deficiency among pregnant women in the plains of Nepal. *J Nutr* **130**, 2527–2536.

Duffy TP (2004) In: Goldman L and Ausiello D (eds). *Cecil Text Book of Medicine* 22nd edn. W.B. Saunders: Philadelphia, USA.

Fishman SM, Christian P, West Jr KP (2000). The role of vitamins in the prevention and control of anaemia. *Pub Health Nutr* **3**, 125–150.

Gibson RS (2005a). *Principles of nutritional assessment*. Oxford University Press: New York, USA. pp 595–640.

Gibson RS (2005b). *Principles of nutritional assessment*. Oxford University Press: New York, USA. pp 443–476.

HKI/IPHN (1999a). *Iron deficiency anaemia throughout the lifecycle in rural Bangladesh. National vitamin A survey, 1997–98*. HKI: Dhaka, Bangladesh.

HKI/IPHN (1999b). *Vitamin A status throughout the lifecycle in rural Bangladesh. National vitamin A survey, 1997–98*. HKI: Dhaka, Bangladesh.

INACG (2002). *Anaemia, iron deficiency and iron deficiency anaemia*. ILSI Human Nutrition Institute: Washington, DC.

INACG (1985). *Iron deficiency in woman. A report of the international nutritional anaemia consultative group 1984*. The Nutrition Foundations: Washington DC.

Jacob RA (1999). Vitamin C. In: Shils ME, Olson JA, Shike M and Ross AC (eds). *Modern Nutrition in Health and Disease* 9th edn. Williams and Wilkins: Baltimore, USA. pp 467–483.

Jahan K, Hossain M (1998). *Nature and extent of malnutrition in Bangladesh. Bangladesh National Nutrition Survey, 1995–96*. Institute of Nutrition and Food Science, University of Dhaka: Dhaka, Bangladesh.

Jansson LT, Kling S, Dallman PH (1986). Anemia in children with acute infection seen in a primary care pediatric outpatient clinic. *Pediatr Infect Dis* **5**, 424–427.

Lonnerdal B (1989). Dietary factors affecting trace element absorption in infants. *Acta Paediatr Scand* **351**, 109–113.

McDonnell SM, White D (1997). Hereditary hemochromatosis. Preventing chronic effects of this underdiagnosed disorder. *Postgradute Medicine* **102**, 83–85.

Mejia LA (1992). Role of vitamin A in iron deficiency anaemia. In: Nutritional anaemias (eds). SF Fomon & S Zlotkin, *Nestle Nutrition Workshop Series* vol. 30, pp 93–101. Vevey: Nestle Ltd./New York: Raven press.

Pepys MB (1981). C-reactive protein fifty years on. *Lancet* **8221**, 653–657.

Powers HJ, Bates CJ, Duerden JM (1983). Effect of riboflavin deficiency in rats on some aspect of iron metabolism. *Int J Vit Nutr Res* **53**, 371–376.

Prasad AP, Lakshmi AV, Bamji MS (1987). Riboflavin and haemoglobin status of urban school boys: relationship with income, diet and anthropometry. *Indian J Pediatr* **54**, 529–533.

Ronnenberg AG, Goldman MB, Aitken IW, Xu X (2000). Anemia and deficiencies of folate and vitamin B6 are common are common and vary with season in Chinese women of childbearing age. *J Nutr* **130**, 2703–2710.

Roos N, Islam MM, Thilsted SH (2003). Small indigenous fish species in Bangladesh: contribution to vitamin A, calcium and iron intakes. *J Nutr* **133**, 4021S–4026S.

Senti FR, Pilch SM (1985). *Assessment of the folate nutritional status of the US Population based on data collected in the Second national Health and Nutrition Examination Survey 1976–1980*. Life Sciences Research Office, Federation of American societies for Experimental Biology: Bethesda, MD, USA.

Stoltzfus RJ, Chwaya HM, Tielsch JM, Schulze KM, Albonico M, Savioli L (1996). Epidemiology of iron deficiency anaemia in Zanzibari schoolchildren: the importance of hookworms. *Am J Clin Nutr* **65**, 153–159.

Suzuki N (1981). *Color Atlas of Human Helminth Eggs*. JAPC and JOICEP: Tokyo.

Thompson D, Milford-Ward A, Whicher JT (1992). The value of acute phase protein measurements in clinical practice. *Ann Clin Biochem* **29**, 123–131.

WHO (1994). *Bench aids for the diagnosis of intestinal parasites*. WHO: Geneva, Switzerland.

WHO (1995). *Physical Status: The Use and Interpretation of Anthropometry* WHO Technical Series No 854 WHO: Geneva, Switzerland.

WHO (1996). *Indicators of assessing vitamin A deficiency and their application in monitoring and evaluating intervention programmes. WHO/NUT/96.10*. WHO: Geneva, Switzerland.

Yu YY, Cho YO (1990). Tissue iron content in riboflavin and pyridoxine deficient rats. *J Nutr Biochem* **1**, 310–314.