

# **Seasonal Variations in Selected Biochemical Indices of Nutritional Status in Adolescent Girls**

*Umme Fatema Chowdhury, Cadi Parvin Banu, Rezaul Karim and Faruk Ahmed*

Institute of Nutrition and Food Science, University of Dhaka, Dhaka Bangladesh

## **Introduction**

Various epidemiological studies have shown that several personal factors may influence the nutritional status, especially the biochemical indices<sup>1,2</sup>. Large variations within persons on the measurement of association between the biochemical indices of nutritional status and an outcome of interest may reduce the strength of association. Thus a major concern in the assessment of association between the outcome variables and the biochemical indices, especially plasma retinol, is the imprecision resulting from variations over a period of time in actual nutrient concentration<sup>3,4</sup>. There has been a suggestion that this may result from the variation in dietary intake<sup>5</sup>.

Seasonal variations in food consumption<sup>6</sup> and childrens' nutritional status<sup>7,8,9</sup> have been identified in rural areas of Bangladesh. Although there is limited choice of food items

in Bangladeshi diet, a recent study has shown that the daily variation of nutrient intake in each individual over a period of time is as large as that has been reported for developed countries<sup>10</sup>.

Since the epidemiological studies involving biochemical indices of nutritional status are typically based on single blood specimen, often collected without regard to season, it is important to determine how well a single measurement reflects subjects usual blood/serum concentrations of various biochemical indices. In this paper we report the results of a study of variation between two seasons in blood/serum concentrations of selected biochemical indices in a group of adolescent girls living in an orphanage.

## **Materials and Methods**

Fifty-one healthy adolescent girls, aged between 13 and 16 years, who were the residents of the Sir Salim-

ullah Muslim Orphanage, Azimpur, Dhaka were selected for the study. None of the subjects took iron tablets or vitamin supplements during the study period. The subjects were not told about the possible dependence of study results on their diets. The purpose of the study was explained to the orphanage administration and permission from the orphanage authority was sought, and informed consent was obtained from all participants. Anthropometric data and blood samples were collected twice: in summer (mid May, 1993) and in winter (early January, 1994).

Body weight, to the nearest 100g, was measured for each girl whilst barefoot and wearing school uniform, with a lever balance (Detecto-Medic, Detecto Scales Inc, Webb city, MO). The school uniform was weighed later (the average weight was 0.5 kg) and the value was subtracted from the weight measured. Height was measured with the subjects standing barefoot, to the nearest 0.1 cm, using a standard scale (Detecto-Medic, Webb city, MO). Weight-for-height was calculated using National Centre for Health Statistics reference population<sup>11</sup>.

Three millilitres of non-fasting blood were drawn from the subject's arm between 09:00 and 10:00 a. m. A sample of 0.5 ml of this blood was

taken into a heparinized tube to measure haemoglobin concentration. The remaining 2.5 ml was placed in an acid washed glass centrifuge tube and immediately wrapped in aluminium foil to protect against degradation of vitamin A by light. The blood was allowed to clot at 25°C for 2 h. After centrifugation at 1000 ×g for 10 min at room temperature, serum samples were separated and kept frozen at -20°C until further analysis. For estimation of zinc and copper, serum samples were kept at -20°C in separate ion free vials.

Haemoglobin and serum protein concentrations were determined by the cyanomethaemoglobin and biuret methods respectively using commercial kits (Boehringer Mannheim, Germany). The serum zinc and copper concentrations were measured by atomic absorption spectrophotometry (SP9; Pye-Unicam, Cambridge, UK) according to the manual provided with the instrument. Serum retinol (vitamin A) was assayed according to the method of Bieri *et al.*<sup>12</sup> with slight modification using high pressure liquid chromatography. More details of the method are described elsewhere<sup>2</sup>. The interassay variation for serum retinol was 2.3%.

Data were analyzed by SPSS/PC (version<sup>4.1</sup>; SPSS Inc, Chicago).

Before analysis, normality of distributions of data were tested using the K-S goodness of fit test, and where necessary, data were normalized using appropriate transformations. Mean and standard deviations were calculated: one way analysis of variance was used to analyze the differences between the two seasons.

