Original Article

Micronutrient status and its relationship with nutritional status in preschool children in urban Sri Lanka

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Objective: To assess the micronutrient status and its relationship with nutritional status in preschool children. **Methods**: In a cross sectional study, anthropometric data and fasting blood samples were obtained from 340 children attending preschool in urban Sri Lanka. Serum concentrations of vitamin D, parathyroid hormone, vitamin A, zinc and haemoglobin were measured. Z-scores of anthropometric indices of height-for-age, weight-for-age and weight-for-height were computed to evaluate the nutritional status. **Results**: Prevalence of stunting, underweight, wasting and anaemia among children were 7.1%, 16.9%, 21.2% and 7.4%, respectively. Deficiencies of zinc and vitamin A occurred among 67% and 38% of children, respectively. Vitamin D deficiency (<10 ng/mL) and insufficiency (10-20 ng/mL) occurred in 5.0% and 29.1% of children, respectively, and12% had parathyroid hormone levels indicative of hypocalcaemia. Nutritional status was significantly correlated ($p<0.05$) with vitamin D status [height-for-age (r=0.10), weight-for-age (r=-0.18), weight-for-height (r=-0.12)], and with haemoglobin status [weight-for-age (r=0.11)]. Zinc and vitamin A levels were lower in severe stunting compared with normal height ($p<0.05$). Significant correlations ($p<0.05$) were observed between vitamin D and parathyroid hormone (r=-0.12) and between haemoglobin and vitamin A (r=0.01), zinc (r=0.02) and vitamin D (r=0.02) levels. **Conclusions**: In the surveyed population, zinc deficiency was high and to a lesser degree vitamin A deficiency and vitamin D insufficiency prevailed. The nutritional status of the children was related to vitamin D status and with haemoglobin status. Zinc and vitamin A levels were low in children with severe stunting. Vitamins A, D and zinc levels were associated with haemoglobin status.

Key Words: micronutrients, nutritional status, vitamin D, preschool children, Sri Lanka

INTRODUCTION

Micronutrient status in children is of particular interest since they can affect health outcomes such as growth, immune competence, cognitive and physical development.[1] Micronutrient deficiencies are common in many developing countries and are typically due to inadequate food intake, poor dietary quality, poor bioavailability and/or the presence of infections.[2,3] While the role of macronutrients in physical growth is well established, the roles of micronutrients are less obvious, thus earning the name "hidden hunger". Micronutrient deficiencies are not clinically identifiable until late stages, which could last for a long period of time and lead to many lasting consequences. Therefore, a comprehensive assessment of the micronutrient status of children is a priority.

In Sri Lanka, childhood under nutrition is still a major public health problem. According to a survey conducted at the national level in 2012, prevalence of stunting, underweight and wasting of children under five years were 13.1%, 23.5% and 19.6%, respectively.[4] Several targeted interventional programmes have been implemented at the national level to improve these indices of nutritional status. Although micronutrient deficiencies are a significant cause of under nutrition, adequate attention has not been given to investigate the micronutrient status among preschool children in Sri Lanka. Most studies on micronutrient status in this age group have often been limited to vitamin A deficiency (VAD),[5,6] and iron deficiency anaemia (IDA).[4,7] While VAD and IDA remain significant public health concerns, it is important to recognize other micronutrient deficiencies such as vitamin D, calcium and zinc which could impair the growth potential of children.[8] To our knowledge, there is only one community based study which had reported co-existent multiple micronutrient deficiencies in Sri Lankan preschool chil-

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dren.[9] The present study was therefore designed to investigate the status of some key micronutrients (vitamin D, vitamin A, zinc, parathyroid hormone as a surrogate marker of calcium), haemoglobin status, their inter-relationships and their relationship with the nutritional status in preschool children in urban Sri Lanka.

METHODS
Study area and subjects
In a cross sectional study, a total of 340 apparently healthy children (172 girls and 168 boys) aged 2-5 years were selected by cluster sampling from the Ragama Medical Officer of Health (MOH) area. Ragama is an urban area located in the Western province of Sri Lanka twenty kilometres to the north of Colombo, the capital city of Sri Lanka. Children with chronic or recent illnesses and consuming micronutrient supplements were not included in the study. Socio-demographic profiles of the children and their families were obtained from parents/guardians at enrolment using an interviewer administered questionnaire.

Ethical consideration
The Ethical Review Committee of Faculty of Medicine, University of Kelaniya, granted approval for the study. Informed written consent was obtained from the parents or guardians of the children registered for the study.

Nutritional assessment
Body weight was determined to the nearest 0.1 kg on an electronic digital scale and height was measured to the nearest 0.1 cm by the anthropometric rod. Age was calculated from the child's birthday taken from the Child Health Development Record (CHDR). Z-scores for weight-for-age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) were derived from Epi info and WHO Anthro Plus softwares.[10] A Z-score <-2.0 from the reference median was used to define underweight, stunting and wasting/thinness respectively.

