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The assessment between IL-6 and IL-8 and anthropometric status in malnourished children

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Abstract

Purpose: To evaluate the correlations between the pro-inflammatory interleukins IL-6 and IL-8 and the anthropometric measurements in malnourished *vs.* non-malnourished children. *Patients and Methods*: We have examined 219 children from Pediatric Clinic I, University of Medicine and Pharmacy of Tirgu Mures, Romania, during January 1, 2012–March 1, 2013 and divided according to Body Mass Index (BMI kg/m^2) in the following two groups: 164 with normal nutritional status – control group (BMI between -2SD and +2SD), and 55 children with malnutrition (BMI <-2SD). All the children were evaluated anthropometric: BMI, weight for age (W/A), height for age (H/A), mid-upper-arm circumference (MUAC), tricipital skinfold (TSF) and paraclinical: IL-6 and IL-8 levels. *Results*: From 219 children, 25.1% were malnourished. The mean age was 5.16 years in malnourished. IL-6 and IL-8 mean levels were 2.54 pg/mL, respectively 6.83 pg/mL in malnourished and 6.02 pg/mL, respectively 9.06 pg/mL in non-malnourished. By statistically comparing IL-6 in malnourished group *vs.* control group, we observed decreased values (p<0.0001) and also significantly lower values for IL-8. We also obtained statistical differences between the two groups in BMI, W/A, MUAC and TSF. The BMI SD have an increasing trend line, from -4SD in newborn malnourished to -2SD in near 18-year-old malnourished; the trend line had only a slight ascension in non-malnourished children. *Conclusions*: The interleukin levels and BMI, W/A, MUAC and TSF are significantly lower in malnourished children than in non-malnourished. This functional impairment may be involved in the malnutrition to develop a specific immune response in these children.

Keywords: interleukins, malnutrition, anthropometric measurements, children.

Introduction

The evaluation of nutritional status can be approached by simultaneous use of case history, clinical, anthropometric and biochemical data. Independently, each part gives little information, and so, a diagnosis of nutritional deficiency that is established simply on clinical data can be tricky. Nowadays, the evaluation of biochemical markers is a necessity, in order to complete the diagnosis. A wide range of markers can be used in this process, starting with serum proteins, blood glucose, to special markers such as Insulin-like Growth Factor 1 or circulating cytokines. Though the initial cost of complex biochemical determinations is high, it could be a real long-term benefit, by improving the future cost of hospitalizations due to malnutrition and its complications such as recurrent infections. It is known that the morbidity and mortality of the malnourished children is high, even one year after treated for moderate acute malnutrition [1, 2].

Cytokines are essential modulators of the immune response. IL-6 is a 26 kDa polypeptidic cytokine that stimulates the immune response during several conditions associated with inflammation, autoimmune or malignant diseases [3]. IL-8 is a chemotactic factor that stimulates the migration of leukocytes to the inflammation site [4]. The determination of IL-6 and IL-8 levels is relevant to several chronic diseases, such as diabetes, cancer, obesity, or autoimmune disease. If any inflammatory condition is present, even in malnourished, the IL-6 levels are predominantly high [5–9].

The release of IL-6 from adipocytes is an important mechanism that is regulated mainly by the fatty food intake; the saturated fats increase the IL-6, whereas the non-saturated vegetable oils decrease its levels. The IL-8 is released by the nonfat cells from visceral adipose tissue and by other tissues [10, 11]. A high IL-6 level leads to a low appetite and reduces the food intake in order to regulate the body weight [12, 13]. It seems that a sudden increase if IL-6 stimulates the lipolysis in the muscles rather than adipose tissues, by increasing fat oxidation and esterification of the fatty acids. The released energy and the activation of COX-2 in the brain also increase the body temperature, IL-6 being one of the most important mediators of fever [14–16].

Purpose

The purpose of this study was to assess the relation between malnutrition and the production of some cytokines and to establish the correlations between interleukins

IL-6/IL-8 and anthropometric measurements in malnourished *vs.* non-malnourished children.

