

Relation of Serum Micronutrients to Growth and Nutritional Habits of School-Aged Children in Egypt

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Proper growth of children depends on several micronutrients. A fundamental role of Zinc and vitamins as A, B12 and D is crucial to prevent a failure to thrive. To study the relationship between Egyptian school aged children growth, nutritional habits and micronutrients deficiencies. Materials and methods: A case control research involved 60 school aged children (age ranged from 6 to 12 years). Group 1 (malnourished) compared with group II (apparently healthy children of matched peers). Serum concentrations of Zinc, Copper, and vitamins as A, B12, and D were measured using ELISA kits. Compared to controls, the cases showed significantly lower serum concentrations of Zinc, vitamin A, B12, and D ($P < 0.000$ in all), while serum concentration of Copper was significantly higher. In the patient's group, serum vitamin B12, and Zinc concentrations showed significant positive correlation with height for age z- score, while serum Copper concentration showed significant negative correlation with BMI for age z- score. Serum Zinc, vitamin B12, and D concentrations were significantly correlated positively with serum vitamin A. Multiple logistic regression showed statistically significant association of high serum Copper and weekly consumption of sweetened beverages among the studied patients ($P < 0.01$). Vitamins and Zinc deficiencies showed high prevalence among the school children. Serum vitamin B12 and Zinc concentrations showed significant positive correlation with height. The significant association between high serum Copper and sugary beverages among the studied patients may be due to contained preservatives.

Keywords: Egyptian Children; Growth; Micronutrient; Nutritional Habits.

Nutrition is essential for growth, particularly in children. Micronutrients are nutrients needed in small amounts, and function in the synthesis of enzymes, hormones and other substances¹. They also support growth activity, cognitive development², and immune and reproductive systems functions³.

Micronutrient malnutrition is a world nutritional problem and significantly affects childhood morbidity and mortality^{4,5}. Its prevalence is high in developing countries (accounts for 7.3% of the global burden of diseases)⁶. Egypt is among the first twenty countries worldwide with elevated chronic childhood malnourishment and growth failure⁷.

Micronutrient deficiencies mainly affect young children although all age groups can be affected⁸. Poor nutrition, high losses and elevated needs especially in children during growth and development phase are some causes of such deficiencies⁹. These micronutrients include Iron (Fe), Iodine (I), Zinc (Zn), Copper (Cu), Selenium (Se), vitamins as A, B2, B6, B12, C, D and E^{10,11}. Vitamins and minerals deficiencies mainly vitamin A, and Zn spread among more than 2 billion people in the world today particularly in Africa^{12,13}.

Although the status of one or two micronutrients among children has been documented by many studies^{14,15}, little is known about several micronutrients at school-age period. Therefore, the current study was conducted to assess age dependent serum levels of micronutrients in Egyptian school aged children, and to study the relationship between their growth, nutritional habits and level of multiple micronutrients deficiencies.

MATERIALS AND METHODS

This case control research involved sixty elementary school children of both sexes; within the age range of 6 to 12 years old (mean age 8.08 ± 0.7). Majority of the children were males (70%). By screening for symptoms and signs of malnutrition, our study group was further subdivided into two subgroups. One subgroup consisted of 30 malnourished children; and the second subgroup consisted of 30 apparently healthy children in the group II of matched age and sex with no signs of malnutrition.

Inclusion criteria: Malnourished children between 6 and 12 years of age who were frequenting the Nutrition Clinic of the National Nutrition Institute. The enrollment was done over a 12 months' duration.

Exclusion criteria: Any child suffering from chronic disease or on supplements (vitamins and/or minerals and/or trace elements) were excluded from the study.

This study was conducted in the National Research center. Written consent was signed by the parents. Approval of National Research center Ethical Committee was fulfilled at the beginning of the study.

Data collection

Demographic data and clinical examination

Demographic data included age, gender and nutritional status. General examination included vital signs (blood pressure, radial pulse, respiratory rate and temperature).

All the children in both groups underwent a clinical and nutritional evaluation, anthropometrical assessment and laboratory tests. Cases of malnutrition were depicted through the presence of symptoms and or signs suggestive of vitamins deficiency, and the presence of anemia in laboratory investigations. Screening for symptoms and signs of vitamins deficiency included night blindness, muscle cramps, cheilosis, dermatitis, diarrhea, poor memory, abdominal pain, numbness in the extremities, bleeding tendency, previous bone fracture and disturbed gait.

Dietary intake

Dietary information was collected using food frequency questionnaire including dietary habits as fast foods, beverages with added sugar, vegetables, fruits, breakfast consumption were asked about. Data of 24-hour and one week dietary recall were recorded.