Determination of micronutrients in serum
Five mL blood samples were collected after an overnight fast from the median-cubital vein under aseptic conditions using disposable needles connected with polypropylene tubes. Hemoglobin (Hb) level was measured at the time of blood collection by Hemocue® field device. All tubes were kept in dark boxes and allowed to clot and centrifuged to extract the sera at 5000 g for 10 mins, within 4 hours. Aliquots of sera were stored at -40°C until analysis. Serum vitamin A levels were measured by reverse phase High Performance Liquid Chromatography (Waters®, UK) according to the method of Bieri et al[11] with a C_{18} column (Waters, UK), mobile phase of methanol: water (95:5) and was detected at 325 nm in a ultra violet detector. Serum vitamin A concentrations were calculated from the standard curve (produced with standard retinol) with the correction of percentage loss using the internal standard (retinyl acetate). Serum vitamin D levels were determined as 25(OH)D in serum by a chemiluminescent immunoassay (Diasporin Liason, USA). Vitamin D analysis was outsourced to a private hospital laboratory with international quality control Certificate of Accreditation

(ISO 15189). Serum parathyroid hormone (PTH) levels were measured on Immulite®/Immulite® 1000 systems for intact PTH by a solid-phase, two-site chemiluminescent enzyme-labeled immunometric assay.[12] The reagents were provided by Siemens Healthcare Diagnostics Products Ltd., U.K. Secretion of PTH is controlled chiefly by serum calcium concentration through negative feedback leading to an inversely proportional relationship with calcium.[13] Serum Zn levels were measured by flame atomic absorption spectrometry (GBC-1312) at 214 nm wavelength using a standard procedure.[14]

The following cut off values were used to define the respective micronutrient deficiency states: vitamin A<20 μg/dL,[6,15] vitamin D deficiency<10 ng/mL, vitamin D insufficiency 10-20 ng/mL),[16] Zinc <9.9 μmol/L.[17] Anaemia was defined as hemoglobin level <11.0 g/dL,[18] and PTH levels >65 pg/mL as an indicator of serum ionic calcium deficiency.[19]

Statistical analysis
Data was analyzed using SPSS version 16 statistical package (SPSS, Inc, Chicago, IL, USA). All micronutrient levels were normally distributed by one-sample Kolmogorov-Smirnov test. Comparisons of serum levels of the micronutrients in different categories of nutritional status were performed using one-way-ANOVA. Linear regression analysis was used to assess the correlation between two continuous variables. Independent sample t-tests were used to compare the prevalence of deficiencies and their differences between groups. The significance level was set at $p<0.05$.

RESULTS
The study sample consisted of 340 children between two to five years of age. There were almost equal proportions of females (50.6%, n=172) and males (49.4%, n=168) and there was no significant difference in the mean age between genders ($p=0.88$).

Table 1 presents the nutritional status of the children. The mean Z-scores of weight-for-height (WHZ), height-for-age (HAZ) and weight-for-age (WAZ) were -1.33±1.05, -0.15±2.78 and 0.82±1.92, respectively, and no significant differences were found between genders ($p>0.05$). The prevalence of wasting, stunting and underweight among the children were 21.2%, 7.1% and 16.9%, respectively. Although stunting was slightly higher among the boys (7.7%) than in girls (6.4%), wasting and underweight were comparable between the genders. None of the children were overweight or obese.

The biochemical indices of micronutrient status are given in Table 2. The mean serum levels of vitamin A, vitamin D, parathyroid hormone (PTH), zinc and haemoglobinin the study population were 23.1±8.89 μg/dL, 23.5±8.97 ng/mL, 26.5±18.4 pg/mL, 9.94±4.58 μmol/L and 11.6±3.24 g/dL, respectively. There was no statistical significance between the mean levels of any of the above parameters between genders ($p>0.05$). Zinc deficiency was the most prevalent micronutrient deficiency among the children (67%), with 69% of the girls and 65% of the boys being zinc deficient when a cut off of 9.9 μmol/L was used ($p>0.05$). 38% each in both genders were found to be vitamin A deficient. Nearly one third of the sample

(32% of boys and 27% of girls) had vitamin D insufficiency (10-20 ng/mL) and 5% of the children (4.8% boys and 5.2% girls) had vitamin D deficiency (<10 ng/mL) (*p*>0.05). This study was the first to document the serum PTH levels in this age group of the Sri Lankan population. Serum PTH level was measured as a surrogate marker of serum ionized calcium level. Using the reference range of 10-65 pg/mL, the majority of the children (88%) had normal serum PTH levels, with mean levels of 25.2±18.4 pg/mL and 27.8±18.4 pg/mL in boys and girls respectively (*p*>0.05). The prevalence of anaemia (Hb<11.0 g/dL) was marginally higher among the boys (7.7% vs 7.0%,

p>0.05). Of the anaemic children, 56% had vitamin D deficiency and vitamin A and zinc deficiencies were found in 60% each (data not shown).

