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Original Research Article

Low plasma ghrelin levels in children with severe protein energy malnutrition

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ABSTRACT

Background: Children with primary protein energy malnutrition (PEM) have significant loss of appetite which makes their nutritional rehabilitation difficult. Trials in patients with anorexia nervosa and cancer cachexia have shown short term efficacy of supplementing ghrelin to increase appetite. However, literature on ghrelin hormone status in children with PEM is scarce. We planned to study plasma ghrelin hormone levels in children with PEM and difference in plasma ghrelin levels among children having PEM of different grades.
Methods: Cross-sectional observational study was conducted over one year period. All hospitalised children during the study period and fulfilling the inclusion criteria for primary PEM (WHO criteria for malnutrition) were enrolled as cases. The cases (59 children) were divided into 2 groups – (Group 1 -severe PEM, group 2 - mild to moderate PEM) and were compared with 19 healthy children as controls (Group 3). Plasma fasting ghrelin levels were measured using enzyme immunoassay. The results were analysed using Mann-Whitney U Test.
Results: Median plasma ghrelin level among severe PEM group was 1.942ng/ml (interquartile range (IQR): 0.064, 9.506), mild to moderate PEM - 17.662 ng/ml (IQR: 1.658, 40.129) and controls - 17.525 ng/ml (IQR: 0.626, 27.361). Median ghrelin value was significantly lower in severe PEM group as compared to mild to moderate PEM (p value-0.027).
Conclusions: Plasma ghrelin levels are significantly reduced in children with severe PEM as compared with mild to moderate PEM and healthy controls.

Keywords: Children, Ghrelin, Protein energy malnutrition (PEM)

INTRODUCTION

Protein-energy malnutrition (PEM) is a major health problem in developing countries. According to Indian National Family Health Survey- 3 (NFHS-3), 46 % children under three years of age were underweight, 38% were stunted and 10% were wasted.[1] Children are specially prone to malnutrition because of the high protein and energy requirement for growth.[2] PEM is a catabolic state in which various factors lead to a net negative energy balance. There are various hormonal factors affecting this catabolic state. Many gastro-intestinal hormones like Ghrelin, adiponectin, leptin, etc. have a significant role in appetite regulation and net energy balance in the body.[2]

Ghrelin is a 28 amino acid orexigenic hormone secreted by neuroendocrine cells lining the fundus of the human stomach and epsilon cells of the pancreas.[3] Besides stimulating hunger and increased food intake, Ghrelin is a

potent stimulator of growth hormone from anterior pituitary and has various other physiological functions. The name Ghrelin is based on "ghre" meaning "to grow" because of its ability to stimulate GH release.[4] It is believed to have a role in various physiological processes including stimulation of lactotroph and corticotroph function, suppression of LH secretion, appetite stimulation, sleep–wake regulation and control of energy balance. Gastrointestinal functions of Ghrelin include promotion of gastric motility and acid secretion, induction of hyperglycemia and suppression of pancreatic exocrine and endocrine function.[5] Ghrelin has anti-inflammatory, para-sympathetic stimulatory and sympatho-inhibitory effects which attribute to its beneficial actions in sepsis and other inflammatory conditions.[6] It also plays significant role in immune modulation and cancer related cachexia.[7] Ghrelin has a proven role in appetite, energy balance and body weight regulation.[8] The secretion of Ghrelin increases under conditions of negative energy balance, such as starvation, cachexia, and anorexia nervosa, whereas its expression decreases under conditions of positive energy-balance such as feeding, hyperglycemia, and obesity.[9] In humans, Ghrelin levels are decreased in chronic and acute states of positive energy balance, whereas plasma levels of Ghrelin are increased by fasting and in cachectic patients with anorexia nervosa.[5]

Children with protein energy malnutrition are in a state of chronic negative energy balance. This is attributed to the poor appetite and the resulting poor food intake. Plasma levels of Ghrelin were found to be elevated in children with PEM in previous studies.[5,10,11] The primary objective of our study was to assess plasma Ghrelin hormone levels in children with severe PEM and to compare it with levels in children with mild to moderate PEM and healthy control population. We hypothesized that in children with severe PEM, the compensatory/adaptive mechanisms could fail and hence plasma Ghrelin levels further be lower in severe PEM in comparison with mild and moderate PEM and healthy control population.

