

Plasma Ghrelin in Marasmic Infants

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Abstract: Objectives: Malnutrition is one of leading health problems in developing countries. Ghrelin, a recently discovered peptide hormone, has been proven to influence appetite and body weight in adults. This work aims to measure plasma ghrelin level in infants suffering from marasmus and to assess its relation with some metabolic and anthropometric measures. Design and Methods: The study included 26 marasmic infants (age ranged from 4-24 months), who's hospital admission was mainly due to chest infection or gastroenteritis. Twenty-seven age and sex matched healthy infants served as a control group. Anthropometric measurements including weight, length, head circumference and body mass index were recorded. Complete blood picture, random blood glucose and plasma insulin were measured. Total plasma ghrelin was determined using Enzyme-linked Immunosorbent assay. Results: Results revealed that the anthropometric measures were significantly lower in marasmic infants. Anemia and leukocytosis were significantly more common in marasmic group. The plasma ghrelin was significantly higher in marasmic infants, while insulin and blood glucose were significantly lower when compared to the control group. Plasma ghrelin correlated positively with the total leukocyte count and negatively with hemoglobin percent and sex in marasmic infants. It did not show any correlation with any of the recorded anthropometric measurements. Conclusion: We postulated that ghrelin release in marasmic infants can be attributed to negative energy balance caused by low insulin and blood glucose.

Key words: Malnutrition- Ghrelin- Insulin- Anthropometric measurements

INTRODUCTION

Malnutrition is one of leading health problems in developing countries. It remains one of the most common causes of morbidity and mortality among infants and children through out the world (Muller and Krawinkel, 2005). Children are especially threatened by malnutrition, because of the high protein energy cost of growth. UNICEF, 1999, estimated that 31% of children fewer than 5 years in developing countries are underweight, 38% are stunted and 11% wasted.

Protein energy malnutrition (PEM) may range in severity from mild to moderate to severe degrees. The severe degree of PEM is characterized by distinct syndromes namely, Marasmus, Kwashiorkor (KWO) and Marasmic- Kwashiorkor. Marasmus is known to be the most common form of PEM. Although PEM is a nutritional deficiency disease, the exact form that develops depend on the age of the child, the duration of breast feeding and also on weaning practice (Dickerson, 1995).

Ghrelin, a recently discovered peptide hormone, is secreted predominantly, but not exclusively, by the X/A oxyntic cells of the stomach (Kojima et al., 1999 and Date et al., 2000). There is now evidence that ghrelin play a prominent role in the physiologic regulation of appetite and body weight in adults (Cummings and Shannon, 2003). This work aims to measure plasma ghrelin level in infants suffering from marasmus and to assess its relation with some metabolic and anthropometric measures.

MATERIALS AND METHODS

The study included 26 infants suffering from marasmus (14 males and 12 females) who were recruited from the pediatric unit at Al-Mataria Teaching Hospital. Patient age ranged from 4-24 months with the mean of 6.76±5.74. Marasmic infants had clinical muscle wasting, loss of subcutaneous fat and absence of edema.

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Hospital admission was mainly due to chest infection or gastroenteritis. Twenty-seven age and sex matched healthy infants served as a control group. All patients were subjected to full history taking, thorough clinical examination. Anthropometric measurements of the studied infants including weight, length, head circumference and body mass index (BMI = Kg/m²) were recorded. Routine laboratory investigation including complete blood picture and random blood glucose was done.

Venous blood samples, 5 ml each, were collected and written informed consent was obtained from parents of studied infants before blood sampling. Plasma was separated and stored at -20°C until further assayed. Plasma insulin was determined using immunoenzymetic assay, the sensitivity was 0.73 uU/ml (Temple *et al.*, 1992).

Total plasma ghrelin was determined using Enzyme-linked Immunosorbent assay, the sensitivity was 30 pg/ml (Pirazzoli, 2005).

Statistical analysis using the SPSS program of personal computer version 10 employed the student's t-test for comparison of the mean values and the simple correlations. Pearson Correlations were calculated to evaluate the relationship between the variables. P-values were two-tailed and values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Results:

The marasmic infants group had a mean weight of 4.7±1.62 kg, mean length of 59.8±7.77 cm, mean BMI of 12.96±1.98 kg/m² and mean head circumference of 39.2±3.92 cm. The mean values of the control group were 9.8±3.16 kg, 74.4±12.41 cm, 17.53±2.38 kg/m² and 44±3.29 cm respectively. Differences in weight, length, BMI and head circumference were statistically significant (table 1).