Table 3 shows the mean levels of the biochemical indices of micronutrient status in relation to the nutritional status (WHZ, HAZ and WAZ) each classified as normal (±2SD), moderate (-2SD>Z-score>-3SD) and severe (<-3SD). The mean vitamin D levels were significantly different among different levels of wasting (*p*<0.05), stunting (*p*<0.05) and underweight (*p*<0.01). The mean vitamin D levels increased with increasing levels of wasting and underweight but decreased with increasing levels of

Table 1. Nutritional status of the children in the study

Nutritional status	Boys (n=168)		Girls (n=172)		Total (n=340)	
	*Mean±SD	Z-score <-2SD n (%)	*Mean±SD	Z-score<-2SD n (%)	Mean±SD	Z-score<-2SD n (%)
WHZ	-1.15±1.05	35 (20.8)	-1.11±1.96	37 (21.5)	-1.33±1.05	72 (21.2)
HAZ	-0.17±2.44	13 (7.7)	-0.13±3.08	11 (6.4)	-0.15±2.78	24 (7.1)
WAZ	0.85±1.68	27 (16.1)	0.79±2.13	29 (16.7)	0.82±1.92	56 (16.9)

*p>0.05 between genders.
WHZ: weight-for-height Z-score; HAZ: height-for-age Z-score; WAZ: weight-for-age Z-score.
WHZ<-2SD (wasting), HAZ<-2SD (stunting), WAZ<-2SD (underweight).

Table 2. Biochemical indices of micronutrient status of the children in the study

Analytes	Boys		Girls		Total	
	*Mean±SD	Deficiency n (%)	*Mean±SD	Deficiency n (%)	Mean±SD	Deficiency n (%)
Vitamin A (µg/dL)	23.3±9.13	64 (38.0)	22.8±8.68	66 (38.4)	23.1±8.89	130 (38.2)
Vitamin D (ng/mL)	23.5±9.31	53 (31.5)[†]	23.5±8.64	46 (26.7)[†]	23.5±8.97	99 (29.1)[†]
		8 (4.8)[‡]		9 (5.2)[‡]		17 (5.0)[‡]
PTH (pg/mL)	25.2±18.4	20 (11.9)[§]	27.8±18.4	21 (12.2)[§]	26.5±18.4	41 (12.06)[§]
Zinc (µmol/L)	9.77±4.12	109 (64.7)	10.1±5.04	118 (68.6)	9.94±4.58	227 (66.7)
Haemoglobin (g/dL)	11.6±3.19	13 (7.7)	11.6±3.29	12 (7.0)	11.6±3.24	25 (7.4)

*p>0.05 between genders in all analytes.
Definitions of deficiency parameters; vitamin A <20 µg/dL; [†]vitamin D insufficiency 10-20 ng/mL; [‡]vitamin D deficiency <10 ng/mL; [§]PTH (parathyroid hormone) >65 pg/mL; n (%) indicative of hypocalcaemia ; zinc <9.9 µmol/L; haemoglobin <11.0 g/dL.

Table 3. Biochemical indices of micronutrient status (mean±SD) in relation to nutritional status of the children in the study

	n (%)	Vitamin A (µg/dL)	Vitamin D (ng/mL)	PTH (pg/mL)	Zinc (µmol/L)	Haemoglobin (g/dL)
WHZ						
Severe wasting[‡]	17 (5)	21.7±9.32	28.7±10.5	22.2±15.7	9.62±1.41	10.1±4.89
Moderate wasting[§]	55 (16.2)	22.9±7.59	24.4±9.14	24.1±18.5	9.80±4.02	11.6±2.92
Normal[¶]	268 (78.8)	23.2±5.87	22.9±8.71	27.2±18.5	10.1±4.92	11.7±3.11
Total	340 (100)	23.1±8.89	23.5±8.96*	26.5±18.4	9.96±4.66	11.6±3.03
HAZ						
Severe stunting[‡]	9 (2.7)	16.0±5.82[†]	23.1±10.7	26.9±18.5	9.18±1.89[†]	9.63±5.63
Moderate stunting[§]	15 (4.4)	21.1±7.66	27.1±7.88	18.9±16.4	9.95±4.84	11.4±3.25
Normal[¶]	316 (92.9)	23.3±8.96	28.9±8.90	23.6±18.7	10.5±1.97	11.7±3.15
Total	340 (100)	23.1±8.89	23.5±8.96*	26.5±18.4	9.96±4.66	11.6±3.03
WAZ						
Severe underweight[‡]	15 (4.4)	22.1±8.25	28.5±8.96	19.9±16.0	9.11±1.39[†]	8.83±5.57
Moderate underweight[§]	41 (12.1)	22.8±7.17	25.2±7.35	23.6±14.5	9.99±3.41	11.5±3.38
Normal[¶]	284 (83.5)	23.2±9.19	23.0±11.3	27.2±19.0	10.1±4.92	11.8±3.00
Total	340 (100)	23.1±8.89	23.5±8.96**	26.5±18.4	9.96±4.66	11.6±3.03*

