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Multiple Micronutrient Deficiencies are Related to the Nutritional Status of Children Living in North and Far North Regions of Cameroon

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ABSTRACT

Several micronutrients are essential for adequate growth of children. However, little information is available on multiple micronutrient status of preschool children in Cameroon. The present study was designed to evaluate the relationship between multiple micronutrient levels and nutritional status among preschool school children. This was a cross-sectional study of 331 children 6-59 months in the North and Far North Regions of Cameroon. Serum concentration of magnesium, calcium, copper, and zinc were measured by inductively coupled plasma mass spectrometer. Ferritin, sTfR, RBP, CRP, AGP were measured by Sandwich ELISA. Weight-for-age, height-for-age and weight-for-height were used to estimate the children's nutritional status. The prevalence of stunting, underweight, wasting among preschool children was 42.0%, 26.0%, and 6.6%, respectively. The mean serum levels of magnesium, calcium, Body Iron Stores, copper, and zinc were 19.9±0.1 (µg/dl), 80.7±0.3 (µg/dl), 1.02±0.25 (mg/kg), 201.5.30±33.7 (µg/dl), and 49.4±0.8 (µg/dl) respectively. The proportion of low plasma Zinc Concentration was 85.6%, Iron Deficiency anemia was 24.2% with 37.9 % of Iron deficiency associated to anemia. Height-for-age showed significant positive correlation with the levels of Zinc ($r=0.093$, $p<0.05$) and body Iron Stores ($r=0.111$, $p < 0.01$) and with the levels of magnesium ($p = 0.05$). 70.4% of the children presented more than one form of micronutrient deficiency. Multiple micronutrient deficiencies and Malnutrition are significantly higher for the Far North Region compared to the North Region. The prevalence of both malnutrition and multiple micronutrient deficiency was significant. Strong Associations were observed between multiple micronutrient deficiencies and Nutritional Status.

Keywords: Preschool children, Nutritional status, Micronutrients, North region, Far North Region, Cameroon

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INTRODUCTION

Micronutrient deficiencies are caused by inadequate dietary intake, increased losses from the body, and/or increased requirements[1]. It has become recognized by the nutrition community that micronutrient malnutrition is very widespread, probably one of the main nutritional problems in the world[1] and a major contributor to childhood morbidity and mortality[2]. Micronutrients of known public health importance include the following: zinc, iodine, iron, selenium, copper, vitamins A, E, C, D, B2, B6, B12 and folate[2]. Besides, micronutrient deficiencies (MD) and especially iron deficiency, is believed to be one of the main underlying causes of anemia[3] Zinc is an essential micronutrient, which is required for normal growth, immune function, neuro-behavioral development, and pregnancy outcomes[4].More than 2 billion people in the world today are estimated to be deficient in key vitamins and minerals, mainly vitamin A, iodine and zinc. Particularly in Africa, MD affect millions of people, especially the most vulnerable groups, which are children and pregnant women[5] Studies in Cameroon have shown that MD are prevalent and likely contribute to increased morbidity and mortality[6]. A national survey conducted in 2001 found that 39% of children 1–5 years of age had serum retinol concentrations < 0.70 µmol/L (without adjustment for elevated acute phase proteins)[7]. The prevalence of anemia among children 6–59 mo (hemoglobin <110 g/L) was 70%, in 2004. Although the prevalence of zinc deficiency has not been specifically measured, the high prevalence of stunting among children 0–59 mo old (32%) suggests that zinc deficiency may also be a public health problem. Though recent studies that have discussed on insufficiencies of some micronutrients in Cameroonian children[8], we are not aware of any recent publication regarding a comprehensive study that includes multiple micronutrients, hemoglobin, as well as Nutritional status among children in the North and Far North Regions of Cameroon. Therefore, the present study was aimed at describing the distribution of selected micronutrients and the presence of anemia among children aged 12-59 months living in rural and urban areas of the North and Far North regions of Cameroon and their relationship with Nutritional status.