Table 1: Characteristics of the studied infants

	Marasmic patients (n=26)	Healthy control (n=27)	P- value
Weight (kg)	4.7±1.62	9.8±3.16	<0.01
Length (cm)	59.8±7.77	74.4±12.41	<0.01
BMI (Kg/m ²)	12.96±1.98	17.53±2.38	<0.01
Head circumference (cm)	39.2±3.92	44±3.29	<0.01
Total leukocyte count	14,030±6,356	9,416±1,203	<0.01
Hemoglobin (%)	9.4±1.64	11.2±0.95	<0.01
Ghrelin (pg/ml)	407.24±166.05	19.92±8.26	<0.01
Insulin (µU/ml)	9.25±7.44	24±13.67	<0.01
Glucose (mg/dl)	76.45±10.30	86.88±4.11	<0.01

Table 2: Correlations between plasma ghrelin, insulin, glucose and anthropometric measurements of marasmic infants

	Age	Sex	Birth order	Weight	Length	BMI	Head circumference
Ghrelin	-.035	-.638*	.202	.118	.253	-.084	-.174
Insulin	-.337	.546*	-.078	-.334	-.193	-.436	-.674**
Glucose	.451*	.340	-.459*	.351	.356	.164	.101

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

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

Table 3: Correlations between different blood parameters in marasmic infants

	Total leukocyte count	Hemoglobin	Ghrelin	Insulin	Glucose
Ghrelin	.807**	-.651*	1.000	-.004	-.400
Insulin	-.250	.539*	-.004	1.000	.579*
Glucose	-.172	.298	-.400	.579*	1.000

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

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

Table 4: Correlation between different parameters in control infants

	Weight	Length	BMI	Head circum- -ference	Total leukocyte count	Hemo -globin	Ghrelin	Insulin	Glucose
Ghrelin	.558**	.637**	-.220	.488**	.590*	.465	1.000	.709**	.510**
Insulin	.282	.337	-.164	.382	.514*	.119	.709**	1.000	.551**
Glucose	.326	.254	.130	.255	.229	.027	.510**	.551**	1.000

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

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

In marasmic infants group, the mean values of plasma ghrelin, insulin and glucose were 407.24±166.05 pg/ml, 9.25±7.44 µU/ml and 76.45±10.30 mg/dl respectively. These figures were 19.92±8.26 pg/ml, 24±13.67 µU/ml and 86.88±4.11 mg/dl respectively in the control group. The mean values of the tested biochemical parameters in plasma of marasmic infants were statistically significant compared to control group (Table 1).

In marasmic infants group, plasma ghrelin correlated positively with the total leukocyte count and negatively with hemoglobin percent and sex. It did not show any correlation with any of the recorded anthropometric measurements. Plasma insulin correlated positively with sex, hemoglobin percent, and blood glucose and correlated negatively with the head circumference. Blood glucose correlated positively with age, insulin and negatively with birth border (Table 2 & 3).

In the control group, plasma ghrelin correlated positively with the body weight, length, and head circumference, and with the total leukocyte count, insulin and blood glucose (Table 4).

Discussion:

Malnutrition is one of the leading causes of morbidity and mortality in infancy and childhood, particularly in developing countries (Kilic *et al.*, 2004). Earlier onset of malnutrition, coincide with higher incidence of permanent brain development impairment (WHO, 1999). Protein energy malnutrition (PEM) is a pathological state resulting from insufficient intake of energy and other nutrients (Ge and Chang, 2001). Marasmus is known to be the most common form of PEM. It is due to severe caloric depletion. Marasmus is diagnosed in the presence of clinical muscle wasting, loss of subcutaneous fat and absence of edema (Gernaat and Voorhoeve, 2000). A child is defined as suffering from marasmus if his weight is below 60% of the expected weight (fiftieth centile) for age (Axton, 1990).