p values (one-way-ANOVA) <0.05*, <0.01** for the comparison between categories of nutritional status.
[†]*p*<0.05 versus normal
WHZ: weight-for-height Z-score; HAZ: height-for-age Z-score; WAZ: weight-for-age Z-score. PTH: parathyroid hormone.
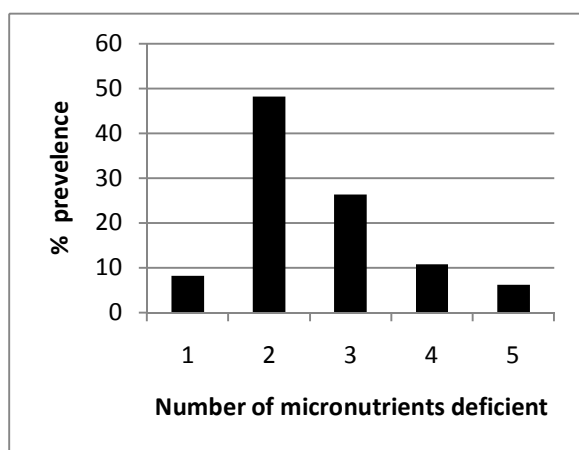[‡]Z-score <-3SD; [§]-2SD >Z-score ≥ -3SD; [¶]Z-score ±2SD.

Figure 1. % prevalence of micronutrient deficiencies among children in the study

stunting. Although not statistically significant the mean levels of serum PTH had a trend opposite to that observed with vitamin D and nutritional status. The mean haemoglobin levels significantly decreased with increasing underweight ($p<0.05$), and 72% of the anaemic children were found to be underweight. With increasing levels of wasting, stunting and underweight, decreasing mean levels of serum vitamin A and zinc were observed, although statistical significance differences ($p<0.05$) were found only between severely stunted and normal height group with respect to vitamin A and severely stunted and severely underweight groups when compared with the respective normal groups for mean zinc levels.

Table 4 shows the correlations between the biochemical indices of micronutrient status and nutritional status. In linear regression analysis vitamin D levels showed a significant positive correlation with HAZ scores ($r=0.10$, $p<0.05$) and significant negative correlations with WAZ ($r=-0.18$, $p<0.05$) and WHZ scores ($r=-0.12$, $p<0.05$). Haemoglobin levels were significantly and positively correlated with WAZ scores ($r=0.11$, $p<0.05$). Intercorrelations between indices of micronutrient status (Table 5) revealed a significant negative correlation between vitamin D and PTH levels ($r=-0.12$, $p<0.05$) and haemoglobin correlated positively and significantly with vitamin D ($r=0.02$, $p<0.01$), vitamin A ($r=0.01$, $p<0.05$) and zinc ($r=0.02$, $p<0.05$).

Figure 1 shows the prevalence of multiple micronutrient deficiencies in the sample studied. None of the children were free of from any micronutrient deficiency investigated. Except for a very small proportion (8%), the majority of the children had two or more micronutrient deficiencies.

DISCUSSION

In general, the majority of preschool children in the sample had normal nutritional status with only 25.3% having some form of malnutrition. Chronic malnutrition was low with 94% of the girls and 92% of the boys having normal height-for-age. Based on the classification criteria for assessing severity of growth deficits,[20] prevalence of underweight (16.7%) and wasting (21.5%) in the sample were "moderate" and "very high" respectively. These data may indicate that acute malnutrition is a more prevalent nutritional problem among preschool children in Ragama MOH area of Sri Lanka. A similar trend was observed at the national level survey conducted in 2013 where the prevalence of stunting showed a decline (13.1%) from previous years, but underweight (23.5%) and wasting (19.6%) remained major public health problems among preschool children.[4] This indicated the need for effective interventional programmes to reduce malnutrition on a priority basis.

Vitamin D deficiency was low (5%), although nearly a one third had had vitamin D insufficiency (29%). This finding was unexpected, since Sri Lanka is a tropical country located at latitude between 6 and 10 degrees in the Northern Hemisphere with sufficient UV-B radiation throughout the year. Studies evaluating the vitamin D status in the Sri Lankan populations are scarce, but there is increasing evidence of hypovitaminosis D from other countries in the South Asian region.[21,22]

A key finding of the present study relating to vitamin D status was its relationship with the height and weight of children (Table 3). Serum vitamin D levels significantly decreased with increasing levels of stunting, which indicated the well recognized role of vitamin D together with calcium in skeletal growth of children. Serum vitamin D

Table 4. Correlation coefficients (r) between nutritional status and indices of micronutrient status

	Vitamin A	Vitamin D	PTH	Zinc	Haemoglobin
WHZ	0.22 (0.23)	-0.12 (0.04)*	0.32 (0.32)	0.11 (0.34)	0.23 (0.11)
WAZ	0.12 (0.21)	-0.18 (0.04)*	0.21 (0.21)	0.19 (0.26)	0.11 (0.03)*
HAZ	0.21 (0.34)	0.10 (0.04)*	0.11 (0.23)	0.13 (0.15)	0.12 (0.12)

Results presented as r (*p*-value), *$p< 0.05$.
WHZ: weight-for-height Z-score; HAZ: height-for-age Z-score; WAZ: weight-for-age Z-score; PTH: parathyroid hormone.

