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Leptin and Nutritional Markers in Childhood Protein-Energy Malnutrition

A.B. ERBAGCI*, M. KILINÇ†, M. TARAKÇIOGLU, S. TAYSI and R. KOCABAS
Department of Biochemistry and Clinical Biochemistry, School of Medicine, University of Gaziantep, TR-27310, Gaziantep, Turkey
Fax: (90)(342)3601617; Tel: (90)(342)3606060-77788
E-mail: aerbagci@gantep.edu.tr

Leptin is an important signal in metabolic/endocrine adaptation to prolonged nutritional deprivation. This cross-sectional study was performed to determine alterations associated with protein-energy malnutrition in serum levels of leptin and classical nutritional markers. Forty-two children with protein-energy malnutrition was classified according to Gomez and 21 healthy controls were recruited. Serum leptin, albumin, prealbumin, fibronectin, transferrin, lipoprotein (a) and whole blood hemoglobin concentrations were determined. Leptin levels were significantly lower in undernourished children, declining with degree of malnutrition. Alteration of albumin, hemoglobin and lipoprotein (a) became apparent at late stages of malnutrition. Gomez classification was the only significant contributor to the variations in leptin concentrations among age, weight, presence of infection, breast-feeding duration and age at feeding with complementary foods. This study provides evidence that leptin is sensitive to stages of malnutrition independent of body weight, probably for it's role in metabolic adaptation. Serum prealbumin, transferrin and fibronectin does not offer a sensitive alternative in the background of increased susceptibility to infection.

Key Words: Protein-energy malnutrition, Leptin, Lipoprotein (a), Prealbumin, Fibronectin, Transferrin.

INTRODUCTION

Protein-energy malnutrition (PEM) is the most frequent type of malnutrition, affecting approximately 800 million people worldwide. It occurs especially in children, the elderly and patients suffering from neoplasia or chronic illnesses[1]. Leptin, the ob gene's encoded protein, is an adipocyte-derived hormone that is essential for normal regulation of body weight and has been considered a signal of energy deficiency and integrator of neuroendocrine function. Leptin regulates adipose tissue mass through hypothalamic effects on satiety and energy expenditure[2,3]. During prolonged PEM, diversion of the substrates away from growth toward metabolic homeostasis leads to altered levels of plasma proteins, lipids and lipoproteins[1-3].

†Department of Pediatrics, School of Medicine, University of Gaziantep, Gaziantep, Turkey.

The use of anthropometric measurement (*e.g.*, of triceps skin-fold thickness or arm-muscle circumference), an early method of nutritional assessment, has been shown to be an inaccurate indicator of nutritional status, is very skill dependent and can result in underestimation of malnutrition risk. The detailed nutritional assessment was used as the reference method to determine PEM. The detailed nutritional assessment can be considered one of the most comprehensive methods, but it is time-consuming, costly and unsuitable for large-scale use[4,5]. The use of serum protein measurements is widespread for the assessment of nutritional status. Albumin was traditionally measured to estimate the nutritional status, but it has the disadvantages of having a long half-life (18-20 days) and that 60 per cent of total body albumin is extravascular[6,7]. Transferrin is the major transport protein for iron and is the second most investigated protein marker in malnutrition. Transferrin is synthesized in the liver and has a much smaller pool than albumin, most of it being intravascular. It has a shorter half-life of 8-9 days[6,7]. Fibronectin is a cold-insoluble globulin, non-specific serum opsonin which has important functions in neutrophil adhesion, bacterial opsonization, T-cell activation and vascular integrity. Fibronectin acts as a potent chemoattractant for monocytes and for neutrophils. It enhances the secretion of interleukin l and also affects wound healing and blood coagulation[6-8]. Prealbumin has been shown to be a useful marker in monitoring malnourished patients, because its serum concentrations are closely related to early changes in the nutritional status and it changes in response to nutritional support. Clinical studies indicate that determination of the prealbumin level may allow for earlier recognition of malnutrition risk and timely intervention[4,5]. Lipoprotein (a) [Lp(a)] is an independent lipoprotein risk factor for cardiovascular disease. It is distinguished from LDL in that, in Lp(a), attached to the LDL particle is another apoprotein, termed apoprotein (a)[8]. Effect of malnutrition on Lp(a) is not well-known.

This study was performed to determine serum levels of leptin and several nutritional markers in children with different grades of non-edematous PEM classified according to Gomez *et al.*[9]. Plasma albumin, fibronectin, prealbumin, transferrin, Lp(a) and whole blood hemoglobin concentrations were investigated, which may be prone to metabolic regulation associated with limited amount of substrates.