MATERIALS AND METHOD

Study area and subjects

The study was designed as a regionally representative, two stage (cluster) sample survey of preschool children (12–59 mo). The population was divided into 2 strata, representing each region. Using the 2005 census data from the Cameroon Central Bureau of Census and Population studies (BUCREP), seventeen clusters (sub-divisions and health districts) were selected in each

stratum, according to the probability-proportional-to-population-size method. In each cluster, 10 households were selected using a random start point and systematic selection of adjacent households to the right. A uniform pre-piloted questionnaire translated into French was administered to the caretaker/head of household of the participant children by trained personnel (nurses and health officers). The questionnaire comprised the following parts: demographic characteristics, nutritional status and Food consumption frequency. The following information regarding health status was collected during the interview: fever in the last 15 days and weight loss. Dietary data were collected through a 24-hour diet recall. Informed oral consent was obtained from the index woman (the child's primary female caretaker), with permission from the head of household. The study was approved by the Cameroon National Ethics Committee.

Anthropometry

For children aged <2 y, length was measured in duplicate to the nearest 0.1 cm using a portable length board (Seca 416, Infantometer; Seca Medical Scales and Measuring Systems). The standing height of caregivers and children ≥2 y was measured in duplicate to the nearest 0.1 cm using a portable stadiometer (Seca Leicester Portable Height Measure; Seca Medical Scales and Measuring Systems). Anthropometric Z-scores for children were calculated according to the WHO standard[9].

Blood collection and processing

Trained state registered Nurses collected 5–7 mL of blood by venipuncture into tubes containing lithium heparin as an anticoagulant (Sarstedt). Hemoglobin was immediately measured in whole blood using a portable photometer (Hb201+, Hemocue). Blood samples were then centrifuged for 10 min at 2500 3 g to separate plasma in the field (Hermle Z206A, Hermle Labor Technik). Plasma was frozen on the day of collection and stored at ≤ - 220 ºC until analysis. Blood collection and processing techniques adhered to the recommendations developed by the International Zinc Nutrition Consultative Group (IZiNCG) for analysis of Plasma Zinc Concentration (PZC).

Determination of levels of Micro-nutrients in serum

Plasma levels of Zinc were determined as previously described[10]. For the plasma pool, the between-run CV was 2.5% and the within-run CV was 4.9%; for the Seronorm samples, the between-run CV was 4.3% and the within-run CV was 5.2%. Samples from a total of 314 children were analyzed in duplicate (mean CV: 8.1%; median CV: 2.9%). Samples were reanalyzed if any of the following were present: PZC greater than or less than the limit of detection, PZC < 30 mg/dL, PZC > means ±3 SDs, or CV > 10% (for samples analyzed in

duplicate). Following extraction of ROH into hexane, the plasma ROH concentration was measured by reversed-phase HPLC with an isocratic 2234 mobile phase of 95:5 methanol: water (v:v) and a photodiode array detector (Shimadzu) [11] Retinyl acetate was used as an internal standard. Values were calibrated using two control sera (fat-soluble vitamin serum 968d and 968e) from the National Institute of Standards and Technology. Duplicate ROH samples were analyzed for 14% of participants; the mean CV of randomly selected duplicates analyzed in different runs was 4.9% (range 1.3–8.5%). The CV of a control sample run in triplicate with each batch of plasma ROH samples was 3.8% within the same run and 5.40% between runs. Ferritin, Serum transferin Receptor (sTfR), retinol binding protein (RBP), C-Reactive protein (CRP), and and alpha (1)-acid glycoprotein (AGP) were analyzed by a combined sandwich ELISA. Ferritin and sTfR were measured as indicators of iron status and used to calculate BIS as previously described [12.]The inter-assay CVs were 4.2% for Ferritin, 5.8% for sTfR, 8.5% for CRP, 4.5% for AGP, and 3.7% for RBP.

Determination of levels of trace elements in serum

Concentration of trace elements in serum was determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (model 8500, Schimadzu, Tokyo, Japan), following previously published procedures[13.]