METHODS

A cross sectional observational study was conducted over a one year period at a tertiary care Paediatric hospital in northern India. All consecutive children in the age group between 6 months to 5 years, hospitalised for primary PEM or one of its complications, were enrolled as cases and age matched healthy children presenting to outpatient department with minor acute illness needing vene-puncture for other investigations were taken as controls. PEM was defined and categorised as per WHO classification and growth standards.[12,13]

Children with concomitant chronic systemic illness including cardiac, renal or any other chronic systemic disorder resulting in secondary protein energy malnutrition were excluded from the study. The study population was classified into 3 groups; Group 1 consisting of children with severe PEM (< 70% weight-for-length or below -3Z scores of the median WHO growth standards), Group 2 consisting of children with mild to moderate PEM (weight for height between -2 to -3 Z scores of the median WHO growth standards) and Group 3 consisting of age matched healthy controls.

The study protocol was approved by Institute Ethics Committee before enrolment of subjects. A written informed consent in local language (Hindi and Punjabi) was taken from the primary care-giver before enrolment of their ward in the study.

All children enrolled in the study were subjected to a detailed clinical evaluation giving importance to history of current illness, past illnesses including systemic infections, gastroenteritis and chronic diarrhoea and hospitalizations, dietary history by 24 hour recall, immunization history, development and socioeconomic history. Socioeconomic status of the family was assessed using modified Kuppuswamy socioeconomic scale which included education, occupation and monthly income of the family.[14] Further, the study subjects underwent a thorough clinical examination including anthropometry (weight, length or height, occipito-frontal circumference, mid- upper arm circumference (MUAC) expressed as standard deviations according to WHO growth standards, vitals (heart rate, respiratory rate, temperature, capillary refill time, blood pressure), signs of PEM and micronutrient and vitamin deficiencies.[13]

The haematological and biochemical parameters were recorded for cases at the time of hospitalisation. It included complete blood count with peripheral smear and indices, renal, liver function tests with total protein and serum albumin, serum electrolytes, and random blood sugar and coagulation profile. The fasting blood samples were taken for Ghrelin hormone assay from the cases and controls in the morning before breakfast to avoid diurnal and prandial variations. Samples for Ghrelin assay were collected into vial containing EDTA, plasma was separated by centrifugation and was stored at -80°C until analysis. All the samples were processed in one batch using a human Ghrelin Enzyme Immunoassay (EIA) Kit (Ray Biotech, GA, USA), as per manufacturer's instructions in Endocrinology laboratory of the Institute. Briefly, the samples were processed in a sandwich assay using standards and controls supplied in the kit. The minimum detection limit of the assay was 161 pg/ml and the intra and inter assay coefficient of variability were 10% and 15% respectively.

Statistical analysis

All the clinical and lab data collected was entered in a pre-designed proforma and transferred to the SPSS software. Preliminary analysis was univariate/ descriptive expressed as Means (±SD), Medians with interquartile ranges (IQR), proportions and frequencies. Descriptive statistics were used for baseline variables. Dichotomous /

categorical variables were analysed by Chi square test with continuity correction or Fisher's exact test as applicable. Numerical variables were compared by Student t test or Mann Whitney U test, depending on distribution of data. Comparison between 2 groups was done using Chi square test and among three groups was done using Mann Whitney U test. Due to skewed data for Plasma Ghrelin levels Median with IQR was calculated for the 3 groups. The p value of less than 0.05 was taken as statistically significant.

RESULTS

A total of 78 children (49 boys and 29 girls) including controls, were enrolled in the study with mean age of 26.71±16.47 months. There were 36 children with Severe PEM (Group 1), 23 children with mild-moderate PEM (Group 2) and 19 children in control group (Group 3). Details of demographic and growth parameters of children in the 3 groups are given in Table 1.

Table 1: Demographic and growth parameters of the study population (n = 78).

Parameter	Subdivisions	Group1[a] (n=36)	Group 2[b] (n = 23)	Group 3[c] (n = 19)
Age in months (mean±SD)		22.4±16.0	27.56±17.06	34.45±14.38
WAZ[$] score (mean±SD)		-4.60±1.40	-2.65±0.53	- 0.22±0.12
HAZ[*] score (mean±SD)		-4.17±1.27	-2.51±0.47	- 1.02±0.62
BMI Z score (mean±SD)		-2.67±0.61	-1.34±0.31	+ 0.61±0.23
Serum Albumin (mean±SD) (g/dl)		2.71±1.09	3.03±0.78	not done
Plasma Hb (mean±SD) (g/dl)		8.20±2.87	8.79±3.05	not done
Socioeconomic status [#]	Upper	0	0	1
	Upper middle	0	4	12
	Lower middle	3	5	3
	Upper lower	33	14	3
Immunization status	Complete	19	18	19
	Partial	15	4	0
	Unimmunized	2	1	0
Gender	Male	23	13	13
	Female	13	10	6

[$]Weight for age; [*]Height for age; [#]Modified Kuppuswamy socio-economic scale; [a]Severe PEM; [b]Mild to Moderate PEM; [c]Controls

Table 2: The prevalence of the clinical signs of malnutrition in children with PEM.

