CHILDHOOD ANEMIA AND VITAMIN A DEFICIENCY IN RURAL BANGLADESH

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Abstract. The study aimed to assess the prevalence of anemia and vitamin A deficiency in preschool children in rural Bangladesh. A cross-sectional study was done on eight randomly-selected sub-districts of rural Bangladesh. Children (n=1,302) aged 2-6 years were studied. Families of 43% of the study participants had a monthly household expenditure of US$ 60 or less. Fifty-six percent of the children were underweight, and 17% were severely underweight; 18% were wasted, and 1% were severely wasted; and 45% were stunted while 20% were severely stunted. The mean ± SD serum retinol of the children was 1.0 ± 0.4 μmol/l, and 3% of them had serum retinol levels of <0.35 μmol/l, about one-fifth (20%) had a serum retinol level of <0.70 μmol/l and 55% had serum retinol levels of <1.05 μmol/l. The mean hemoglobin concentration of the children was 110 ± 11 g/l, and 48% had a Hb of <11 g/l signifying anemia in this age group. Thirty-one percent (31%) of children had low serum ferritin (<12 μg/l), and 14% had elevated CRP (≥5 mg/l) indicating the presence of a sub-clinical infection. Male and female children had similar nutritional status and biochemical profiles although boys tended to be heavier than girls (p=0.013). The proportion of children with anemia and iron deficiency anemia (IDA) declined significantly (p<0.001) with advancing age. Five percent of the study children had IDA and concomitant low serum retinol. The proportion of children with IDA and serum retinol also declined significantly with increasing age from 8% in children aged 35 months or younger, to 3% in children aged 60 months and more (p=0.025). Results of our study clearly demonstrated the public health importance of anemia and vitamin A deficiency among children of rural Bangladesh.

INTRODUCTION

Childhood nutritional deficiencies (which include deficiencies in micronutrients), and its associated illnesses – diarrhea, respiratory track infections, and debilitation – are major public health concerns of developing countries such as Bangladesh (Tomkins, 1986; Underwood and Smitasiri, 1999; Udall *et al,* 2002). It has been estimated that globally 150 million children under the age of five are malnourished; of this number, 72% live in Asia, mostly in South Asia (Udall *et al,* 2002). A recent countrywide survey in Bangladesh (n=4,527) found that 49, 47, and 11% of rural children aged 0-59 months were underweight, stunted, and wasted, respectively (National Institute of Population Research and Training, 2001). Micronutrient-malnutrition is a deficiency of one or more vitamins or minerals. Iron deficiency in children impairs physical

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growth, mental development, and academic achievements. Deficiency of vitamin A results in night blindness, growth retardation, mucosal damage of tracts, and ultimately blindness (Underwood and Smitasiri, 1999; Singh, 2004). Micronutrients play an active role in the promotion of physical growth and neuromotor development (Guesry, 1998; Underwood and Smitasiri, 1999; Singh, 2004). There is a lack of substantial evidence regarding multiple-micronutrient deficiency in preschool children in Bangladesh (Ahmed, 2000; Rahman *et al,* 2002). The aim of the present study was to quantify the prevalence of anemia and vitamin A deficiency in preschool aged children in rural Bangladesh.

MATERIALS AND METHODS

Data sources/Site/Design/Participants

Data for analysis were derived from a database comprising information collected for the evaluation of an ongoing home-gardening program in rural Bangladesh. A cross-sectional survey design was applied for evaluation, and data were collected from areas where the home-gardening program was in operation for more than two years. The study was conducted in randomly-selected (using multistage sampling) eight *thanas* (sub-districts) of rural Bangladesh from October 1997 to December 1999. Two groups were studied: the first group included children 24-72 months of age (n=1,302) permanently living in the area and their mothers constituted the second group (n=1,091). Pregnant women or participants with known or suspected chronic and congenital diseases were excluded. Eligible women were requested to participate and a written consent was obtained if they agreed to participate in the evaluation. The response rate varied from 80 to 90% in each village. The study was approved by the Research Review Committee and the Ethical Review Committee of ICDDR,B: Centre for Health and Population Research. For this study, we analyzed data that were obtained from children.