Statistical analysis

Data were analyzed using SPSS version 19 statistical package. Descriptive statistics were calculated for all variables. Continuous variables were examined for adherence to a normal distribution and all micronutrients values in serum were normally distributed and hence no transformation was done. Comparisons of serum values of the trace elements among children were made using one-way-ANOVA. Cut off value for magnesium, calcium, and copper, was defined at their serum levels of 18 µg/dl, 84 µg/dl, 75 µg/dl, and 7 µg/dl respectively[14.] The cutoffs used to define deficiency for the other micronutrient indicators were as follows: Unadjusted and adjusted ferritin, <12 mg/L; unadjusted ferritin, <30 mg/L; unadjusted and adjusted BIS, <0 mg/kg; sTfR, >8.3 mg/L; hemoglobin, <110 g/L; plasma zinc, <65 µg/dl. Body iron Stores (BIS) were calculated using the ratio of sTfR and ferritin concentrations according to a formula derived by Cook et al. 14. Plasma concentrations of ferritin were adjusted for the presence of inflammation using the method previously published by Thurnham[15] and involves first stratifying individuals into categories based on elevated CRP and/or AGP: apparently healthy reference group (CRP ≤5 mg/L and AGP ≤1 g/L), incubation (CRP >5 mg/L and AGP ≤1 g/L), early convalescence (CRP >5 mg/L and AGP >1g/L), and late convalescence (CRP ≤5

mg/L and AGP >1 g/L). Individual values were then adjusted by multiplying by the ratio of the median of the apparently healthy group to the median of that individual's inflammation group. Pearson's test was used to assess the correlation between two continuous variables. Statistical significance was assigned for p values less than 0.05. The z score values for height-, weight- and BMI-for-age relative to the WHO 2006 reference were calculated using Epi Info and WHO Anthro Plus software[16]

RESULTS AND DISCUSSION

The study sample consisted of 331 children aged 6-59 month (mean age 28.8 mo ±1.3). Majority of the children were males (53.2%). A great proportion (42.6% poorest, 26.3% poor) of the children were Poor as per the Socio-Economic Status Quintile (SES Quintile). 47.4% of the households (HH) heads were unemployed and 43.2% of the caregiver did seasonal jobs. The mail source of drinking water was Dug wells (33.6%) followed by Boreholes (33.5%). Though up to 36.9% had electricity as source of lighting, 24.8% are using oil/gas lantern and 28.1% flash lights (Table 1).

Table 1: Socio-Demographic data of children in the North and Far North regions of Cameroon

Variable	Far North Region	North Region	Total
N	164	167	331
SES Quintile			
Poorest	64.0%	21.6%	42.6%
Poor	23.2%	29.3%	26.3%
Average	9.1%	26.3%	17.8%
Rich	3.0%	6.6%	4.8%
Richest	0.6%	16.2%	8.5%
Primary House Hold Religion			
Catholic	9.1%	21.0%	15.1%
Protestant	15.9%	23.4%	19.6%
Muslim	59.8%	50.9%	55.3%
Others	15.2%	4.8%	10.0%
Marital Status of Caregiver			
single	0.6%	5.4%	3.0%
Married	96.3%	82.0%	89.1%
Living with Partner	0.0%	7.2%	3.6%
Divorced/widowed	3.0%	5.4%	4.2%
Care giver Level of Education			
No schooling	72.0%	58.1%	65.0%
Primary	23.2%	25.1%	24.2%
Secondary	4.9%	15.6%	10.3%
Higher Education	0.0%	1.2%	0.6%

Variable	Far North Region	North Region	Total
N	164	167	331
Head of House Hold Level of Education			
No schooling	54.3%	46.1%	50.2%
Primary	23.2%	25.1%	24.2%
Secondary	21.3%	24.0%	22.7%
Higher Education	1.2%	4.8%	3.0%
Marital Status of Head of House Hold			
single	0.6%	1.8%	1.2%
Married	98.2%	89.2%	93.7%
Living with Partner	0.0%	6.0%	3.0%
Divorced/widowed	1.2%	3.0%	2.1%
Type of toilet			
Modern toilet	33.5%	24.6%	29.0%
Pit Latrine (Improved with slab)	60.4%	65.9%	63.1%
Pit Latrine without slab/open pit	1.8%	8.4%	5.1%
Other	4.3%	1.2%	2.7%
Source of energy for cooking			
Wood/Charcoal	98.8%	83.8%	91.2%
kerosene/Gas	0.0%	15.6%	7.9%
Other	1.2%	0.6%	0.9%
Source of energy for Lighting			
Electricity	30.5%	43.1%	36.9%
Oil/Gas lantern	20.1%	29.3%	24.8%
Flashlight/tube light (Battery)	43.3%	13.2%	28.1%
no lights	1.8%	6.6%	4.2%
Other	4.3%	7.8%	6.0%