## Results

Table 1 shows the personal characteristics of the study participants. Mean ( $\pm$ SD) age of the respondents at recruitment was 13.8 ( $\pm$ 0.9) years, with a median of 14.0 years. Mean ( $\pm$  SD) body weight and height of the girls was 41.5 ( $\pm$  5.1) kg and 148.7 ( $\pm$  4.6) cm respectively. The mean ( $\pm$  SD) weight-for-height was 103.9 ( $\pm$  13.7) %, with a median of 102.7% and ranged from 81.0 to 146.5%. Mean. ( $\pm$  SD) serum protein

concentration of the study girls at recruitment was 8.4 ( $\pm$  1.2) g/dl.

The mean concentrations of the selected biochemical indices of the girls in summer and in winter are given in Table 2. Mean concentrations of haemoglobin, serum vitamin A and zinc were significantly lower in winter than those in summer. Serum copper level remained unchanged.

The number and percentage of the study participants with low levels of various biochemical indices in summer and winter are shown in Table 2. In winter, greater proportion of the girls had low haemoglobin ( $<$ 12 g/dl), serum vitamin A ( $<$  30  $\mu$ g/dl) and zinc levels ( $<$  70  $\mu$ g/dl) compared with those in summer. There was a slight reduction in winter in the proportion of the girls with subnormal serum copper level ( $<$  70  $\mu$ g/dl).

**Table 1. Personal Characteristics of the Study Participants.**

Variable	Mean	$\pm$ SD	Range
Age (years)	13.8	$\pm$ 1.0	13-16
Body Weight (kg)	41.5	$\pm$ 5.1	32.8-56.4
Height (cm)	148.7	$\pm$ 4.6	138.0 -159.0
Weight for Height (%)	103.9	$\pm$ 13.7	81.0-146.5
Serum Protein (g/dl)	8.4	$\pm$ 1.2	6.0 -10.9

**Table 2. Mean concentrations of the selected biochemical indices of the study participants in summer and in winter.**

Variable	Summer		Winter		Decrease in Level (%)	P* value
	Mean	± SD	Mean	± SD		
Haemoglobin (g/dl)	14.1	± 1.3	13.3	± 1.4	5.5	0.001
Serum retinol (µg/dl)	64.7	± 20.9	47.9	± 17.9	25.9	0.0001
Serum zinc (µg/dl)	83.3	± 18.1	68.1	± 14.7	18.2	0.0001
Serum copper (µg/dl)	95.8	± 22.1	95.5	± 19.5	0.3	0.94

\* calculated using oneway analysis.

**Table 3. Distribution of the study participants with low levels of the selected biochemical indices in summer and in winter.**

Variable	Summer		Winter	
	Number	(%)	Number	(%)
Haemoglobin	4	8	7	13.7
Serum retinol	1	2	7	13.7
Serum zinc	11	21.6	27	52.9
Serum copper	8	15.7	7	13.7

### Discussion

The present study shows the seasonal variation of a number of biochemical indices of the nutritional status in a group of adolescent girls, aged between 13 and 16 years, living in an orphanage in Dhaka city. All girls, who fell in

the selected age group, of Sir Salimullah Muslim Orphanage, Azimpur, Dhaka were invited to participate in the study. About 44% of eligible girls completed the study.

The growth of the girls as expressed by weight-for-height was not significantly different between summer and

the following winter (data not shown). The ability of anthropometric indicators to detect the short-term seasonal changes in the nutritional status of a population depends on the age group and the ability of the indicators to measure changes in body size or composition that are truly nutritional in character<sup>13</sup>.

There were substantial variations between the two seasons in the biochemical indices of the nutritional status: values being higher in summer than subsequent winter. In winter, mean concentration of haemoglobin of the study participants was lower by 5.5% compared with that of summer value. Serum retinol was about 26% lower in winter. Serum zinc level was decreased by about 18%, while there was only a little change in serum copper level. In both seasons, however, the average blood/serum concentrations of the biochemical indices were within the normal range, except serum zinc which fell below the normal value in winter.