Table 5. Correlation coefficients (r) between indices of micronutrient status

	Vitamin D	PTH	Zinc	Haemoglobin
Vitamin A	0.25 (0.54)	-0.32 (0.80)	0.21 (0.45)	0.01 (0.04)*
Vitamin D		-0.12 (0.04)*	0.13 (0.34)	0.02 (0.003)**
PTH			-0.20 (0.23)	-0.32 (0.82)
Zinc				0.02 (0.04)*

Results presented as r (*p*-value), *$p< 0.05$, **$p<0.01$. PTH: parathyroid hormone.

status significantly increased with increasing thinness (wasting) and underweight as indicated by the decreasing WHZ and WAZ scores respectively. Overall, children with higher serum vitamin D levels were taller and thinner. In recent literature many studies have reported that overweight and obesity are associated with low vitamin D status in both children and adults.[23,24] Although the mechanistic basis is not clearly defined, it is hypothesized that vitamin D after absorption is sequestered and stored in adipose and muscle tissues and then released slowly into the circulation.[25,26] However, vitamin D may increase lean body mass and inhibit the development of adipocytes.[27] Leaner individuals have been shown to have higher serum vitamin D status than heavier individuals.[28] Further to this, serum vitamin D levels had a significant inverse correlation with serum PTH, the surrogate marker of serum ionic calcium. This indicated that with increasing vitamin D levels, serum calcium levels also increased. Emerging evidence suggests a mechanistic role for calcium and vitamin D in the regulation of body weight and composition through thermogenesis, fat oxidation and satiety control.[29,30] While it is clear that both calcium and vitamin D contribute to an anti-obesity effect they also play many other biological roles in the body. Therefore, defining the adequacy of each nutrient alone, or in combination, for nutrients facilitate the full growth potential in childhood warrants further investigation.

The present study also reported a high prevalence of zinc deficiency (67%) among preschool children, which may have far-reaching implications on growth and development. Although information on zinc status in Sri Lankan children is limited, in two other studies conducted in southern Sri Lanka, 50% of the preschool children and 55% of adolescents were found to be zinc deficient using the same cut-off used in the present study.[9,31] These figures are much higher than the level set by the International Zinc Nutrition Consultative Group (IZiNCG) as an indicator for the need of a national interventional programme to improve zinc status.[32] Children with normal zinc status had higher z-scores for WHZ, WAZ and HAZ than zinc deficient children, although no statistically significant correlation was found between zinc status and anthropometric indices. The importance of zinc in growth and development of children is well known, and several previous studies have demonstrated that zinc supplementation improved growth in children.[33,34] Low intake of dietary zinc *per se* rather than poor bioavailability was probably the main factor responsible for low zinc status of these children. Rice-based diets with few animal source foods are likely to be low in absorbable zinc and may not be adequate to improve the zinc status to support growth. Our findings highlight the need for intervention strategies to improve the zinc status in children through zinc supplementation and fortification.

The prevalence of vitamin A deficiency in the study sample (38%) was higher than the value reported for this age group (30%) in the national survey of vitamin A status.[6] In the present study, mean serum vitamin A levels decreased with increasing levels of stunting, underweight and wasting although statistically significant correlations were not found. Severely stunted children had the lowest mean level of serum vitamin A (16 µg/dL) when compared with the mean vitamin A levels of all other categories of nutritional status. This finding is in agreement with several other cross sectional studies which have linked vitamin A deficiency to a greater risk of stunting.[35,36] In some other studies, effects of supplementation of vitamin A on linear growth has been found to be variable indicating that vitamin A needs to be considered as a part of a group of coexisting factors which modify growth.[37-39] Although vitamin A mega dose supplementation at 6 month intervals among preschoolers was a component of the national child health programme since 2008 in Sri Lanka, it had not yet reached the desirable outcome. Therefore the importance of more effective interventional strategies in improving the vitamin A status in children is emphasized.