Anthropometric assessment

Body weight was assessed to the nearest 0.1kg on an electronic digital scale. Height was measured to the nearest 0.5 cm on a Holtain portable stadiometer. Body mass index (BMI) was calculated as Weight (kg)/Height (m²)¹⁶. Then Z-scores (weight, height and BMI) were evaluated referring to WHO growth criteria¹⁷, and Anthro 2007© software¹⁸. Z-scores for BMI were used-as recommended by the WHO-to assess thinness/wasting in school-aged children¹⁹.

Biochemical measurements

Five milliliters venous blood was withdrawn from all children. Blood was centrifuged and sera were kept at -70°C. Serum concentrations of vitamin A, B12, and D were measured by using a commercial enzyme linked immunosorbent assay (ELISA) kit, produced by Glory Science Co., Ltd. 2400 www.glorybioscience.com. Serum concentrations of Cu and Zn were measured by colorimetric method with 5-Brom-PAPS, according to the method of Johnsen and Eliasson²⁰.

Statistical Analysis

SPSS (Statistical Package of Social Sciences, Chicago, IL, USA) version 21 was used

for statistical analyses. The Mean \pm SD were used to express continuous data and were compared using Student's *t*-test. Frequencies and percentages were used to express categorical data and were compared between groups by Chi-square test. Evaluation of the association between continuous exposure and continuous covariates was carried out by Pearson's correlation analysis. A multiple logistic regression analysis was performed to study the effect of presumed risk factors on serum concentrations of micronutrients. A *p* value less than 0.05 was considered as statistically significant.

RESULTS

This study group included 60 children (30 cases and 30 controls). Their age ranged from 6 to

12 years old with the mean age 8.08 ± 0.7 . Majority of the children were males (70%). Stunting was detected in 20% of malnourished children. On comparing mean value of height, and height for age z-scores between cases and controls, a significantly lower mean values were detected in cases group ($P < 0.01$ in all) (Table 1).

The weekly dietary behavior revealed a significantly higher difference of low vegetables intake in cases group compared to controls ($P = 0.000$).

Having sugar sweetened beverages and irregularity of having three meals were significantly commoner among cases ($P = 0.000$).

No statistical difference was noted between both groups as regards having breakfast ($P = 0.078$) (Table 2).

Table 1. Anthropometric measurements in cases compared to controls

Variables	Control (n=30) Mean \pm SD	Patients (n=30) Mean \pm SD	P-value
Weight (kg)	34.45 \pm 15.60	32.68 \pm 11.32	0.617
Height (cm)	135.50 \pm 21.60	125.03 \pm 12.38	0.04*
BMI (kg/m ²)	17.44 \pm 2.48	18.89 \pm 3.04	0.47
Weight/ age z-score	-0.13 \pm 0.91	0.36 \pm 1.63	0.164
Height/ age z-score	0.68 \pm 1.80	-0.34 \pm 1.06	0.037*
BMI z-score	0.33 \pm 1.38	0.61 \pm 1.01	0.395

*Significant difference at $p < 0.05$

Table 2. Dietary behaviors in cases versus control groups

Variables	Answers	Control (n= 30)	Patients (n= 30)	P value
Fast foods / week	Yes	3	4	0.735
	No	9	11	
	Sometimes	18	15	
Regular 3 meals/ week	Yes	2	12	0.000*
	No	10	13	
	Sometimes	9	5	
Beverages with sugar/ week	Yes	0	17	0.000*
	No	15	0	
	Sometimes	15	13	
Vegetables consumption / week	Yes	30	8	0.000*
	No	0	15	
	Sometimes	0	7	
Breakfast consumption / week	Yes	21	19	0.078
	No	3	9	
	Sometimes	6	2	

* $P < 0.05$ is significant

On comparison with controls, the studied cases showed highly significantly lower serum Zn, vitamin A, B12, and D with a $P=0.000$ for the three vitamins and $P=0.028$ for Zn, while serum concentration of Cu was significantly higher in cases (Table 3) and (Table 4).

The cases' serum vitamin B12 and Zn concentration showed significantly positive correlation with height for age z- score, while serum Cu concentration showed significantly negative correlation with BMI for age z- score.

The correlations of anthropometry and serum levels of micronutrients of the studied cases are shown in Table 5.

In the patient's group, serum vitamin B12, and D concentrations showed significant positive correlation with serum vitamin A, as well as between serum Zn and serum B12 ($p < 0.05$).

Serum Cu showed a significant negative correlation with serum vitamin D ($p < 0.05$) as shown in Table 6.

Multiple logistic regression showed a statistically significant association between high serum Cu and consumption of sweetened beverages per week among the studied patients ($P < 0.01$), as shown in Table (7)

DISCUSSION

Micronutrient and vitamin deficiencies endanger health in developing countries. Malnourishment is prevalent in sub-Saharan Africa with a percent ranging from 4% to 46% including 1% to 10% severe cases²¹. Worldwide, it is considered a high risk for morbidity and mortality in childhood²².