According to WHO<sup>14</sup>, adolescent girls with a haemoglobin level below 12.0 g/dl are considered to be anaemic. Using this criterion, about 8% of the girls in summer and about 14% in winter were found to be anaemic. Serum retinol level of 30.0 µg/dl or above is considered to be adequate vitamin A nutrition<sup>15</sup>. In the present

study, only 2% was found to have serum retinol level below 30.0 µg/dl in summer, while in winter this figure was raised to about 14%. Serum zinc and copper levels below 70.0 µg/dl are indicative of marginal deficiency<sup>16</sup>. Our data indicate that about 22% and 16% of the girls were marginally deficient in zinc and copper respectively in summer, while in winter the figures were 53% for zinc and about 14% for copper. Sandstead<sup>16</sup> suggested that mild zinc deficiency might be related to poor zinc availability in foods combined with the accelerated growth spurt that occurs during adolescence. Kinard *et al.*<sup>17</sup> worked on American adolescent girls and reported that mean dietary zinc intake was 81% of recommended dietary allowances for this population, which was reflected in plasma zinc level. They also reported that about 70% of their study subjects had relatively poor dietary copper intake, however, plasma and red blood cell copper levels were within normal range<sup>17</sup>. The interpretation of rather high prevalence of marginal zinc and copper deficiency among the orphan girls in both seasons in this study needs further investigation using several different parameters including dietary intake for assessment of zinc and copper status.

Apart from seasonal variations, the orphan girls appeared to have good nutritional status. Our findings are consistent with the work reported by Hassan and Barua<sup>18</sup> who worked on orphan boys, aged 5-15 years, in Dhaka city. The possible explanation of good nutritional status is that in addition to the regular meal provided by the orphanage authority, most of the orphanage children in Dhaka city frequently receive generous gifts of sweets, seasonal fruits and prepared foods from the local benevolent people, which supplement their regular diet. Thus not surprisingly, these orphans get an adequate amount of foods<sup>18</sup> which in turn results in good nutritional status found in our study.

There may be two explanations to higher blood/serum concentrations of the biochemical indices in summer; first, the blood samples of the girls were drawn after two weeks of Eid-ul-Azha (muslim festival) when the intakes of meat and rich foods (gift from benevolent people) were much higher than any other time of the year. However, we do not have any quantitative data on dietary intake. Secondly, the period of blood collection (in summer) was the peak time for seasonal fruits in the country and the consumption of fruits such as mango, pineapple,

jackfruit (*Artocarpus heterophylla*) and various citrus fruits were also reported to be higher than any other season of the year. These fruits contain high levels of carotenes and ascorbic acid (vitamin C). Bates *et al.*<sup>3</sup> worked on the seasonal variations in plasma retinol and carotenoid levels in rural Gambian women and reported a maximum level of both serum carotene and retinol in May and June, which is the peak time for mango, a major contributor to carotene intake<sup>19</sup>. Vitamin C was reported to be associated with increased absorption of iron<sup>20</sup>. The meat intake of the girls was high in summer and it is not only an excellent source of bioavailable heme iron, it also promotes absorption of non-heme iron from the common pool<sup>21</sup>. Further, there is considerable evidence which suggests that vitamin A (retinol) is associated with the iron metabolism and thereby enhance the utilization of iron for erythropoiesis<sup>22</sup>. High intake of carotenoids and ascorbic acid in summer might have influenced the concentration of hemoglobin in summer.

Our findings reveal seasonal variations in the levels of biochemical indices of the nutritional status of adolescent orphan girls. The influence of seasons on the blood levels of various biochemical indices

in the present study may have important implication on the design and interpretation of biochemical epidemiological studies, which generally rely on a single serum measurement of biochemical indices.

### Summary

The influence of seasonal variations in the concentrations of the selected biochemical indices of nutritional status was examined in a group of fifty-one apparently healthy adolescent girls, aged between 13 and 16 years, living in an orphanage in Dhaka city. Blood samples were collected in two seasons: in summer (mid May, 1993) and in winter (early January, 1994). Mean concentrations of haemoglobin, serum vitamin A (retinol) and zinc in winter were significantly lower than those in summer. No change was observed for serum copper level. A greater proportion of the girls had low haemoglobin, serum vitamin A and zinc levels in winter than in summer. The results suggest that seasonal factors may seriously influence the levels of biochemical indices of nutritional status in adolescents.

### Acknowledgements

The authors express their sincere thanks to all the participants of this study.