EXPERIMENTAL

The study was conducted at Gaziantep University, Faculty of Medicine, Department of Pediatrics and Department of Biochemistry and Clinical Biochemistry. Informed consent was obtained from all subjects according to the Helsinki declaration as revised in 1996. Forty-three children with non-edematous PEM (18 without/25 with mild to moderate upper respiratory tract infections, mean ± SEM age: 27 ± 5 months, 20M/23F) and 21 age- and gender-matched control subjects (12 without/9 with mild to moderate upper respiratory tract infections, 27 ± 6 months, 13M/8F) were recruited to this study. Twenty of the children with PEM were grade 1, 15 were grade 2 and 8 were grade 3 according to Gomez classification[9]. Subjects receiving

any systematic or topical therapy within at least 1 month preceding the study or having an established diagnosis of any systematic disease other than PEM with either clinical or laboratory signs were excluded.

Blood samples were collected using standard venipuncture technique between 9:30 to 11:00 am after 12 h fast. Serum samples were separated immediately after centrifugation at 4 °C, 2000 g for 10 min and stored at -20 °C until analysis, which were performed in the same run to avoid inter-run analytical variation. Serum prealbumin, fibronectin, transferrin and Lp(a) concentrations were determined using BN II nephelometer (Behring Diagnostics GmBH, Marburg, Germany). Control sera were included in analytical run. Intra-assay and inter-assay precision performances of the assay was determined on 10 replicates in a single run and in 10 different runs.

Leptin levels were determined with human leptin ELISA reagent (Diagnostic Systems Laboratories, Inc, Texas, USA). Intra-assay precision study yielded CV of 4.3 %.

Statistical analyze: Data are presented as mean ± SEM. Mann Whitney U test was used to consider case-control differences. Case-control differences in nominal data were evaluated with Chi-squared test. Significant influences of variables on leptin concentrations were evaluated with general linear model. Two tailed p values < 0.05 were considered significant. Analyses were performed with SPSS 9.0 (SPSS Inc., Chicago, IL, USA) statistical software program.

RESULTS AND DISCUSSION

The baseline characteristics of the study participants are presented in Table-1. A statistically difference was not noted in age and gender. Body mass index (BMI) was lower in PEM group. Although statistically insignificant per cent of mild upper respiratory infections was higher in patients with PEM.

TABLE-1
CHARACTERISTIC FEATURES OF THE STUDY GROUPS

	PEM (n = 43)	Controls (n = 21)	p
Gender	20M/23F	13M/8F	NS
Age (months)	21.0±3.2	26.8±4.6	NS
BMI (kg/m^2)	13.4±0.3	16.5±0.4	0.0001
Upper respiratory infections	n:25, 58%	n:9, 43%	NS

The nutritional markers, leptin and Lp(a) levels in patients and controls are presented in Table-2. Leptin levels were significantly lower in undernourished children, declining with degree of malnutrition ($r = -0.7$, $p < 0.001$). Albumin levels were comparable in the control and Gomez group 1, but were significantly decreased in groups 2 and 3 compared to controls and group 1. Lp(a) levels were lower in Gomez group 3 than in the healthy controls. Hemoglobin levels were also decreased in children with PEM than in the controls. A significant difference was not observed for serum prealbumin, transferrin and fibronectin concentrations.

TABLE-2
LEPTIN AND NUTRITIONAL MARKERS IN PEM CLASSIFIED ACCORDING TO GOMEZ AND THE HEALTHY CONTROL GROUPS

	Grade 1 n:20	Grade 2 n:15	Grade 3 n:7	Controls n:21
BMI (kg/m^2)	14.50±0.4[b]	13.30±0.5[b]	11.1±0.6[b]	16.50±0.4
Leptin (ng/mL)	1.10±0.09[c]	0.80±0.08[c]	0.50±0.01[c]	1.60±0.15
Albumin (g/dL)	3.90±0.9	3.60±0.2[a]	3.50±0.2[a]	4.00±0.8
Transferrin (g/L)	2.70±0.2	2.30±0.2	2.30±0.3	2.70±0.2
Prealbumin (g/L)	0.17±0.02	0.14±0.01	0.16±0.03	0.15±0.01
Lp(a) (g/L)	0.15±0.05	0.08±0.03	0.02±0.01[a]	0.09±0.02
Hemoglobin (g/dL)	10.20±0.5[a]	10.20±0.4[a]	9.10±0.9[a]	11.60±0.4

a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.005$, *vs.* control group.