Table 1: Socio-Demographic data of children in the North and Far North regions of Cameroon (*Continuation)*

Variable	Far North Region	North Region	Total
N	**164**	**167**	**331**
Source of drinking water			
Piped water inside/outside	11.0%	24.0%	17.5%
Tube well/Borehole	53.0%	14.4%	33.5%
Dug Well	32.3%	40.7%	36.6%
Spring	0.0%	4.8%	2.4%
Surface water	1.2%	8.4%	4.8%
Other	2.4%	7.8%	5.1%
Principal Economic activity of Caregiver			
Business	44.5%	35.3%	39.9%
Unemployed	11.6%	12.0%	11.8%

Variable	Far North Region	North Region	Total
N	**164**	**167**	**331**
Farmer	43.9%	49.1%	46.5%
Other	0.0%	3.6%	1.8%
Principal Economic activity of Head of Household			
Business	40.2%	29.9%	35.0%
Unemployed	45.1%	49.7%	47.4%
Farmer	4.9%	10.2%	7.6%
Other	9.8%	10.2%	10.0%
Employment status of Caregiver over the past year			
Worked	37.8%	33.5%	35.6%
Seasonal	37.8%	48.5%	43.2%
Occasional	23.8%	15.0%	19.3%
Other	0.6%	3.0%	1.8%
SEX of Child			
Male	51.2%	55.1%	53.2%
Female	48.8%	44.9%	46.8%
SEX of Head of Household			
Male	98.2%	94.0%	96.1%
Female	1.8%	6.0%	3.9%
Mean HH size			
Mean±SEM	9.2 ± 0.4	7.5 ± 0.3	8.3 ± 0.2
Average distance from source of drinking Water			
Mean (minutes) ±SEM	165.2 ± 28.1	101.6 ± 22.1	133.0 ± 17.9

The means z-scores of HAZ, WAZ and WHZ of the study participants were −1.66±0.17, -0.32±0.11 and −0.32±0.13, respectively (Table 2). The means were not significantly different between females and males but were significantly different between the two regions ($p< 0.05$) in all anthropometric indices used to evaluate their nutritional status. This study also showed that 42.0%, 26.0% and 6.6% of the school children were respectively stunted, underweight and wasted (Table 2).

Table 2: Nutritional status of children in the North and Far North regions of Cameroon

Variable	Far North Region	North Region	Total
N	**160**	**159**	**319**
Age, months (Mean, 95% CI)	29.4 (27.7, 31.1)	28.2 (26.3, 30.1)	28.8 (27.5, 30.1)
HAZ (Mean, 95% CI)	-1.84 (-2.07, -1.59)	-1.49 (-1.73, -1.28)	-1.66 (-1.83, -1.50)
Stunted, HAZ<-2SD; % (CI)	46.3 (38.8, 53.8)	37.7 (30.2, 45.3)	42.0 (37.3 - 47.3)
WHZ (Mean, 95% CI)	-0.51 (-0.68, -0.35)	-0.12 (-0.29, 0.06)	-0.32 (-0.43, -0.19)
Wasted, WHZ<-2SD; % (CI)	8.8 (4.4, 13.1)	4.4 (1.9, 8.20	6.6 (4.1, 9.4)
WAZ (Mean, 95% CI)	-1.36 (-1.55, -1.15)	-0.91 (-1.10, -0.74)	-0.32 (-0.43, -0.20)
Underweight, WAZ<-2SD; % (CI)	33.1 (26.3, 41.3)	18.9 (12.6, 25.2)	26.0 (21.3, 31.0)

The mean serum retinol (ROH) concentration for all children surveyed was slightly lower than the cut-off point of 0.70 µmol/l and the mean RBP was equal to the cut-off point of 0.83 µmol/l

(Table 3). After adjusting for infection, both the mean ROH and RBP concentrations were higher than the unadjusted mean (Adjusted plasma ROH = 0.75± µmol/l, Adjusted Plasma RBP = 0.90±0.01 µmol/l). When the data were disaggregated by Region, the unadjusted mean serum ROH and RBP concentrations were significantly lower in children living in the North Region ($p < 0.001$) compared to those living in the Far North Region. Adjusted RBP concentrations were also significantly lower ($p<0.001$) while the result of adjusted ROH did not differ by region (Table 3).