### References

1. Comstock GW, Menkes MS, Schober SE, Vuilleumier JP and Helsing KJ. Serum levels of retinol, beta-carotene and alpha-tocopherol in older adults. *Am. J. Epidemiol.* 1987; **127**: 114-123.
2. Ahmed F, Mohiduzzaman M, Barua S, Shaheen N, Margetts BM and Jackson AA. Effect of family size and income on the biochemical indices of urban school children of Bangladesh. *Eur. J. Clin. Nutr.* 1992; **46** : 465-473.
3. Bates CJ, Villard L, Prentice AM, Paul AA and Whitehead RG. Seasonal variation in plasma retinol and carotenoid levels in rural Gambian women. *Trans. Roy. Soc. Trop. Med. Hyg.* 1984; **78** : 814-817.
4. Nierenberg DW and Stukel TA. Diurnal variation in plasma levels of retinol, tocopherol and beta-carotene. *Am. J. Med. Sci.* 1987; **30**: 187-190.
5. Ziegler RG, Wilcox HB, Mason TJ, Bill JS and Virgo PW. Seasonal variation in intake of carotenoids and vegetables and fruits among white men in New Jersey. *Am J. Clin. Nutr.* 1987; **45** : 107-114.
6. Institute of Nutrition and Food Science. Nutrition survey of rural Bangladesh, 1975-76. Institute of Nutrition and Food Science, University of Dhaka. 1977.
7. Chen LC, Chowdhury AKMA and Huffman SL. Seasonal dimensions of energy protein malnutrition in rural Bangladesh. The role of agriculture, dietary practices, and infection. *Ecol. Food. Nutr.* 1979; **8**: 175-187.
8. Bairagi R. Is income the only constraint on child nutrition in rural Bangladesh? *Bull WHO.* 1980. **58**: 767-772.

9. Brown H.K, Robert EB and Becker S. Seasonal changes in nutritional status and the prevalence of malnutrition in a longitudinal study of young children in rural Bangladesh. *Am. J. Clin. Nutr.* 1982, **36** : 303-313.
10. Torres A, Willet W, Orav J, Chen L and Huq. Variability of total energy and protein intake in rural Bangladesh: implications for epidemiological studies of diet in developing countries. *Food Nutr. Bull.* 1990, **12** : 220-228.
11. National Center for Health Statistics. NCHS growth chart, 1976. Rockville MD: NCHS. 1976.
12. Bieri JG, Tolliver TJ and Catignani GL. Simultaneous determination of alpha-tocopherol and retinol in plasma or red cells by high pressure liquid chromatography. *Am. J. Clin. Nutr.* 1979, **32**: 2143-2149.
13. Yarbrough C, Habicht JP, Martorell R and Klein RE. Anthropometry as an index of nutritional status. In : *Nutrition and malnutrition*, ed. A F Roche and F Falkner, pp 15-26. New York: Plenum Press. 1974.
14. World Health Organization. Nutritional anaemias *Tech. Rep. Ser. No. 580*: p. 6. WHO, Geneva. 1972.
15. Olson AJ. Recommended dietary intakes (RDI) of vitamin A in humans. *Am. J. Clin. Nutr.* 1987, **45** : 704-716.
16. Sandstead HH. Nutritional role of zinc and effect of deficiency. In : *Adolescent nutrition*, ed. M. Winick, pp-97-124. New York: John Wiley and Sons. 1982.
17. Kinard MD, Liu-Wu SM and Bazzarre TL. Zinc and copper status of adolescent females. *Nutr. Res.* 1989, **9**: 1207-1216.
18. Hassan N and Barua S. Nutrition profile of the orphans: A case study in the Dharmarajika Orphanage of Dhaka city. *Bangladesh. J. Nutr.* 1990 **3** : 43-52.
19. Bates CJ. Vitamin A in pregnancy and lactation. *Proc. Nutr Soc.* 1983, **42**: 65-79.
20. Dallman PR. Iron. In: *Present knowledge in Nutrition*, 6th edn. ed. M. L Brown. International Life Science Institute, Nutrition Foundation. Washington, D. C. 1990.
21. Bothwell TH, Charlton RW, Finch CA and Cook JD. *Iron metabolism in man*. p 1-576. Oxford: Blackwell Scientific Publications. 1979.
22. Bloem MW, Wedel M, van Agtmaal EJ, Speek AJ, Saowakontha S and Schreurs WHP. Vitamin A intervention: short term effects of a single oral, massive dose on iron metabolism. *Am. J. Clin. Nutr.* 1990, **51**: 76-79.