The present study reported a prevalence of anaemia of 7.4%, which is lower than the national average prevalence (15.1%) reported in 2012. Haemoglobin level had a positive and a significant correlation with WAZ scores, with severely underweight children having the lowest mean haemoglobin level (8.8 g/dL). The positive association between haemoglobin level and growth of children has been shown in many studies.[40,41] The majority of anaemic children in our study were deficient in vitamin A, vitamin D and zinc indicating the importance of multiple micronutrients in maintaining haemoglobin status. The mechanisms by which zinc and vitamin A may affect haemoglobin concentrations have been discussed in previous studies.[42,43] It has been shown by intervention studies that supplementation of either nutrient alone or in combination with iron relative to iron alone improved the hematological response in young children.[44-46] Although weak, a significant correlation (r=0.02, *p*=0.003) was observed between haemoglobin and vitamin D levels in our study, which may indicate a role of vitamin D in haemoglobin synthesis. This finding is supported by other studies in which vitamin D deficiency has been shown to be associated with increased risk of anaemia in children,[47] and thereby suggesting a role of vitamin D in the bone marrow and in erythopoiesis.[48]

Of concern in this study is that the majority of preschool children (92%) suffered from two or more co-existing micronutrient deficiencies. Although nearly a 75% of the children had normal nutritional status with respect body weight and height, micronutrient malnutrition is a hidden but a major health problem among these children. Several other studies including one from Southern Sri Lanka have also reported that two thirds or more of preschoolers suffer from co-existing micronutrient deficiencies, indicating the wide-spread nature of the issue.[9,49] Multiple micronutrient deficiencies or "hidden hunger" in early childhood can lead to lasting and damaging consequences not only on health but also on learning ability and productivity later in life. A limitation of the present study was that serum ferritin levels were not determined in anaemic children. Although the prevalence of anaemia was low in the study, such data would have been useful in determining the aetiology of anaemia in children.

In conclusion, our findings reveal that nutritional status of preschool children is affected by micronutrient and haemoglobin status. Significant relationships were found between serum vitamin D status and height-for age,

weight-for age, weight-for-height of children, and between haemoglobin level and weight-for age of children. Vitamin A and zinc levels may affect linear growth. The findings also highlight affects of vitamin D, vitamin A and zinc on the plasma haemoglobin level. The study identifies a high prevalence of zinc deficiency and to a lesser degree prevalence of vitamin A deficiency and vitamin D insufficiency, either individually or concomitantly among urban preschool children in Sri Lanka. The results presented here calls for coordinated and sustainable interventional programmes to reduce multiple micronutrient deficiencies towards optimizing full growth potential among preschool children.