Table 3: Serum Vitamin A concentrations of children in the North and Far North regions of Cameroon

Region	**Far North**	**North**	**Total**
N	**160**	**154**	**314**
Plasma RBP (µmol/l) (Mean±SEM)	0.86±0.02[a]	0.79±0.02 [b]	0.83±0.01 [a]
Adjusted Plasma RBP (µmol/l) (Mean±SEM)	0.93±0.02[d]	0.87±0.02[e]	0.90±0.01[e]
Plasma RBP <0.83 µmol/L, %	27.50%	38.30%	32.80%
Adjusted Plasma RBP <0.83 µmol/L, %	35.60%	46.10%	40.80%
N	**20**	**15**	**35**
Plasma ROH (µmol/l) (Mean±SEM)	0.69±0.05 [a]	0.65±0.04 [a]	0.67±0.03 [c]
Adjusted Plasma ROH (µmol/l) (Mean±SEM)	0.74±0.05	0.77±0.06	0.75±0.04
ROH:RBP molar Ration (Mean±SEM)	0.80±0.02	0.81±0.01	0.81±0.01
Adjusted ROH:RBP molar Ration (Mean±SEM)	0.81±0.02	0.81±0.02	0.81±0.01
***Apo*-RBP (µmol/l) (Mean±SEM)**	0.15±0.01	0.14±0.01	0.15±0.01
Adjusted ***Apo*-RBP (µmol/l) (Mean±SEM)**	0.17±0.01	0.17±0.01	0.17±0.01
Plasma ROH <0.70 µmol/L, %	50.00%	60.00%	54.30%
Adjusted Plasma ROH <0.70 µmol/L, %	40.00%	33.30%	37.10%

[a] data with different superscript letters significantly differ $p<0.05$

The prevalence of Iron Deficiency (ID) and Iron Deficiency anaemia (IDA) and the proportion of anaemia associated with ID varied widely by indicator and adjustment for inflammation (Table 4). The prevalence of ID was lowest according to unadjusted ferritin (22.7%) whereas the prevalence of ID was highest when defined by elevated sTfR (77.3%). Adjustment of ferritin and BIS for inflammation increased the measured prevalence of ID, regardless of the method of adjustment. The proportion of anaemia associated with ID ranged from 30.3 to 88% in the target population, depending on the indicator applied. The prevalence of ID was positively related to the proportion of anaemia that was associated with ID. The Mean PZC was lower than the cut off of 65µg/dL (Table 5). The adjusted PZC was significantly higher than the unadjusted values. Region wise, the PZC was significantly higher for the north Region compared to the Far North Region ($p<0.001$). 85.8% of the overall population presented PZC < 65µg/dL. Table 6 shows the concentrations of serum magnesium, calcium, iron, copper, and zinc in the study population in

relation to nutritional status. Serum concentration of calcium was significantly higher in moderately wasted children ($p<0.05$) compared to normal. There was a significant difference among different classification of height-for age and copper-to-zinc ratio ($p<0.05$). However, serum concentration of zinc was significantly lower in severely wasted children ($p<0.05$) compared to normal. On the contrary, moderately wasted children had significantly higher concentration of copper, although not statistically significant. As a result, the copper-to-zinc ratio was significantly lower in wasted children ($p<0.05$) compared to normal children.

Table 4 Prevalence of ID, IDA and the proportion of anaemia associated with ID by iron indicator and adjustment for inflammation1

Anaemia , Heamoglobin <110g/L, %(95% CI)					**63.7 (58.3-68.9)**
	ID		**IDA**		**Anaemia associated with ID[2]**
	%	***CI***	***%***	***CI***	***%***
Unadjusted ferritin <12 µg/L	22.70	18.1-27.2	19.30	15.1-23.9	30.30
Unadjusted ferritin <30 µg/L	51.70	46.2-56.8	32.60	27.8-37.5	51.18
Adjusted Plasma ferritin <12 µg/L	29.30	24.8-34.1	24.20	19.3-28.7	37.99
Unadjusted Body Iron < mg/kg	33.80	28.4-39.3	26.90	22.7-31.7	42.23
Adjusted Body Iron < 0mg/kg	63.10	57.7-68.0	49.50	44.4-54.7	77.71
sTfR>8.30 mg/L	77.30	72.5-81.9	56.20	51.1-61.6	88.23