Results of general linear model in the whole study group, serum leptin levels as the dependent variable is shown in Table-3. The model explained 47.7 % of the variation in leptin concentrations ($p < 0.0001$). In the model that included, age, weight, Gomez classification, presence of infection, breast-feeding duration and age at feeding with complementary foods, only Gomez classification yielded a significant result. Compared to controls, leptin concentration decreased 0.11 ± 0.23 ng/mL ($p = 0.64$) in Gomez group I, 0.66 ± 0.26 ng/mL ($p < 0.01$) in Gomez group II and, 0.91 ± 0.35 ng/mL ($p < 0.01$) in Gomez group III.

TABLE-3
GENERAL LINEAR MODEL IN THE WHOLE STUDY GROUP, SERUM LEPTIN LEVELS AS THE DEPENDENT VARIABLE

Dependent variable	Leptin (ng/mL)	
	B±SEM	p
Age (months)	0.04±0.06	0.644
Weight (g)	0.0±0.0	0.899
Gomez classification (*vs.* controls)		
Gomez group I	-0.11±0.23	0.641
Gomez group II	-0.66±0.26	0.014
Gomez group III	-0.91±0.35	0.012
Infection	0.34±0.22	0.124
Breast-feeding duration (months)	0.01±0.01	0.318
Age at feeding with complementary foods (months)	-0.01±0.02	0.544
R^2	0.477	0.000
Intercept		0.003

In present study, leptin seems to be the earliest biochemical signal of nutritional deprivation among the molecules investigated. Leptin levels not only correlate with Gomez classification, but are significantly different between stages of PEM. However such a relationship was not observed between leptin and body weight, after correcting for co-variants such as age and presence of infection. Therefore it's possible to assume that observed decrease in leptin may be a sign of metabolic adaptation to nutritional deficiency state rather than reflecting decreased total body

fat. Previously decreased leptin levels in PEM and a rebound elevation much higher than in healthy controls after nutritional recovery have been reported[16-18]. It is well known that the basic function of leptin is to regulate body weight by reducing food intake and increasing energy consumption through corticotrophin-releasing hormone and neuropeptide Y (NPY); an important appetite hormone of the hypothalamus[10,11]. It is known that glucocorticosteroids and insulin stimulate NPY levels, whereas leptin inhibit NPY levels[12,13]. During prolonged PEM, low level of insulin and/or its decreased effect due to an insulin resistant status in the presence of high circulating growth hormone and glucocorticoid hormone levels ensure the diversion of substrates away from growth toward metabolic homeostasis. In this condition low leptin levels help to increase appetite and food intake and stimulate hypothalamo-hypophyser-adrenal axis necessary for effective catabolism[14]. Suppressed leptin levels may be advantageous for its adaptive role during the period of limited nutrient supply[15]. However, studies have demonstrated that leptin is a pleotropic molecule and has profound effects on regulation of reproduction, neuroendocrine and immune functions, hematopoiesis, angionesis, blood pressure control and bone development too, which may have further implications on poor growth and development[16].

It is not surprising that a reduction of circulating albumin and hemoglobin levels follow leptin as they compromise the bulky amount of protein that can be used as an amino acid store in such instances[4,5,14]. However in contrast to the previous studies a significant difference was not observed for serum prealbumin, transferrin and fibronectin[4,6,7]. These proteins are acute phase proteins and in addition to nutritional status their concentrations are prone to alterations associated with metabolic stress. Multiple abnormalities in the inflammatory-immune response have been described in protein energy malnourished children and may account for increased severity and frequency of infection[17]. Therefore, presence of mild infections and/or an uncontrolled inflammatory response may lead non-specific differences.

The decrease in Lp(a) in children with severe PEM does not support the idea that Lp(a) concentrations are highly heritable and not markedly affected by environmental factors such as nutrition, weight and lipid lowering drugs[8].

Conclusion

These results provide evidence that leptin is an early sign of PEM and is sensitive to clinical stages of malnutrition, probably for its role in metabolic adaptation to limited nutrient supply.

Alteration of albumin, hemoglobin and Lp(a) become apparent at late stages of malnutrition and/or are not correlated with grade of PEM. Serum prealbumin, transferrin and fibronectin do not offer any sensitive alternative as a nutritional marker in the background of increased susceptibility to infection and an uncontrolled inflammatory response.

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