Patients and Methods

We have examined 219 children from Pediatric Clinic I, University of Medicine and Pharmacy of Tîrgu Mureş, Romania, during January 1, 2012– March 1, 2013. We have included children without signs of infection or inflammatory disease, whose parents agreed and signed the consent form. A number of 17 children were not included because they did not respect the inclusion criteria or their parents did not agreed with the study. The study was approved by the Ethics Committee within the University of Medicine and Pharmacy of Tîrgu Mureş.

All the included children were anthropometrically evaluated, using common methods such as Body Mass Index (BMI), weight for age (W/A), height for age (H/A), mid-upper-arm circumference (MUAC), tricipital skinfold (TSF). For anthropometric measurements, we have used standard deviation (SD) according to *WHO Z*-score or standard deviation classification system.

Paraclinically, we have followed the IL-6 and IL-8 serum levels. The serum cytokine levels were determined within the Central Clinical Laboratory, Emergency County Hospital of Tîrgu Mureş.

In order to compare the data in malnourished *vs.* non-malnourished children, we have divided the included children, according to BMI, in the two groups. The first group included children with BMI (kg/m^2) <-2SD, considered according to *WHO* specifications as malnourished or underweighted children. The second group (control group) consisted in children with normal nutritional status, with BMI (kg/m^2) between -2SD and +2SD.

In order to analyze the cytokine levels in nutritional status context, we had to reject from the statistics all the cases with values over the maximum accepted limit, as any high level of interleukins (IL) could be associated with undiagnosed/clinically absent inflammatory response. Including the high values will have altered the true mean level of interleukins in both groups. We considered the upper accepted limit in IL-6 as 10 pg/mL and 15 pg/mL for IL-8 respectively. In the cases with IL levels under the detection limit, we substituted the values by the one-half limit for less-than value statistical analysis.

We have used Kolmogorov and Smirnov normality test for checking the Gaussian distribution between the compared groups. If the normality test was passed, the unpaired *t*-test was used for statistics; otherwise, non-parametric Mann–Whitney test was applied. In all statistic tests, we calculated a two-tail *p*-value, with 95% confidence interval. For correlation of interleukins with and between the anthropometric measurements, we used Pearson's *r*-test. All statistic tests were calculated in GraphPad InStat 3 and spreadsheet software.

Results

From the 219 included children, 164 (74.9%) were found with normal nutritional status, and 55 (25.1%) children were evaluated with malnutrition.

The mean overall age was 7.2 years. The mean age in malnourished was 5.16±4.6 years (3.9–6.4 at 95% CI), with a median of 3.67 years, ranging between two months to 17 years. In control group, the mean age was 7.94±4.9 (7.2–8.7 at 95% CI), with a median of 7.31, ranging between two months to 18 years. The unpaired *t*-test showed a statistically significant difference between the median ages in malnourished *vs.* normal children (p=0.0003), with no difference between the two SD of age (±4.6 years and ±4.9 years respectively).

Most children were girls (n=126; 57.5%). In malnourished children, the two genders were represented in equal proportions; in the control group, 59% were girls. No statistical difference was found in the two groups regarding the genders (p=0.27).

We have excluded from statistics 19% (42 cases) of IL-6 values and 67% (148 cases) of IL-8 values from the 219 cases, as they have exceeded the maximum accepted limit. The calculations for interleukins were made on 177 cases of IL-6 and 71 cases of IL-8. IL-6 and IL-8 mean levels were 2.54 pg/mL (1.92–3.17 at 95% CI), respectively 6.83 pg/mL (5.48–8.19 at 95% CI), in malnourished, and 6.02 pg/mL (5.68–6.36 at 95% CI), respectively 9.06 pg/mL (7.51–10.61 at 95% CI), in control group. By statistically comparing the interleukins mean levels in malnourished group *vs.* control group, we observed significantly decreased values in malnourished for IL-6 (p<0.0001), and also lower values for IL-8 (p=0.0024). There is a high number of cases with IL-6 <2 pg/mL (p<0.0001) in malnourished (52.6%) *vs.* control group (8.6%). Following the number of cases with IL-8 <2 pg/mL, there is no difference between the two groups (10.8% in malnourished, 14.7% in control group, p=0.89) (Table 1).