[1]Values are percent (95% CI) or percent, n=331 [2]Calculated as the prevalence of IDA divided by the prevalence of all anaemia

Table 5: Serum Plasma Zinc Concentrations (PZC) of children in the North and Far North regions of Cameroon

Region	**Far North**	**North**	**Total**
N	**159**	**155**	**314**
Unadjusted PZC, (µg/dL) (Mean±SEM)	48.28±1.33[a]	50.24±0.83[b]	49.25±0.79[a]
APP adjusted PZC, (µg/dL) (Mean±SEM)	50.15±1.36[b]	52.63±0.85[c]	51.38±0.81[c]
Low Unadjusted PZC, (<65µg/dL), %	90.9%	85.0%	87.9%
Low adjusted PZC, (<65µg/dL), %	89.0%	82.6%	85.8%

APP = Acute Phase Proteins (referring to CRP and AGP); Means different superscript letters significantly differ, $p<0.001$.

Table 6: Levels of Serum Micro-nutrients (Mean±SEM) in relation to Nutritional status of children of the North and Far North regions of Cameroon

		N	**Ca**	**Mg**	**Ca/Mg Ratio**	**Cu**	**Zinc**	**Cu/Zn Ratio**
Height-for-Age	Normal	176	81.1±0.4	20.0±0.1	4.1±0.03	225.4±58.3	50.3±0.8	4.5±0.9
	Moderate	75	80.8±0.6	20.0±0.4	4.1±0.04	163.1±4.8	48.9±1.3	3.5±0.1
	Severe	54	79.6±0.9	19.6±0.2	4.1±0.04	176.9±11.7	47.1±3.2	4.0±0.2
	total	305	80.7±0.3	19.9±0.1	4.1±0.02	201.5±33.7	49.4±0.8	4.1±0.5
Weight-for Height	Normal	285	80.7±0.3	19.9±0.1	4.1±0.02	204.1±36.1	49.2±0.7	4.2±0.6
	Moderate	17	82.6±1.1	20.0±0.4	4.2±0.10	170.8±8.8	55.0±9.2	3.6±0.3

	Severe	3	76.2±1.5	21.1±0.2	3.6±0.07	133.3±34.3	36.3±1.7	3.6±0.8
	total	305	80.7±0.3	19.9±0.1	4.1±0.02	201.5±33.7	49.4±0.8	4.1±0.5
Weight-for - Age	Normal	225	80.7±0.4	19.9±0.1	4.1±0.03	213.1±45.6	49.9±0.8	4.3±0.7
	Moderate	8	83.0±0.7	20.4±0.6	4.1±0.10	190.5±10.7	48.2±3.0	4.1±0.4
	Severe	19	82.6±1.2	19.9±0.3	4.2±0.10	150.3±7.6	52.8±8.4	3.3±0.2
	total	252	80.9±0.4	19.9±0.1	4.1±0.02	207.6±40.8	50.1±0.9	4.2±0.7

The prevalence of multi-micronutrient deficiency was high with up to 70.4% of the children presenting two or more forms of micronutrient deficiency. The proportion of children presenting two, three, four, five and six micronutrient deficiencies were respectively 20.5%, 26.3%, 29.3%, 14.8%, 3.0% and 0.3% (Table 7). Of the children who presented between two and five types of MD, the most prevalent of MDs were Vitamin A, Iron and Anemia.

