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Evaluation of pro-inflammatory cytokines in nutritionally stunted Egyptian children

Awatif M. Abd El-Maksoud [a], Sahar A. Khairy [a], Hayat M. Sharada [b], Mohga S. Abdalla [b], Nehal F. Ahmed [a,*]

[a] Nutritional Requirements and Growth Department, National Nutrition Institute, Cairo, Egypt
[b] Chemistry Department, Faculty of Science, Helwan University, Cairo, Egypt

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ABSTRACT

Objectives: Stunting affects 32% of children living in developing countries and has a major impact on child health and development. In this study, we aimed to assess the pro-inflammatory cytokines and some micronutrients among the healthy control and nutritionally stunted Egyptian children.
Subjects and methods: A total of 88 children were enrolled; 60 stunted children and 28 non-stunted of matched age and sex. Clinical, demographic characteristics, serum levels of calcium, magnesium, and zinc were measured. Plasma levels of interleukin-6 (IL-6), tumor necrosis factor alpha (TNFα) and C – reactive protein (CRP) were measured in these subjects.
Results: Stunted children showed a marked decrease in weight and weight-for-age Z score (WAZ) versus healthy one. Significant reduction in the serum level of Ca, Mg, and Zn was detected in stunted children compared to those of the healthy control subjects. Moreover, the mean serum levels of IL-6 (pg/ml) (1.5 Vs 1.6), TNFα (pg/ml) (1.7 Vs 1.8) and CRP (mg/l) (0.7 Vs 1) were significantly higher among the stunted group compared to control.
Conclusion: Nutritionally stunted Egyptian children have lowered serum levels of Zn. Ca, and Mg, in addition to elevated serum levels of proinflammatory cytokines (Il-6, TNFα, and CRP).

Introduction

Stunting is an intractable public health problem affecting around one-third of children in developing countries. Stunting underlies 14–17% of child deaths globally.[1] The important cause of child stunting beside inadequate dietary is exposure to chronic, low-grade inflammation during fetal and postnatal life, which suppresses production of insulin-like growth factor-1 (IGF-1), perturbing the growth hormone axis early in life.[2]

Deficiencies of some micronutrients may play a role in delayed growth. This is because certain micronutrients are essential for physical growth, sexual maturity, neuromotor development and the integrity and function of the immune system.[3] Mineral deficiencies usually occur among fewer than 5 years of age due to low dietary nutrients intake, increased need of nutrients for rapid growth and development of infectious diseases.[4] Calcium is an element that is primarily related to bone health, structure and rigidity of the skeleton. Reduced intake of Ca and vitamin D during periods of growth can cause not only rickets but also interfering with attainment of genetically programmed height.[5] Also, Mg is an essential mineral that plays a key role in the maintenance of healthy bones.[6] Lower serum Mg levels in malnourished children can lead to stunting.[7] Moreover, Zn is vital for the formation of bones as it plays a role in Ca uptake in bones and modulates the effect of growth hormones. Zinc deficiency has been associated with poor growth in childhood, reduced immuno-competence, persistent diarrhea, anemia, and increased infectious disease related morbidity.[8] In this context several studies observed that there was a significant negative correlation between minerals deficiencies and height-for-age.[7,9]

Micronutrient deficiencies and infectious disease in malnourished children often coexist and show complex interactions that promote acute inflammation that is associated with activation of macrophages, natural killer lymphocytes, and the complement system to produce cytokines.[10] It involves temporal suppression of acquired, and amplification of innate immune responses, with secretion of positive acute phase proteins like CRP. An uncontrolled inflammatory response due to an imbalance between the pro-inflammatory mediators and anti-inflammatory mediators plays an important role in the development of clinical syndrome of malnutrition.[11]

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* Corresponding author at: Nutritional Requirements and Growth Department, National Nutrition Institute, 16 Al-Kasr Al-Aini Street, Cairo 11562, Egypt.
E-mail address: NFA_2085@yahoo.com (N.F. Ahmed).

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TNF represents two homologous proteins primarily derived from mononuclear phagocytes (TNF-α) and lymphocytes (TNF-β). The active form of both cytokines is a homotrimer.[12] The evaluation of TNF-α production in malnourished children is important because a deficiency in TNF-α production may contribute to the immune deficits occurring in malnutrition. Alternatively, excess production of TNF-α, by inducing anorexia and cachexia, may aggravate the nutritional status.[13] IL-6 is considered the most important inducer of hepatocyte synthesis of acute phase proteins.[14] In childhood; chronic inflammation and overproduction of IL-6 causes growth defect which was leading to stunting. It acts negatively on liver IGF-I gene expression and increased the proteolytic degradation of insulin-like growth factor binding protein-3 (IGFBP-3).[15] Sederquist et al.[16] found that several inflammatory cytokines including TNF-α and IL-6 may act individually or in combination to affect child growth. These molecules may act through systemic mechanisms and/or local action at the level of the growth plate of long bones. CRP is produced mainly in the liver in response to IL-6. It is belonging to family of proteins that increase in concentration during the occurrence of an injury, inflammation or tissue death.[17] In this context Prendergast et al.[2] suggested that an extensive enteropathy occurs during infancy may impair infant growth and induce low-grade chronic inflammation that mainly associated with low maternal IGF-1 levels and higher levels of CRP and Alpha-1-acid glycoprotein during infancy.