REFERENCES
1. Viteri FE, Gonzalez H. Adverse outcomes of poor micronutrient in childhood and adolescence. Nutr Rev. 2002; 60:S77-83. doi: 10.1301/00296640260130795.
2. Ramakrishnan U. Prevalence of micronutrient malnutrition worldwide. Nutr Rev. 2002;60:S46-52. doi: 10.1301/002966 40260130731.
3. Rayhan I, Khan SH. Factors causing malnutrition among under five children in Bangladesh, Asian Network for scientific information. Pak J Nutr. 2006;5:558-62. doi: 10.39 23/pjn.2006.558.562.
4. Department of Nutrition, Medical Research Institute, Ministry of Health Care and Nutrition, Sri Lanka in collaboration with UNICEF. National nutrition and micronutrient survey 2012. 2013/02/28 [cited 2014/01/15]; Avialable from: www.unicef.org/srilanka/MNS.
5. Vitamin A deficiency status of children in Sri Lanka 1995/1996 – A Survey Report. Sri Lanka: Medical Research Institute, Ministry of Health; 1998.
6. Jayatissa R, Gunatilleka MM. Vitamin A nutritional status in Sri Lanka. Department of Nutrition, Medical Research Institute, Ministry of Health Care and Nutrition in collaboration with UNICEF. 2006:1- 49.
7. Prevalence of anaemia among children and women. Demographic and Health Survey 2006/7, Department of Census and Statistics, Sri Lanka. Sri Lanka: Ministry of Healthcare and Nutrition; 2009.
8. Rivera JA, Hotz C, Gonzalez-Cossio T, Neufeld L, Garcia-Guerra A. The effect of micronutrient deficiencies on child growth: a review of results from community-based supplementation trials. J Nutr. 2003;133:4010S-20.
9. Hettiarachchi M, Liyanage C. Coexisting micronutrient deficiencies among Sri Lankan preschool children; a community-based study. Matern Child Nutr. 2012;8:259-66. doi: 10.1111/j.1740-8709.2010.00290.x.
10. WHO Anthro Plus for personal computers manual: software for assessing growth of the world's children and adolescents. Geneva, Switzerland: WHO; 2009. [cited 2010/06/25] Avialable from: http:// www.who.int/growthref/ tools/en/.
11. Bieri JG, Tolliver TJ, Catignani GL. Simultaneous determination of alpha tocopherol and retinol in plasma and red cell by high pressure liquid chromatography. Am J Clin Nutr. 1979;32:2243-9.
12. IMMULITE®/IMMULITE® 1000 Intact PTH operators' manual. UK: Siemens Healthcare Diagnostics; 2008.
13. Rahman MH, Hossain MM, Sultana S, Jamal CY, Karim MA. Correlation of serum parathomone level with biochemical parametres in chronic renal failure. Indian Pediatr. 2005;42:250-4.
14. Butrimovitz GP, Purdy WC. The determination of zinc in blood plasma by atomic absorption spectrometry. Anal Chim Acta. 1977;94:63-73. doi: 10.1016/S0003-2670(01)83 632-1.
15. WHO/UNICEF/CANADIAN Micronutrient Report, 2001. [cited 2010/08/30]; Avialable from: www.micro nutrient.org.
16. Lips P. Relative value of 25(OH)D and 1,25(OH)2D measurements. J Bone Min Res. 2007;22:1668-71. doi: 10. 1359/jbmr.070716.
17. Hotz C, Brown K. Assesment of risk of zinc deficiency in populations and options for its control. Food Nutr Bull. 2004; 25:S99-199.
18. Stoltzfus RJ, Dreyfuss MI. Guidelines for the use of iron supplements to prevent and treat iron deficiency anaemia. INAG/WHO/UNICEF: Washington.
19. Aloia JF, Feuerman M, Yeh JK. Reference range for serum parathyroid hormone. Endocr Pract. 2006;12:137-44. doi: 10. 4158/EP.12.2.137.
20. De Onis M, Blossner M. WHO global database on child growth and malnutrition. Geneva: WHO; 1997.
21. Masood SH, Iqbal MP. Prevalence of vitamin D deficiency in South Asia. Pak J Med Sci. 2008;24:891-97.
22. Arabi A, El Rassi R, Fuleihan GE.Hypovitaminosis D in developing countries-prevalence, risk factors and outcomes. Nat Rev Endocrinol. 2010;6:550-61. doi:10.1038/nrendo. 2010.146.
23. Khor GL, Chee WS, Shariff ZM, Poh BK, Arumugam M, Rahman JA, Theobald HE. High prevalence of vitamin D insufficiency and its association with BMI-for-age among primary school children in Kuala Lumpur, Malaysia. BMC Public Health. 2011;11:95. doi:10.1186/1471-2458-11-95.
24. Weng FL, Shults J, Leonard MB, Stallings VA, Zemel BS. Risk factors for lowserum 25-hydroxyvitamin D concentrations in otherwise healthy children and adolescents. Am J Clin Nutr. 2007;86:150-8.
25. Lumb GA, Mawer EB, Stanbury SW. The apparent vitamin D resistance of chronic renal failure: a study of the physiology of vitamin D in man. Am J Med. 1971;50:421-44.
26. Wortsman J, Matsuoka LY, Chen T, Lu Z, Holicvk MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr. 2000;72:690-3. doi: 10.1016/0002-9343(71)90332-9.
27. Reid IR. Relationships between fat and bone. Osteoporos Int. 2008;19:595-606. doi: 10.1007/s00198-007-0492-z.
28. Yetley EA. Assesing the vitamin D status of the US population. Am J Clin Nutr. 2008;88:558S-64.
29. Soares MJ, Murhadi LL, Kurpad AV, Chan She Ping-Delfos WL, Piers LS. Mechanistic roles for calcium and vitamin D in the regulation of body weight. Obes Rev. 2012;13:592-605. doi: 10.1111/j.1467-789X.2012.00986.x.
30. Ping-Delfos WCS, Soares M. Diet induced thermogenesis, fat oxidation and food intake following sequential meals: influence of calcium and vitamin D. Clin Nutr. 2011;30:376-83. doi: 10.1016/j.clnu.2010.11.006.
31. Hettiarachchi M, Liyanage C, Wickremasinghe R, Hilmers DC, Abrams SA. Prevalence and severity of micronutrient deficiency: a cross sectional study among adolescents in Sri Lanka. Asia Pac J Clin Nutr. 2006;15:56-63.
32. Brown KH, Rivera JA, Bhutta Z, Gibson RS, King JC, Lonnerdal B, Ruel MT, Sandtrom B, Wasantwisut E, Hotz C.

International Zinc Nutrition Consultative Group (IZiNCG) technical document 1. Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull. 2004;25:S99-203.

33. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2002;75:1062-71.

34. Brown KH, Peerson JM, Baker SK, Hess SY. Preventive zinc supplementation among infants, preschoolers, and older prepubertal children. Food Nutr Bull. 2009;30:S12-40.

35. Muhilal, Permeisih D, Idjradinata YR, Muherdiyantiningsih, Karyadi D. Vitamin A – fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. Am J Clin Nutr. 1988;48:1271-6.

36. Arroyave G, Aguilar JR, Flores M, Guzman MA. Evaluation of sugar fortification with vitamin A at the national level. Washington, DC: Pan American Health Organization; 1979.

37. Lie C, Ying C, Wang EL, Geissler C. Impact of large-dose vitamin A supplementation on childhood diarrhoea, respiratory disease and growth. Eur J Clin Nutr. 1993;47:88-96.

38. Ramakrishnan U, Latham MC, Abel R. Vitamin A supplementation does not improve growth double-blind field trial in South India. J Nutr. 1995;125:202-11.