Table 1 – *IL values under and over the detection limit in malnourished and control groups*

	Malnourished		Control	
	<2 pg/mL	*>2 pg/mL*	*<2 pg/mL*	*>2 pg/mL*
IL-6	20 (52.6%)	18 (47.4%)	12 (8.6%)	127 (91.4%)
IL-8	4 (10.8%)	33 (89.2%)	5 (14.7%)	29 (85.3%)

A very week positive correlation was found between IL-6 and the BMI of malnourished (r=0.0546) or the BMI of control group (r=0.0321), and between IL-8 and BMI of control group (r=0.1597). A weak negative correlation was found between IL-8 and the BMI of malnourished (r=-0.1187) (Figures 1 and 2).

Other details regarding the correlation between interleukins and anthropometric measurements are presented in Table 2.

Table 2 – *Correlations between interleukins and anthropometric measurements*

	IL-6 (correlation coefficient *r*)		IL-8 (correlation coefficient *r*)	
	Malnourished	*Control*	*Malnourished*	*Control*
BMI	0.054	0.032	-0.118	0.159
W/A	0.098	0.192	0.127	0.314
H/A	0.023	0.150	0.156	0.311
MUAC	0.013	0.133	0.096	-0.008
TSF	0.034	0.123	-0.153	0.076

BMI – Body Mass Index; W/A – Weight for age; H/A – Height for age; MUAC – Mid-upper-arm circumference; TSF – tricipital skinfold.

The mean level of IL-6 in malnourished boys (1.94 pg/mL) is lower than in girls (3.02 pg/mL), p<0.0001, in control group the mean levels being approximately even (5.9 pg/mL). In IL-8, there is no difference in mean levels in boys and

girls (6.81 and 6.86 pg/mL respectively), but differences are present in the control group, where the boys presented a higher value then the girls (10.04 *vs.* 8.58 pg/mL), p=0.0245.

Regarding the anthropometric measurements, we obtained statistical differences between the two groups in BMI, W/A, MUAC and TSF (p<0.05 for each of the parameters). No statistical differences were found in H/A (Table 3).

There is a good positive association both between the BMI and MUAC (r=0.469) and between BMI and TSF (r=0.559). The BMI SD has an increasing linear trend, from -4SD in newborn malnourished to -2SD in near 18-year-old malnourished; the trend line had only a slight ascension in non-malnourished children, from -0.5 to -0.1SD.

Table 3 – ***Anthropometric measurements***

Anthropometric measurements	Malnourished (SD)	Control (SD)	*P*
BMI	-3.35	-0.44	<0.0001
W/A	-3.20	-0.66	<0.0001
H/A	-1.30	-0.31	0.1068
MUAC	-2.99	-1.06	<0.0001
TSF	-2.51	-0.66	<0.0001

BMI – Body Mass Index; W/A – Weight for age; H/A – Height for age; MUAC – Mid-upper-arm circumference; TSF – Tricipital skinfold.

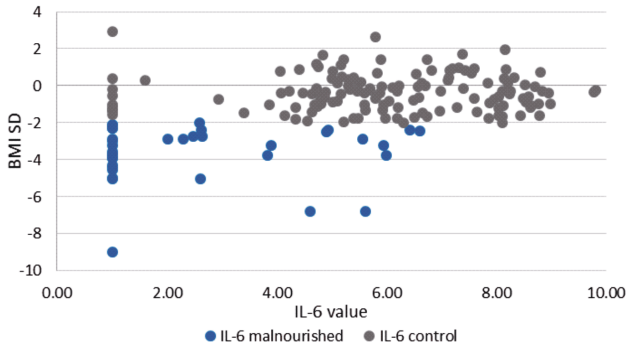

Figure 1 – ***IL-6 values in malnourished and control groups, in relation with BMI.***