In view of that, the present study aimed to determine the levels of some minerals as Zn, Mg and Ca and some inflammatory markers (IL-6, TNFα and CRP) in nutritionally stunted Egyptian children.

Subjects and methods

Subjects

A cross-sectional study was conducted on, 60 children (28 male and 32 female) aged from 5 to 10 years with delayed linear growth, proportionate stunted, determined by Height < −2SD for matched age and sex according to WHO height for age Z score growth chart reference for school-aged children and adolescents released by WHO in 2007.[18] The prevalence of stunting in Egypt was low, so the controls were less than cases to collect more information about the stunted children.

The stunted children were recruited from the stunting clinic of National Nutrition Institute (NNI) in Cairo and the control healthy children who were chosen, came with their parents to be periodically checked up, were recruited from outpatient general clinic of NNI. The study protocol was approved by the Ethics Committee of the National Organization for Teaching Hospitals and Institutes, Cairo, Egypt.

Selection was done according to the following inclusion and exclusion criteria:

Inclusion criteria

Children (male and female), their age ranges from 5 to 10 years, with nutritional cause of retarded linear growth, height below −2 curve according to WHO 2007 standard growth curve of both sexes.[18]

Exclusion criteria: Children with signs of puberty, endocrinal disorders, chronic or systemic diseases, genetic disorders, familial short stature, and disproportion short stature. Children taking any medications that alter growth or hormones were also excluded.

All Children were subjected to full history including age, sex, parental heights and family history. Clinical examination, routine investigation including complete blood picture weight and height were monitored, followed by the calculation of body mass index (BMI) using the formula BMI = weight (kg)/height^2 (m). Height-for-age, weight-for-age and BMI for age z-scores were calculated using Anthro plus program depending on WHO cutoffs (2007).[19]

Preparation of samples and biochemical analyses

Blood samples were drawn by venipuncture in the morning after an overnight fast. Serum was separated by centrifugation. Serum levels of Ca (mg/dl), Mg (mg/dl), and Zn (μg/dl) were determined colorimetrically according to Gindler and King,[20] Mann and Yoe,[21] and Makino et al.,[22] using kits supplied by Bio-Systems (Spain), Spectrum (Egypt), Quimica clinica aplicada (Spain) respectively. Serum levels of IL-6 (pg/ml) and TNFα (pg/ml) were estimated using ELISA Kit provided by Boster (U.S.A). CRP (mg/l) was determined by ELISA kit provided by immunospec (Spain).

Statistical analysis

The data were analyzed using version 16.0 of the computer based statistical package of Statistical Product and Service Solutions (SPSS), 2007. All the data are expressed as mean ± standard deviation. To evaluate the differences between stunted and control children, independent samples *t* test and Mann–Whitney test were performed, respectively, in continuous variables with normal distribution and without normal distribution. Inflammatory parameters were skewed so that the data were log transformed to obtain a more normally distributed data. Correlations were carried out by the bivariate correlation using the Spearman correlation coefficient.

Results

Data in Table 1 showed marked decreases in weight, weight-for-age Z score (WAZ), height, and height-for-age Z score (HAZ) of stunted children versus healthy one. Table 2 showed that stunted children exhibited a marked reduction in the levels of Ca, Mg, and Zn as compared to normal ones. Significant elevations in serum IL-6, TNFα and CRP were also detected in stunted children as compared to control values, as shown in Table 2 and Fig. 1. Furthermore, Table 3 and Fig. 2 showed that there were significant negative correlation between IL-6 levels and Zn ($r = -0.248$, $p = 0.020$) among stunted children. Also, TNFα correlated negatively with Ca ($r = -0.235$, $p = 0.028$), and Mg ($r = -0.266$, $p = 0.012$) (Table 3). CRP correlated negatively with Ca ($r = -0.468$, $p = 0.000$), Mg ($r = -0.291$, $p = 0.006$) and Zn ($r = -0.310$, $p = 0.003$) (Table 3). Moreover, in Table 4 Zn showed

Table 1
The demographic and anthropometric characteristics for healthy control and stunted children.

Parameters	Control (N = 28) Mean ± SD	Stunted children (N = 60) Mean ± SD	P-value
Sex (male/female)	15/13 (53.5%: 46.4%)	28/32 (46.6%: 53.3%)	
Age (years)	7 ± 1.5	6.9 ± 1.8	0.837
Weight (kg)	21.4 ± 4.5	17.4 ± 3.4*	0.000
WAZ	−0.6 ± 0.7	−1 ± 0.7*	0.000
Height (cm)	116.5 ± 10	104.5 ± 9 *	0.000
HAZ	−0.9 ± 0.9	−3 ± 0.5 *	0.000
BMI (kg/m^2)	15.5 ± 1	15.5 ± 1.4	0.381
BMIAZ	−0.1 ± 0.5	−0.1 ± 0.9	0.381

Data are expressed as mean ± SD.
N: the number of subjects in each group (WAZ: Weight-for-age Z score, HAZ: Height-for-age Z score, BMI: Body mass index, BMIAZ: BMI-for-age Z score).
* Significant difference from control at P < 0.05.