Our results revealed that the anthropometric measurements including mean weight, length, BMI and head circumference were significantly lower in marasmic infants when compared to the control group. The laboratory results revealed that total leukocyte count was significantly higher in marasmic infants than control group. This was due to high incidence of infection in malnourished infants, especially respiratory tract infection and gastroenteritis, which may be severe up to death, as proved by (Talboom *et al.*, 1986, Fakhir *et al.*, 1989 and Matta, 1992). Hemoglobin level was significantly lower in marasmic infants than control group as anemia is an established fact in PEM. Sive *et al.*, 1997, reported that iron deficiency anemia is a frequent feature in patients with PEM while McDougall *et al.*, 1982, demonstrated folate deficiency and hemolysis as causes of anemia in patients with PEM.

The present study revealed that the plasma ghrelin was significantly higher in marasmic infants when compared to the control group. While plasma insulin and blood glucose were significantly lower in marasmic infants. Thus the negative energy balance in marasmic infants may be the cause of the ghrelin release.

Ghrelin, the endogenous ligand of the growth hormone secretagogue receptor (Kojima *et al.*, 2001), exerts potent growth hormone releasing activity but also has other endocrine and non-endocrine actions among which is a remarkable influence on the control of food intake (Inui, 2001 and Muccioli *et al.*, 2002). Ghrelin treatment has been shown able to induce appetite, food intake, and reduce fat utilization leading to weight gain in both animals and humans (Tschop *et al.*, 2000). Circulating ghrelin levels are increased by fasting and energy restriction while decreased by food intake, glucose and insulin (Inui, 2001, Tschop *et al.*, 2001, Cummings *et al.*, 2002 and Ukkola, 2003). Tomomi *et al.*, 2002, measured plasma ghrelin concentration after oral and intravenous glucose load in normal subjects. They reported that hyperglycemia resulted in suppression of plasma ghrelin concentration when compared with oral administration of equivalent volume of water which did not change plasma ghrelin level. They reported also a diurnal pattern of plasma ghrelin level with pre-prandial increase and post-prandial decrease. Thus the pre-prandial rise of human plasma ghrelin suggests a possible role of ghrelin as a hunger signal triggering meal initiation.

Our results revealed that anemia and leukocytosis were significantly more common in marasmic group. Ghrelin correlated positively with the total leukocyte count in marasmic infants. This could be attributed to secondary malnutrition that occurs in infants with diseases associated with increased caloric requirements as infection or increased caloric loss as diarrhea (Curran and Barness, 2000). Thus ghrelin behaves as an orexigenic factor and could be considered an adaptive mechanism, promoting energy intake and increasing body fat stores in response to a deficit in energy balance.

The present study revealed that plasma ghrelin in marasmic infants group, did not show any correlation with any of the recorded anthropometric measurements. While in the control healthy infant group, plasma ghrelin correlated positively with the body weight, length, and head circumference. Savino *et al.*, 2006, studied the relationship between ghrelin concentration, fasting time and anthropometry in healthy infants in the first months of life. They concluded that fasting influence on serum ghrelin concentration confirms the role of this hormone as a physiological meal initiator also in infancy. They reported correlation between ghrelin, anthropometrical parameters (Weight, length and head circumference) and age. These findings support the hypothesis that this hormone could exert an important influence on growth in the first months of life.

Conclusion:

Ghrelin rises in marasmic infants and correlates with leukocytosis and anemia commonly seen among these infants. It does not correlate with their anthropometric measurements.