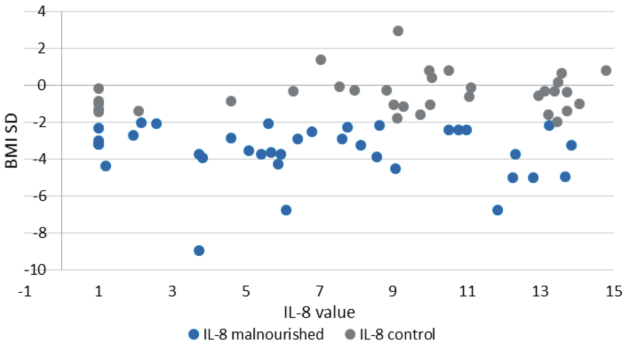

Figure 2 – ***IL-8 values in malnourished and control groups, in relation with BMI.***

Discussion

The nutritional deficiencies are a real problem throughout the world, especially in developing countries and must be properly evaluated.

In our study, the genders of malnourished children were approximately equally represented, with a slightly higher proportion of girls. Regarding the nutritional disorders, no statistical difference was found in the two groups regarding the genders, though other studies report more boys with nutritional deficiencies [17]. According to the mean age, most nutritional-deficient were preschool children (<6-year-old), whereas in control, most children were in school (>7-year-old).

The evaluation of some biochemical markers can be useful tools for appreciating the nutritional status, but they must be evaluated as a whole, together with the clinical data, because an associated inflammation may influence the protein metabolism, including the interleukin levels [18]. The studies show a correlation between interleukins and malnutrition. Studies on animals showed that the lack of proteins lead to a slow IL-6 response [19]. The IL-6 levels are significantly lower in low-fat children compared to the obese, so it is suggested that the proinflammatory markers must be considered in the classification of malnutrition or obesity [20, 21].

High levels of IL-6 are correlated with obesity, but rare data are presented in under-nutrition children [22]. Genetic studies showed that the expression of IL-6, aside IL-2 and γ-interferon are significantly decreased in malnourished, and aside other factors such as the complement loss through inflammation, is leading to immunological disorders with incapability of eradicating infections [7, 23]. Indeed, we have found a significantly high number of cases with IL-6 under the lowest detectable value of 2 pg/mL in malnourished children; for IL-8, there was no difference between the two groups in this manner.

In healthy children, the IL-6 level is decreased in both sexes [24]. This is consistent with our finding in the control group, but we found a significantly lower level of IL-6 in malnourished boys.

The correlation of IL-8 with the BMI is not clear, some studies showing an increase in IL-8 in underweight children [25], other in overweight subjects [26]. The low level of cytokines in malnourished children was explained in some studies as the inability of the leukocytes to secrete normal quantities of cytokines, and it is proved that a high protein intake improves the cytokine production in these patients [27, 28]. Also, the altered immune functionality of these may be the predominant cause of the immune impairment observed in malnourished children [29].

The anthropometric measurements are true indicators of nutritional disorders, as our study shows: the BMI, W/A, MUAC or TSF can be successfully used as anthropometric indicators for nutritional deficiency. Other studies showed that there are some reliability issues with MUAC and TSF in these evaluations [30].

We consider that the increasing trend line of BMI from very low values in newborn (specific for acute malnutrition) to near normal values in adolescents can be due to the adjustment of nutritional habits and medical surveillance [31].

This study has some limitations, as it was conducted on a limited number of cases, especially as we excluded the cases with high level of interleukins, due to a possible inflammatory process for a more exact evaluation of our purpose. Also, in this study we did not take into account the nutritional habits (glucose, lipid and protein intake) and social status of the included children, these variables being of great value in future research. More data need to be collected to clarify this situation, especially on IL-8 side.

Conclusions

It seems that there is no direct correlation between IL-6 or IL-8 and the anthropometrical evaluation. Nevertheless, the IL-6 and IL-8 levels, BMI, W/A, MUAC and TSF are significantly lower in malnourished children than in non-malnourished, in many cases with values under the detection limit. This functional impairment within the malnutrition may lead to a low immune response in these children, with high susceptibility for infections. Further studies are necessary to clarify these relations by studying the correlations with other factors such as genetic predisposition, IL-1, TNF-α, or nutritional habits.

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