Questionnaire and data collection

Pre-coded sets of questionnaire were developed, tested in the field, and finalized to collect relevant information, including demography, socioeconomic status, individual characteristics, recent morbidity, anthropometry, and some selected nutritional biochemical profiles. Mothers were visited on fixed dates for interview. Research assistants were trained to apply standard sets of questionnaires for the accurate collection of data. Investigators monitored the performance of field personnel through regular observation at the household level, regular data-completion checks, and the random resampling of 5% of contacts. Errors were corrected immediately in the field. Identical forms, equipment, definitions, and methods were used throughout the study period. The education level and total household monthly expenditures of mothers were used as proxy indicators of their socioeconomic status. Mothers were asked to recall their own and their children's recent diarrhea, dysentery and morbidity within the past week. Diarrhea was defined as three or more abnormally loose or watery stools without blood, and dysentery was defined as any number of stools in a 24-hour period containing visible blood in the last seven days.

Anthropometry

Anthropometric measurements (weight, height, and mid-upper arm circumference) of the children and the mothers were recorded to determine their nutritional status (Waterlow *et al,* 1977; Hamil *et al,* 1979). Weights of the children were obtained to the nearest 100 g using an electronic digital scale (Seca model 770; Seca, Hamburg, Germany), and its accuracy was periodically verified using reference weights. Recumbent length or standing height was determined with a locally constructed instrument in which a metal tape was extended

between a footplate and a head bar, and the mean of two consecutive measurements to the nearest 0.1 cm was recorded as the observed value. The nutritional status of the children was subsequently compared with the National Center for Health Statistics standard using the z-scores (Waterlow *et al,* 1977; Hamil *et al,* 1979). Weights of the mothers were measured with a Seca electronic scale (Heavy Duty Floor Digital Professional Scale: Model 770) with a digital display and an accuracy of 100 g. Their heights were measured by locally-made wooden height stick to the nearest 0.1 cm. Mid-upper arm circumference (MUAC) was measured to the nearest millimeter on the bare left arm using a locally made non-elastic MUAC tape. Trained and experienced research assistants followed standard procedures in performing anthropometric measurements with an interworker reliability of nearly 95% (Cogill, 2003). Anthropometric measurements were collected on a separate day of the week.

Biochemical measurements

About 2.0 ml of venous blood without any stasis was collected from children by venipuncture using aseptic technique, and an aliquot was immediately put into a vial wrapped with aluminium foil to prevent exposure to light and kept in a foam rack at room temperature until clotted. Another aliquot 50 μl was put in tubes with EDTA for estimation of hemoglobin. All samples were transported to a nearby peripheral laboratory for centrifugation. Separated serum was transported to the Nutritional Biochemistry Laboratory of ICDDR,B and stored at -20ºC until assay. Hemoglobin (Hb) was measured by spectrophotometry (SIGMA kit, St Louis, MO, USA) after conversion of Hb to cyanmethemoglobin (Rice, 1967). Serum retinol was determined by a modified High-Performance Liquid Chromatography (HPLC) technique (Bieri *et al,* 1979). Ferritin concentration was measured by the double antibody sandwich ELISA technique using a commercially-available kit (Ramco Laboratories, USA) (Alfred, 1978). CRP concentration was measured by immunoturbidimetric assay adapted for the Hitachi-902 analyzer using a commercial kit (Roche, Germany) (Titz, 1976). Like anthropometric measurements, blood specimens were collected on a separate day of the week. Laboratory standardization routine coefficient of variations were 2.6, 5.0, 0.7, and 1.7 for C-reactive protein, serum ferritin, hemoglobin, and serum retinol, respectively.

Data management

The data collection forms were visually scanned soon after each interview and marked for omissions, inconsistencies, and mistakes. Corrected data were entered into a personal computer using StatPac Gold Version 3.2 (Walonick Associates, Minneapolis, MN, USA) after creating a template for each data entry file along with appropriate logical and consistency checks as soon as they became available, and all data were entered twice. Summary statistics and tables were produced for inspection. Backup data were immediately copied on two other storage media as soon as data verification was complete.

Data analysis

Data analysis was performed using the Statistical Package for Social Sciences, version 10.01 Windows, (SPSS, Chicago, IL, USA) and Epi Info (version 6.0, USD, Stone Mountain, GA, USA). Statistical analyses included descriptive as well as analytical methods. Univariate analysis consisted of simple frequency description of selected variables. Normally-distributed continuous variables were presented as mean±standard deviation, and those with non-normal distribution were described as median (range).