39. West KP Jr, LeClerq SC, Shrestha SR, Wu LSF, Pradhan EK, Khatry SK et al. Effects of vitamin A on growth of vitamin A-deficient children: field studies in Nepal. J Nutr. 1997;127:1957-65.

40. Awasthi S, Das R, Verma T, Vir S. Anaemia and undernutrition among preschool children in Uttar Pradesh, India. Indian Paediatrics. 2003;40;985-90.

41. Gunnarsson BS, Thorsdottir I, Palsson G. Iron status in 6-y-old children: associations with growth and earlier iron status. Eur J Clin Nut. 2005;59:761-7. doi: 10.1038/sj.ejcn.1602137.

42. Thurlow RA, Winichagoon P, Green T, Wasantwisut E, PongcharoenT, Bailey KB et al. Only a small proportion of anemia in North East Thai school children is associated with iron deficiency. Am J Clin Nutr. 2005;8:380-7.

43. Arruda SF, Siqueira EM, de Valência FF. Vitamin A deficiency increases hepcidin expression and oxidative stress in rat. Nutrition. 2009;25:472-8. doi: 10.1016/j.nut.2008.11.030.

44. Alarcon K, Kolsteren PW, Prada AM, Chian AM, Velarde RE, Pecho IL, Hoeree TF. Effects of separate delivery of zinc or zinc and vitamin A on hemoglobin response, growth, and diarrhea in young Peruvian children receiving iron therapy for anemia. Am J Clin Nutr. 2004;80:1276-82.

45. Zimmermann MB, Biebinger R, Rohner F, Dib A, Zeder C, Hurrel RF, Chaouki N. Vitamin A supplementation in children with poor vitamin A and iron status increases erythropoietin and hemoglobin concentrations without changing total body iron. Am J Clin Nutr. 2006;84:580-6.

46. Chen L, Liu YF, Gong M, Jiang W, Fan Z, Qu P, Chen J, Liu XY, Li YT. Effects of vitamin A, vitamin A plus zinc, and multiple micronutrients on anemia in preschool children in Chongqing, China. Asia Pac J Clin Nutr. 2012;21:3-11.

47. Atkinson MAA, Melamed ML, Kuma J, Roy CN, Miller ER, Furth S, Fadrowski J. Vitamin D, race and risk of anaemia in children. J Pediatr. 2014;164:153-8. doi:10.1016/j.jpeds.2013.08.060.

48. Sim JJ, Lac PT, Liu IL, Meguerditchian SO, Kumar VA, Kujubu DA, Rasgon SA. Vitamin D deficiency and anemia: a cross-sectional study. Ann Hematol. 2010;89:447-52. doi 10.1007/s00277-009-0850-3.

49. Nhien NV, Khan NC, Ninh NX, Huan PV, Hop LT, Lam LT et al. Micronutrient deficiencies and anaemia among preschool children in rural Vietnam. Asia Pac J Clin Nutr. 2008;17:48-55.

Original Article

Micronutrient status and its relationship with nutritional status in preschool children in urban Sri Lanka

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斯里兰卡城市学龄前儿童微量营养素的状况及其与营养状况的关系

目的：评估学龄前儿童微量营养素的状况及其与营养状况的关系。**方法**：采用横断面研究，收集斯里兰卡城市 340 名学前班儿童的人体测量数据和空腹血样。测定血清中维生素 D、甲状旁腺激素、维生素 A、锌和血红蛋白的浓度。计算人体测量指标身高年龄比、体重年龄比和体重身高比的 Z-评分进行了计算来评价营养状况。**结果**：儿童中发育迟缓、低体重、消瘦和贫血的发生率分别为 7.1%、16.9%、21.2%和 7.4%。儿童中锌和维生素 A 缺乏的发生率分别为 67%和 38%。儿童中维生素 D 缺乏（<10 ng/mL）和不足（10-20 ng/mL）的发生率分别为 5.0%和 29.1%，并且甲状旁腺激素水平提示 12%的儿童有低钙血症。营养状况与维生素 D 状况显著相关（$p<0.05$）（身高年龄比，r=0.10；体重年龄比，r=-0.18；体重身高比，r=-0.12）和血红蛋白的状况（体重年龄比，r=0.11）。严重发育迟缓儿童的锌和维生素 A 水平低于正常身高儿童（$p<0.05$）。维生素 D 和甲状旁腺激素显著负相关（r=-0.12），血红蛋白和维生素 A（r=0.01）、锌（r=0.02）和维生素 D（r=0.02）水平显著正相关（$p<0.05$）。**结论**：在被调查的人群中，锌缺乏率略高于维生素 A 缺乏和维生素 D 不足的发生率。儿童的营养状况与维生素 D 和血红蛋白的状况有关。严重发育迟缓儿童的锌和维生素 A 水平低。维生素 A、维生素 D 和锌水平与血红蛋白的状况有关。

关键词：微量营养素、营养状况、维生素 D、学龄前儿童、斯里兰卡