Table 7: Correlation between Micro-nutrients and Nutritional in children of the North and Far North regions of Cameroon

Sex	***Far North***	***North***	***Total***
N	***164***	***167***	***331***
No Micronutrient	3.0%	8.4%	5.7%
1 Micronutrient	21.3%	19.8%	20.5%
2 Micronutrients	25.6%	26.9%	26.3%
3 Micronutrients	30.5%	28.1%	29.3%
4 Micronutrients	16.5%	13.2%	14.8%
5 Micronutrients	3.0%	3.0%	3.0%
6 Micronutrients	0.0%	0.6%	0.3%

Bivariate correlation analysis (Table 8) showed a significant correlation between z-scores of height-for-age and the levels of Zinc ($r=0.093$, $p<0.05$), body iron Stores ($r=0.11$, $p<0.01$) and Ferritin ($r=0.105$, $p<0.01$). Significant negative correlations were found between weight-for-height and Magnesium ($r=-0.101$, $p<0.01$) and between weight-for-height and Cu/Zn ratio ($r= -0.085$, $p<0.05$). Significant correlations were found between weight-for-height and Zinc ($r=0.094$, $p<0.05$), Body Iron Stores ($r=0.140$, $p<0.01$), ferritin ($r=0.168$, $p<0.01$).

Table 8: Correlation between Micro-nutrients and Nutritional in children of the North and Far North regions of Cameroon

	Weight for Age	**Height for Age**	**Weight for Height**
Zinc (µg/dL)	0.094*	0.093*	0.05
Copper (µg/dL)	-0.041	-0.015	-0.048
Cu/Zn Ratio	-0.085*	-.088*	-0.034
Calcuim (mg/dL)	-0.017	0.029	-0.071
Magnesium (mg/dL)	-0.053	0.008	-0.101**
Ca/Mg Ration	0.053	0.024	0.051
Body Iron Stores (mg/Kg)	0.140**	0.111**	0.106**

Serum Ferritin (µg/l)	0.168**	0.105**	0.166**

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Malnutrition and micronutrient deficiencies continue to be major health burdens in developing countries, particularly in sub-Saharan Africa. It is globally one of the most common risk factor for illness and death, with hundreds of millions of pregnant women and young children particularly affected[17]. For children in developing countries, malnutrition is a considerable health problem with prevalence rates estimated to range from 4% to 46% with 1% to 10% severely malnourished[18] The results of this study show that the prevalence of stunting observed among school children was 42.0% (46.3% for the Far North Region and 37.7% for the North Region). These findings are in agreement with data from the 2011 study [19] that found the prevalence of stunting of 43.1% (44.9% for the Far north and 40.2% for the north). Similar prevalence of stunting were observed among school children in Tanzania (42.5%)[20] and in Malaysia (40.2%) [21]. The prevalence of stunting remains high in the area and the fact that the prevalence of stunting is much higher than that of underweight and wasting confirms that the major problem is chronic malnutrition. Since, stunting is a type of chronic malnutrition which begins in childhood, supplementing the infants and children with quality complementary food after 6 months of age and at least until age 36 months is required so as to minimize the long-term negative consequences of chronic under nutrition. Our study also found a significant increase in the expression of acute phase proteins (CRP and AGP) as the nutritional status of the children change from normal to severely stunted and the proportion of Dietary diversity score was lowest amongst the severely stunted. (Data not shown). In this study, the prevalence of underweight (26.0%) was lower than previous reports this region. Both stunting and underweight and infection category grew worse as the study population got older. This may lead to delayed onset of puberty in the boys. We also found out that wasting which is usually caused by a relatively recent illness or food consumption (Food consumption score was lowest in the severely underweight group) was significantly lower than stunting or underweight ($p<0.05$) indicating that chronic malnutrition is more prevalent in this part of Cameroon than acute malnutrition. Our study found a significant negative correlation between Stunting and the SES quintile ($r= - 0.148$, $p<0.01$) thus suggesting that the prolonged poverty status witnessed by the study population might have induced early growth defaulting in the children as early as six months of age. Stunting in children can lead to increased severity of diarrheal episodes, higher susceptibility to infectious disease [22] and greater risks of pneumonia [23.] Poor growth has also been linked to impaired cognitive ability and reduced school performance. More recently, children stunted