RESULTS

In total, 1,082 households in 42 villages were surveyed. A number of basic socioeconomic and demographic characteristics were studied. Health and nutrition-related informa-

tion was collected from 1,302 children, and blood samples were collected from them for the estimation of retinol, ferritin, hemoglobin, and CRP concentrations. Forty-three percent of the children were from families having a monthly household expenditure of US$ 60 or less. Underweight was the dominant form of malnutrition (56%) in study children, and 17% were severely underweight. In terms of prevalence, stunting (45%) ranked second and 20% were severely stunted. Wasting was relatively uncommon: 18% were wasted, and 1% was severely wasted. Male and female children had similar nutritional status and biochemical profiles except boys tended to be heavier than girls (p=0.013) (Table 1). Nutritional biochemistry profile of the study children is presented in Table 2. The mean ± SD serum retinol of the children was 1.0±0.4 μmol/l, and 3% of them had serum retinol levels of <0.35 μmol/l, about one-fifth (20%) had a serum retinol level of <0.70 μmol/l and 55% had serum retinol levels of <1.05 μmol/l. Mean± SD of hemoglobin concentration of the children was 110 ± 11 g/l, and 48% had a Hb of <110 g/l signifying anemia in this age group. Thirty-one percent of children had low serum ferritin (<12 μg/l) while 14% had elevated CRP (≥5 mg/l) indicating the presence of an inflammatory process. In the preceding week, 24% and 5% of the children had a history of at least one episode of diarrhea or dysentery. Eighteen per-

Table 1
Characteristics of the study children (n=1,302).

Characteristic	Overall (n=1,302)	Male (n=660)	Female (n=642)
Age (month), %			
24-35	19.2	18.3	19.8
36-47	21.3	23.2	19.5
48-59	20.8	21.2	21.3
60-71	38.7	37.3	39.4
Mean ± SD Age (month)	50.2±15.9	50.5 ± 15.7	51.0 ± 16.0
Male, %	50.1	50.7	49.3
Anthropometry			
Mean ± SD weight (kg)	12.8±2.7	13.0 ± 2.6	12.7 ± 2.7
Mean ± SD height (cm)	94.9±10.4	95.7 ± 9.9	95.2 ± 10.6
Mean ± SD MUAC (cm)	14.2±1.1	14.2 ± 1.1	14.2 ± 1.1
Weight-for-age Z Score, %			
<-2 SD (underweight)	56.2	57.1	55.3
<-3 SD (severe underweight)	17.4	16.8	17.9
Weight-for-height Z Score, %			
<-2 SD (wasted)	18.2	16.7	19.8
<-3 SD (severe wasted)	1.1	1.2	0.9
Height-for-age Z Score, %			
<-2 SD (stunted)	44.9	46.7	43.1
<-3 SD (severe stunted)	20.4	20.3	20.6
Monthly household expenditure (US$), %			
≤ 60	43.1	43.7	42.5
61-120	42.2	43.6	40.8
>120	14.7	12.7	16.7

Table 2
Nutrition biochemistry profile of the study children (n=1,302).

Characteristic	Overall	Male	Female
Serum retinol (μmol/l), %			
<0.35	2.5	1.4	3.2
0.35-0.69	17.1	15.3	18.6
0.70-1.049	35.8	37.5	34.6
≥1.05	44.6	45.8	43.6
Mean ± SD serum retinol, μmol/l	1.0±0.4	1.1 ± 0.4	1.0 ± 0.4
Anemia (Hb <110 g/l), %	47.5	46.8	48.3
Mean ± SD Hb, g/l	11.0±1.1	11.0 ± 1.1	11.0 ± 1.1
Serum ferritin (μg/l), %			
<12 μg/l	30.5	28.0	31.9
<20 μg/l	50.9	51.0	49.6
Median (range) serum ferritin, (μg/l)	19.0 (0.0-388.5)	19.0 (0.0-260.0)	20.0 (0.0-388.5)
Indicator of inflammation			
C-reactive protein (CRP; mg/l), %			
CRP ≥5 mg/l	13.6	12.5	14.7
Median (range) CRP, mg/l	0.2 (0.0-188.5)	0.1 (0.0-188.5)	0.4 (0.0-166.5)
IDA (Hb <110 g/l + SF < 12 μg/l)	18.4	16.2	19.6
Iron depletion ((Hb ≥110 g/l + SF < 12 μg/l)	11.4	10.9	11.4
IDA and low (<0.70 μmol/l) serum retinol	5.1	4.5	5.5
Morbidity, %			
Diarrhea in last 7 days	23.7	26.8	25.1
Dysentery in last 7 days	5.3	5.5	3.9

Table 3
Anemia, IDA, possible IDA, and iron depletion of children (n=1,302).