Symptom/Sign	Group 1a (n= 36)	Group 2b (n=23)
Pallor	33	17
Pedal edema	15	5
Flaky paint dermatosis	10	7
Flag sign	6	0
Lustreless hair	29	16
Cheliosis	19	8
Angular stomatitis	24	9
Baggypant appearance	20	11
Monkey facies	1	3
Clinical features of Vit. A deficiency	3	4
Clinical features of rickets	13	4

[a]Severe PEM; [b]Mild to moderate PEM

Authors found that educational status of parents, monthly income, socioeconomic status, immunization status were lower in children with PEM as compared to controls. The prevalence of pallor, edema, signs of PEM in form of flag sign, lustreless hair, cheliosis, angular stomatitis, baggy pants appearance and clinical signs of rickets were more prevalent in children with severe PEM (Group 1) as compared to mild-moderate PEM (Group 2) as anticipated, except for monkey facies and signs of Vitamin A deficiency (Table 2) which were more common in mild to moderate PEM group.

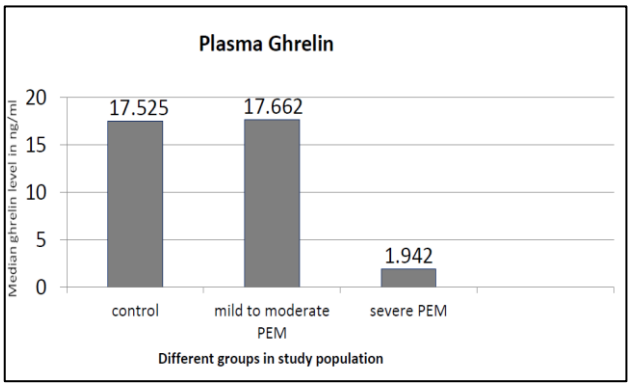

Figure 1: Median plasma Ghrelin levels among PEM groups and healthy controls.

Children with severe PEM had low median plasma Ghrelin levels as compared to healthy controls and mild

to moderate PEM. Median plasma Ghrelin level among severe PEM group was 1.942 ng/ml (IQR: 0.064, 9.506), in mild to moderate PEM was 17.662 ng/ml (IQR: 1.658, 40.129) and among controls was 17.525 ng/ml (IQR: 0.626, 27.361) (Figure 1).

The difference of median plasma Ghrelin level between severe PEM group and mild to moderate PEM was statistically significant (p value = 0.027). However, there was no statistically significant difference in median levels of Ghrelin between mild to moderate PEM group and healthy controls.

DISCUSSION

In present study, it is evident that the median plasma Ghrelin levels were significantly lower in children with severe PEM as compared to children with mild to moderate PEM. The median plasma Ghrelin levels in children with mild to moderate PEM were also slightly higher when compared to healthy controls. The elevated plasma Ghrelin levels in mild to moderate PEM were attributed as an adaptation to the negative energy balance in PEM children. The increase in plasma Ghrelin concentration in mild to moderate PEM is secondary rather than a primary event. This increase of Ghrelin levels will not be able to reverse the deleterious effects of PEM unless nutritional deficiency is corrected. However, plasma Ghrelin levels were significantly reduced in children with severe PEM. We attribute these very low levels of plasma Ghrelin in severe PEM to the failure of the adaptation mechanism and the severe degree of gastric mucosal atrophy (Ghrelin being secreted by gastric mucosal cells) associated with severe PEM as also suggested by Altinkaynak et al.[10]

Most of the previous studies have shown elevated levels of plasma Ghrelin levels in children with PEM and similar results were seen in present study in mild to moderate PEM. In a study by El-Hodhod et al, 30 infants with PEM with their age ranging between 5 and 20 months were enrolled.[5] The children were divided in to four groups namely marasmus, kwashiorkor, marasmic kwashiorkor and controls on the basis of the classification suggested by Gernaat and Voorhoeve, based on the presence or absence of edema and z-score of weight for height (as a measure of wasting).[15] Children were recruited from the inpatient department and nutrition clinic of their hospital as done in present study. They used radioimmunoassay (RIA) in contrary to enzyme immunoassay for plasma Ghrelin measurement in present study. In their study, the mean serum Ghrelin levels were significantly higher among marasmic (6062.08±921.85 pg/ml), kwashiorkor (5394.5±1000.78 pg/ml) and marasmic kwashiorkor (5591.25±1057.86 pg/ml) patients compared to controls (1969.27±610.76 pg/ml). As per the authors, this was attributed to the negative energy balance; a constant factor which exists in the PEM patients. They concluded that plasma Ghrelin levels were elevated in PEM and this elevation was an adapting consequence of the malnutrition rather than a primary event. There was no significant difference in the mean serum Ghrelin level between males and females. Present study also showed similar results; there was no significant difference in Ghrelin level between male and female children (p = 0.199). However, in present study along with slightly increased Ghrelin hormone levels in mild to moderate PEM, there was significant fall in Ghrelin levels in severe PEM group. A study done by Bellone et al, also showed that Ghrelin levels were independent of gender and pubertal status, as there are no differences between males and females in the nutritional status or energy balance.[16]