In this study, the parameters of anthropometry (weight-for-age, height-for-age and BMI-for-age z- scores) served to evaluate the state of nutrition of the participants. Stunting was detected in 20% of malnourished children, which was near to a finding of 24% in a research enrolling preschool Ethiopian children²³. Another Ethiopian study conducted in Gumbrit showed a prevalence of stunting of 50% which is higher than that in the present study²⁴. Also, high prevalence of stunting was documented in Tanzanian children (44.2%)²⁵. Childhood stunting is a type of chronic malnutrition with higher prevalence than underweight and wasting.

A significant difference was revealed between the eating's behaviors of cases compared to controls. Low vegetables' consumption was

Table 3. Levels of serum micronutrients among cases and controls

Micronutrients		Control group Mean \pm SD	Cases Group Mean \pm SD	P
Serum vitamins	Vitamin A (ug/dl)	95.4 \pm 57.86	47.37 \pm 18.12	0.000*
	Vitamin B12 (ug/dl)	19.60 \pm 16.78	0.63 \pm 2.73	0.000*
	Vitamin D (ug/dl)	38.0 \pm 13.97	6.79 \pm 9.31	0.000*
Serum minerals	Zn (umol/l)	23.96 \pm 9.34	18.43 \pm 9.61	0.028*
	Cu (ug/dl)	102.68 \pm 4.86	135.8 \pm 75.52	0.048*

*Significant difference at $p < 0.05$, highly significant difference at $p \leq 0.01$

Table 4. Deficiency percent of micronutrients among both groups

Micronutrients	Deficiency %		p	
	Controls	Cases		
Serum vitamins	Vitamin A (ug/dl)	40%	83.3%	0.001*
	Vitamin B12 (ug/dl)	20%	96.7%	0.000*
	Vitamin D (ug/dl)	30%	93.3%	0.000*
Serum minerals	Zn (umol/l)	0	30%	0.001*
	Cu (ug/dl)	40%	60%	0.004*

*Significant difference at $p < 0.05$, highly significant difference at $p \leq 0.01$

more prevalent in patients group. An irregularity of the three main meals and a high intake of sugar sweetened beverages per week were commoner practices in cases ($P < 0.05$). This matches with the high prevalence of sugary drinks' intake by more than one third of the Egyptian children participating in the study conducted by Abd El-Shaheed *et al.*²⁶. However, there was no statistical difference between patients group compared to controls as regards of having breakfast.

Poor growth, reduced immuno-competence, and increased infectious disease

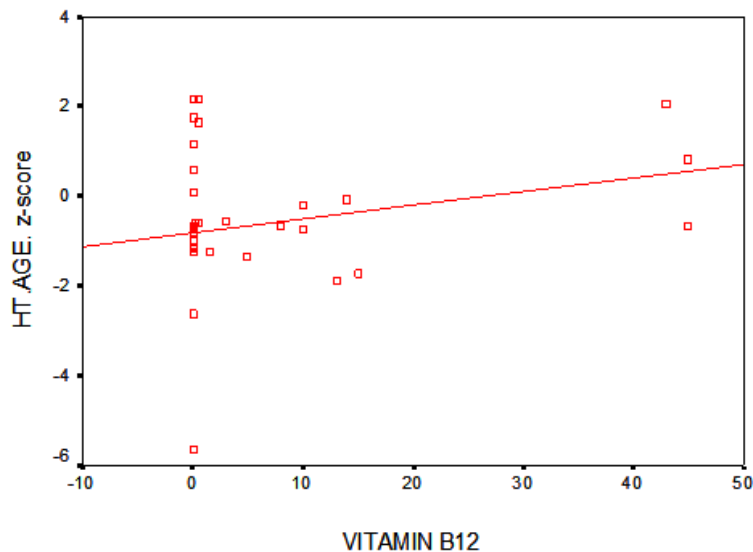
related morbidity are associated with Zn deficiency in children²⁷. Intervention to improve Zn status is recommended when the prevalence of Zn deficiency is more than 20%, according to WHO²⁸.

The cases showed a statistically highly significant low Zn level compared to healthy controls. This finding was in harmony with studies^{29,30}, which detected Zn deficiencies among school age children. Low intake of Zn rich foods and low bioavailability of Zn leads to Zn deficiency which is more common in children from low-income groups³².