before the age of two have been shown to have poorer emotional and behavior outcomes later in adolescence, including increased symptoms of anxiety and depression. Because childhood stunting leads to a reduction in adult size, it has also been associated with reduced work capacity in adulthood. Ultimately, stunting can lead to adverse intergenerational patterns of impaired growth and development, propagated by poverty and disease. In the present study, a statistically significant positive correlation was observed between height-for-age z-score and body iron stores ($r=0.111$, $p<0.01$). It was also demonstrated that severely stunted school children had low serum concentrations of iron when compared to normal children (Data not shown). Less intake, poor absorption and the systemic effect of infection and utilization of iron by microorganisms for its growth and multiplication may be responsible for their lower iron status[24]. This study found a prevalence of anemia (<110g/L) of 63.7% and the proportion of anemia associated to iron deficiency (defined as Adjusted Plasma ferritin <12µg/L) was 37.9% 9 (Table 7). A recent study in Cameroon[25] found out that the Iron and Vitamin A intakes were below the expected levels. Magnesium is important in maintaining several cellular functions as it is a natural activator of most enzymes. Magnesium deficiency frequently develops in a wide variety of clinical conditions such as protein–energy malnutrition malabsorption, hypo-albumin anemia, sepsis, hypothermia, etc., conditions that are commonly seen in children in developing countries[26] In the current study, 9.9% of the population had magnesium <1.80 mg/dl. This prevalence is consistent with previous study in India [27,] serum magnesium levels had non-significant positive correlations with height-for-age and a significant negative correlation with Weight-for-height ($r=-0.101$, $p<0.01$). Soft tissue magnesium functions as a co-factor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis, and maintenance of the electrical potential of nervous tissues and cell membranes. Of particular importance with respect to the pathologic effects of magnesium depletion is the role of this element in regulating potassium fluxes and its involvement in the metabolism of calcium. Magnesium depletion depresses both cellular and extracellular potassium and exacerbates the effects of low-potassium diets on cellular potassium content. Muscle potassium becomes depleted as magnesium deficiency develops, and tissue repletion of potassium is virtually impossible unless magnesium status is restored to normal. Low plasma calcium develops frequently as magnesium status declines. It is not clear whether this occurs because parathyroid hormone release is inhibited or, more probably, because of a reduced sensitivity of the bone to parathyroid hormone, thus restricting withdrawal of calcium from the skeletal matrix. Lower serum magnesium levels in malnourished children may be due to inadequate intake, malabsorption, diarrhea, and infection.

Mean Food consumption and Dietary diversity scores were found to be lowest in children who had plasma magnesium <1.80 mg/dl. The principal clinical features of severe zinc deficiency in humans are growth retardation, a delay in sexual and skeletal maturation, the development of orificial and acral dermatitis, diarrhoea, alopecia, a failure of appetite and the appearance of behavioral changes. Zinc is a co-factor for the synthesis of a number of enzymes, DNA, and RNA. Zinc deficiency has been associated with poor growth in childhood, reduced immuno-competence, and increased infectious disease related morbidity. The findings of this study were in agreement with previous studies which have demonstrated the existence of zinc deficiencies among children and early adolescence. Several studies globally have documented the relationships between lowered zinc concentrations during childhood and morbidity from infectious diseases and the effect on cognitive development. Our study found significant correlation between PZC and WAZ and HAZ. Thus the high levels of stunting could be attributed to long term PZC observed in moderately and severely stunted children. In the present study, the ratio of copper/zinc was higher in serum of children with severe stunting (Table 6).According to WHO, when the prevalence of zinc deficiency is greater than 20%, intervention to improve zinc status is recommended[28]. As a result, the study recommends planning of sustainable community-based intervention strategies to improve the zinc status of school children through zinc supplementation and fortification of staple foods with zinc are recommended. These interventions are imperative in view of the well-known adverse consequences of zinc deficiency to the health and quality of life of school-aged children, particularly in terms of academic performance.

CONCLUSION

This is the first time to our knowledge that a study has demonstrated the relationship between multiple micronutrient deficiencies and malnutrition in this targeted population. Strong associations were found between low PZC and WHZ, HAZ and WAZ. Body iron stores were also strongly associated with HAZ, while plasma magnesium was significantly associated with WAZ. Also, we document for the first time that Vitamin A deficiency was higher in the Far North region compared to the North Region. In summary, this study shows that the serum concentration of micronutrients in pre-school children with different nutritional status was altered. The findings of the present study also reveal a high prevalence of zinc, Vitamin A and Iron deficiencies, among the children aged 12-59 months with the Far North Region presenting higher prevalence compared to the north Region. A limitation of the present study is lack of

detailed data on dietary intake. Such data may provide useful information to explain the situation of micronutrient status and deficiency in the population studied.

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