REFERENCES

- Muller O, and M. Krawinkel, 2005. Malnutrition and health in developing countries. *C M A J*, 173(3): 279-286.
- UNICEF, 1999. The state of the world's children. New York: UNICEF.
- Dickerson, J.W.T., 1995. Nutritional disorders: protein-energy malnutrition. In: *Biochemistry in Clinical Practice*. Williams DL. and Marks V. (eds) 2nd edition. Reed Educational and Professional Publishing Ltd, Oxford, Boston, pp: 1-5.
- Kojima, M, H Hosoda, Y Date, M Nakazato, H Matsuo and K. Kangawa, 1999. Ghrelin is a growth hormone-releasing acylated peptide from stomach. *Science*, 402: 656-658.
- Date, Y, M Kojima and H. Hosoda, 2000. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in gastrointestinal tracts of rats and humans. *Endocrinology*, 141: 4255-4261.
- Cummings, D.E. and M.H. Shannon, 2003. Roles for ghrelin in the regulation of appetite and body weight. *Arch Surg*, 138: 389-396.
- Temple, RC, PM Clark and C.N. Hales, 1992. Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. *Diabetic Medicine*, 9: 503-512.
- Pirazzoli, P., 2005. Active and total ghrelin concentrations in the newborn. *J Pediatr Endocrinol Metab*, 18: 379-384.
- Kilic, M, E Taskin, B Ustundag and A. Aygun, 2004. The evaluation of serum leptin level and other hormonal parameters in children with severe malnutrition. *Clin Biochem*, 37(5): 382-387.
- WHO, 1999. Management of severe malnutrition: A manual for physicians and other health workers. WHO, Geneva, pp: 1-26.
- Ge, K.Y. and S.Y. Chang, 2001. Definition and measurement of child malnutrition. *Biomed Environ Sci.*, 14(4): 283-291.
- Gernaat, HB and W.H. Voorhoeve, 2000. A new classification of protein energy malnutrition. *J Trop Pediatr*, 46: 97-105.
- Axton, J., 1990. Pediatrics in developing countries. In: *Diseases of Children, Textbook of Pediatrics*, edited by Levene M. I. (eds.) 6th edition. Churchill Livingstone, New York, London, pp: 538.
- Talboom, J.M., H Kabir and J. Anderson, 1986. Severe PEM, death and survival in hospital, clinical finding. *Trop Geogr Med.*, 38: 351-358.
- Fakhir, S, P Ahmed and M.A. Fairdi, 1989. Cell mediated immune responses in malnourished host. *J Trop Pediatr*, 35(4): 175.
- Matta, A., 1992. Diarrheal diseases as a cause of malnutrition. *Am J Trop Med Hyg.*, 47(1): 16-27.
- Sive, AA, WS Dampster, H Malan and H. Heese, 1997. Plasma free iron: a possible cause of anemia in KWO. *Arch Dis Child.*, 76(1): 54-56.
- McDougall, L, G Moudly, C Eyberg and M. Quirk, 1982. Mechanisms of PEM in Johannesburg. *Am J Nutr.*, 35: 229-235.
- Kojima, M, H Hosoda, H Matsuo and K. Kangawa, 2001. Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. *Trends in Endocrinology and Metabolism*, 12: 118-122.
- Inui, A, 2001. Ghrelin: an orexigenic and somatotrophic signal from the stomach. *Nature Reviews Neuroscience*, 2: 551-560.
- Muccioli, G, M Tschop, M Papotti, R Deghenghi, M Heiman and E. Ghigo, 2002. Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity. *European Journal of Pharmacology*, 440: 235-254.
- Tschop, M, DL Smiley and M.L. Heiman 2000. Ghrelin induces adiposity in rodents. *Nature*, 407: 908-913.
- Tschop, M, C Weyer, PA Tataranni, V Devanarayan, E Ravussin and M.L. Heiman, 2001. Circulating ghrelin levels are decreased in human obesity. *Diabetes*, 50: 707-709.
- Cummings, DE, DS Weigle, RS Frayo, PA Breen, MK Ma, EP Dellinger and J.Q. Purnell, 2002. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *New England Journal of Medicine*, 346: 1623-1630.

Ukkola, O., 2003. Ghrelin and insulin metabolism. *European Journal of Clinical Investigation*, 33: 183-185.

Tomomi, S, N Masamitsu, M Masanari, D Yukari, S Muhtashan, T Muneki, N Shin-Ichi, H Hiroshi, K Kenji and M. Shigeru, 2002. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol & Metabol*, 87(1): 240-244.

Curran, SJ and LA Barness, 2000. Nutritional requirements and malnutrition. In: *Nelson Textbook of Pediatrics*. Beharman R E, Kliegman R M, and Jenson H B, (eds) 5th edition. W.B. Saunders. Philadelphia, London, Toronto, Chapter 42: 169-172.

Savino, F, EC Grassino, MF Fissore, C Guidi, SA Liguori, L Silvestro, R Oggero and R Miniero, 2006. Ghrelin, motilin, insulin concentration in healthy infants in the first months of life : relation to fasting time and anthropometry. *Clin Endocrinol.*, 65(2): 158-162.