Table 5. Correlations between micronutrients levels and anthropometry

Variables		Height for age z- score	Weight for age z- score	BMI for age z-score
Serum vitamin A (ug/dl)	Pearson Correlation	-.153	-.242	-.146
	Sig. (2-tailed)	.244	.063	.293
Serum vitamin B12 (ug/dl)	Pearson Correlation	.311	.102	.127
	Sig. (2-tailed)	.015*	.439	.361
Serum vitamin D (ug/dl)	Pearson Correlation	-.008	-.222	-.214
	Sig. (2-tailed)	.953	.088	.121
Serum Zn (umol/L)	Pearson Correlation	.329	.183	.036
	Sig. (2-tailed)	.010*	.161	.797
Serum Cu (ug/dL)	Pearson Correlation	.188	-.109	-.272
	Sig. (2-tailed)	.151	.408	.046*

*Significant difference at $p < 0.05$.



Correlation between Vitamin B12 and Ht.age z-score

Fig. 1. Correlation between serum vitamin B12 concentrations and height-age Zscore

Table 6. Correlations between serum vitamins and minerals among cases

Variables		Serum Vitamin B12 (ug/dl)	Serum Vitamin D (ug/dl)
Serum Vitamin A (ug/dl)	Pearson Correlation	0.347	0.522
	Sig. (2-tailed)	0.007**	0.000**
Serum Cu (ug/dL)	Pearson Correlation	-0.036	-0.419
	Sig. (2-tailed)	0.784	0.001**
Serum Zn (umol/L)	Pearson Correlation	0.313	0.236
	Sig. (2-tailed)	0.015*	0.070

*Significant difference at $p < 0.05$, highly significant difference at $p \leq 0.01$

Table 7. Multiple logistic regression for the presumed risk factor for high serum Cu

Variables	B	Std. Error	Wald	Sig.	Exp(B)
Intercept	82.85	6.45	164.76	0.000	
Fast foods/ week	-0.527	0.862	0.374	0.388	0.59
Breakfast consumption/ week	0.968	1.121	.745	0.388	2.63
Regular three meals/ week	0.467	0.839	0.309	0.578	1.59
Beverages with sugar/ week	8.841	1.306	45.825	0.000*	1.44
Vegetables consumption/ week	0.852	0.846	1.014	0.314	2.34

Highly significant difference at $p \leq 0.01$

The mean serum concentration of Cu in children of this study was higher than healthy controls. Similarly, Amare *et al.*¹⁵ and Jumaan *et al.*³³, explained that the elevation of the Cu binding protein, Ceruloplasmin as an acute-phase reactant in infectious diseases and inflammations leads to increased Cu levels in serum.

Fat-soluble vitamins as vitamins A and D are adequately absorbed in fatty meals³⁴. In opposition to controls, our studied patients showed highly significant lower serum levels of vitamin A, B12, and D ($P < 0.000$ in all). The serum mean value for vitamin A was 47.37 ± 18.12 ug/dl, and 83.3% of our studied patients had vitamin A deficiency. Both percentage and mean value is higher than the one reported in Wukro in Northern Ethiopia^{35,36}.

Insufficient serum vitamin D concentration is a common problem worldwide³⁷. Several reports show that low vitamin D is common in sunny regions although sun exposure is the major source of vitamin D³⁸, and 1 billion people worldwide have vitamin D deficiency or insufficiency especially children and young adults^{39,40}.

In the present study, the serum mean value for vitamin D (6.79 ± 9.31 ug/dl) was

statistically significantly lower as compared to healthy controls (38.0 ± 13.97 ug/dl) and 93.3% of this studied patients had vitamin D deficiency. This is in concordance with what have recently been reviewed by Park *et al.*⁴¹.

In the current study, there is a positive correlation between height for age z-score and serum Zn concentration in children. This is in agreement with Prentice *et al.*⁴² who found slow physical growth in children with Zn deficiency.

In our study, the positive correlation between height for age z- score and serum vitamin B12 concentration in children and the negative correlation between serum Cu concentration and BMI for age z-score need to be explained. This is in agreement with Pedraza *et al.*⁴³ who found growth delay in children with multiple micronutrients deficiencies.

In the patient's group, serum vitamin B12, and D concentrations showed significantly positive correlation with serum vitamin A, as well as between serum Zn and vitamin B12 concentrations. Serum Cu concentration showed significant negative correlation with vitamin D concentration. Multiple logistic regressions showed

that those consuming more beverages with sugar were more at risk of having high serum Cu and it may be due to contained preservatives.

CONCLUSION

This study showed that school children in Egypt with different nutritional habits had different serum concentrations of micronutrients with high prevalence of Zn and vitamins deficiencies. The significant association between high serum Cu and sugary beverages among the studied patients may be due to contained preservatives. Appropriate and beneficial strategies for micronutrient supplementation should be planned. More studies are needed to investigate the levels of other vitamins and minerals to have a more holistic perspective of the current nutritional problems.

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Ethical statement

Approval of National Research center Ethical Committee was fulfilled at the beginning of the study. An informed consent was signed by the legal guardian of each child before enrollment.

Conflict of interest

The authors declare having no conflict of interest.

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