In the study conducted by Altinkaynak et al, serum Ghrelin level was higher in patients with PEM, especially in those with marasmus, compared to healthy children.[10] Mean serum Ghrelin level of children with moderate malnutrition was higher than that of children with severe malnutrition. The possible explanation given for higher Ghrelin levels in moderate PEM was mucosal atrophy usually found in severe malnutrition. Similar results were seen in present study with more marked and significant difference in moderate and severe PEM in present study.

In a study conducted by Mohsen et al comparing marasmic infants with normal controls, plasma Ghrelin was significantly higher in marasmic infants when compared to the control group.[11] The negative energy balance in marasmic infants was attributed as the cause for the higher levels of Ghrelin in marasmic children.

Table 3: Variability of plasma total Ghrelin hormone levels (mean±SD) reported in literature in children with malnutrition and healthy controls.

Author	Analytical assay	Control (no of subjects)	Severe malnutrition (no of subjects)
El-Hodhod et al	RIA[$]	1969.27±610.76 pg/ml (15)	6062.08± 921.85 pg/ml (30)
Altinkaynak et al	RIA[$]	107.7±40.1 pg/ml (10)	98.4±74.3 pg/ml (18)
Mohsen et al	ELISA[#]	19.92±8.26pg/ml (27)	407.24±166.05 pg/ml (26)
Tannenbaum et al	RIA[$]	1812±125 pg/ml (5)	2469± 149 pg/ml (9)
Wali et al	ELISA[#]	1484.1±643.2 pg/ml (33)	1241.8±743.4 (13)
Index study median (IQR)	EIA*	17520 pg/ml (620, 27360) (19)	1940 pg/ml (60, 9500) (36)

[$]Radio-immunoassay; [#]Enzyme-linked immunosorbent assay; *Enzyme immunoassay

Interestingly, the total Ghrelin hormone levels in previously conducted studies in children with primary PEM and healthy controls is highly variable as summarised in Table 3. In studies by El-Hodhod et al, Altinkaynak et al, and Tannenbaum et al, although the method of Ghrelin estimation has been radio-immunoassay in all, the mean values of Ghrelin have shown 10-100 times variability across the studies.[5,10,17] Similarly, studies by Mohsen et al and Wali et al have shown nearly 100 times variability in mean total Ghrelin levels despite using ELISA for the assay.[11,18] All these studies have estimated Ghrelin in the morning hours in fasting state, except in study by Tannenbaum et al where blood sample was taken between 0900 to 1200 hours without consideration to meal time.[17] Present study has shown even more marked difference (nearly 100-1000 times higher) in Ghrelin levels of healthy controls and PEM when compared to above studies. This could be related to the different assay (EIA) used in present study. Also, we believe that our patients were more acutely sick compared to other studies where estimation is done on either out-patient or less sick children who were in nutritional rehabilitation stage for PEM. Further, like other hormones in the body, the Ghrelin hormone is a dynamic hormone which could vary significantly in various physiological states including fasting, acute or chronic stress, time of the day etc.[19]

Present study has given further insight to the Ghrelin hormone status in children with severe PEM. There may be a possible therapeutic role of Ghrelin analogues in stimulating appetite as proven by studies in other conditions.[20-22] Further studies are required for establishing therapeutic role of Ghrelin supplementation in PEM.

CONCLUSION

Authors conclude that the median plasma levels of Ghrelin were significantly lower in children with severe PEM when compared to children with mild to moderate PEM and healthy controls. Further, the median plasma Ghrelin levels in children with mild to moderate PEM were higher than healthy controls. The elevated plasma Ghrelin levels in children with mild to moderate PEM is an adaptation to the negative energy balance which exists in children with PEM and low plasma Ghrelin levels in severe PEM could be due to loss of this physiological adaptation.

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