Age (months)	Anemia	IDA	Possible IDA	Iron depletion
24-35 (n=248)	60 (149/248)	24 (60/246)	13 (32/246)	11 (28/246)
36-47 (n=278)	53 (146/278)	23 (64/274)	10 (27/274)	10 (28/274)
48-59 (n=277)	45 (124/277)	18 (48/270)	9 (24/270)	13 (35/270)
60^{+} (n=499)	40 (200/499)	12 (61/495)	9 (44/495)	11 (54/495)
Total (n=1,302)	48 (619/1,302)	18 (233/1,285)	10 (127/1,285)	11 (145/1,285)

Data presented as % (n); IDA, iron deficiency anemia; Hb, hemoglobin; SF, serum ferritin.
* Anemia : Hb < 110 g/l; IDA : Hb < 110 g/l and SF < 12 μg/l
Possible IDA : Hb < 110 g/l and SF = 12-20 μg/l; Iron depletion : Hb ≥ 110 g/l and SF < 12 μg/l

cent of children had confirmed iron deficiency anemia (IDA) (Table 3). There was a significant declining trend (p<0.001) in proportion of anemia, and IDA in children with advancing age. Five percent of the study children had IDA and low serum retinol. Proportion of children with IDA and serum retinol decreased significantly with increasing age from 8% in children aged 35 months or younger to 3% in children aged 60 months or more (p=0.025).

DISCUSSION

Micronutrient-malnutrition is associated with changes in many biochemical indices (Waterlow, 1976). Some of these parameters were evaluated among children in rural Bangladesh. Vitamin A plays an important role in human growth, development, and immunity (Tomkins, 2000). In developing countries, low maternal intake of carotenoids and vitamin A results in sub-normal plasma retinol concentrations. That, in turn, causes not only very low liver stores of vitamin A in neonates but also low concentrations of vitamin A in mother's breast milk (Truswell, 1985). Moreover, absorption and transportation of vitamin A are poor in children with inadequate nutrition. Furthermore, childhood infections can precipitate vitamin A deficiency (Tomkins, 2000). A cross-sectional study in Brazil reporting lower concentrations of vitamin A in the cord-blood of smaller/shorter babies in comparison to heavier/longer babies suggests a role for vitamin A in the promotion of growth (Rondo *et al,* 2001). Certain infections, such as diarrhea and dysentery, result in inadequate dietary intake because of anorexia, reduced intestinal absorption due to damaged epithelia, or increased intestinal and urinary loss of endogenous stores of vitamin A (Tomkins, 1986). Retinol contributes to health by maintaining the integrity of epithelial cells and the promotion of immune functions whereas provitamin-A carotenoids are known to have antioxidant properties (Campos *et al,* 1987; West *et al,* 1999). In rural Bangladesh, diets of children are primarily cereal based and devoid of carotenoids. Frequent illnesses among the children further aggravate the deficiency of vitamin A (Hussain and Kuale, 1996). Iron and other micronutrient deficiencies may occur simultaneously because of high rates of infections, particularly diarrhea with associated anorexia, and poor-quality diet or reduced nutrient bioavailability (Allen *et al,* 2000). It has been postulated that the coexistence of these micronutrient and iron deficiencies may increase the risk of anemia and also limit the hematological response to iron supplementation. Inflammation can influence iron-status indices, and may decrease the effect of additional dietary iron by reducing absorption and iron metabolism (Asobayire *et al,* 2001). The findings of our study should be interpreted with caution, as serum ferritin levels may not in fact be raised, but may still remain within normal limits despite iron deficiency in conditions of inflammations, infection, or significant parenchymal disorders (Abshire *et al,* 1983). However, the strength of our study is derived from the venous blood which we were able to isolate for hemoglobin estimations. Blood obtained by venipuncture as opposed to fingertip skin punctures is known to be more precise (Linpisarn *et al,* 1996). The relatively large study population and the diversity of the geographical sites from which subjects were drawn further add to the strength of our study. The findings of our study are likely to be useful in developing strategies to improve nutritional status, especially the design of effective interventions for children with IDA and low serum retinol concentrations in rural Bangladesh.

ACKNOWLEDGEMENTS

This study was conducted at ICDDR,B: Center for Health and Population Research with the support of the United States Agency for International Development (Cooperative agreement number # HRN-A-00-96-90005-00). ICDDR,B acknowledges with gratitude the commitment of USAID to the Center's research efforts.

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