Websites:
http://www.sciencepub.net/nature
http://www.sciencepub.net

Emails:
naturesciencej@gmail.com
editor@sciencepub.net

Nature and Science

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Serum Ghrelin Level in Children with Growth Hormone Deficiency and Those with Idiopathic Short Stature

Taha O[1], EL Rifaey S[1], Nosair N[2], El Hawary A[3], Erfan A[1]

[1]Department of Pediatrics, Faculty of Medicine, Tanta University, Egypt
[2]Department of Clinical Pathology, Faculty of Medicine, Kafr El-Shaikh University, Egypt
[3]Department of Pediatrics, Faculty of Medicine, Mansoura University, Egypt

Abstract: Short stature is defined as height below 3rd centile or less than two standard deviations (SDs) below the median height for that age and sex according to the population standard. The role of endogenous ghrelin in the growth process of children is unclear. OBJECTIVES: to assess serum ghrelin levels in children with short stature who were growth hormone deficient (GHD) and idiopathic short stature (ISS). PATIENTS AND METHODS: This study was carried out on 60 children with short stature and 30 controls. The children were divided into two groups: Group A: 30 patient; Children with short stature due to GHD. Group B: 30 patients; Children with idiopathic short stature. For all patients and controls, the following were done: Karyotyping for female patients, thyroid function, coeliac disease screening, GH provocation test by insulin or clonidine, serum IGF1, fasting plasma ghrelin and bone age. RESULTS: Fasting ghrelin level was highly elevated in GHD children and ghrelin level in ISS was higher than in Controls. IGF-1was very low in GHD children and no significant deference between Idiopathic short stature and controls. Ghrelin showed an inverse correlation with IGF-1. CONCLUSIONS: The presence of features increased ghrelin secretion in children of idiopathic short stature so ghrelin may play role in pathogenesis of ISS.
[Taha O, EL Rifaey S, Nosair N, El Hawary A, Erfan A. **Serum Ghrelin Level in Children with Growth Hormone Deficiency and Those with Idiopathic Short Stature.** *Nat Sci* 2020;18(4):24-28]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 5. doi:10.7537/marsnsj180420.05.

Key Words: Idiopathic short stature _ Ghrelin _ Growth hormone secretagogue receptor_ Insulin growth factor -1

1. Introduction

Short stature is defined as height below 3rd centile or less than two standard deviations (SDs) below the median height for that age and sex according to the population standard [(1)]. Growth hormone deficiency (GHD) is the most common cause in comparison to other causes of short stature [(2)].

Growth hormone (GH) is the key mediator of childhood growth, and acts primarily through stimulation of insulin-like Growth Factor-1(IGF-I) production and secretion [(3)]. It is recommended that assessment of short children for finding GHD started with direct and indirect methods. Direct methods measure serum GH after stimulation and indirect methods assess action of GH including IGF1, insulin-like Growth Factor-II (IGF-II) and insulin growth factor binding protein-3 (IGF-BP3) which are more reproducible than direct assessment of GH [(2)].

Idiopathic short stature (ISS) is defined as a condition in which the height of an individual is more than 2 SD score (SDS) below the corresponding mean height for a given age, sex, and population group without evidence of systemic, endocrine, nutritional, or chromosomal abnormalities [(4,5)].

Ghrelin is a 28 amino-residue peptide. Ghrelin is a hormone produced mainly by the endocrine P/D 1 cells of the stomach submucosa [(6)]. Ghrelin is the endogenous ligand which is also expressed in the hypothalamus, pituitary, kidney and pancreas. Among its several endocrine activities, ghrelin also exerts orexigenic and adipogenic effects by modulating the expression of orexigenic and anorexigenic neuropeptides in the hypothalamus. Ghrelin was initially identified as the endogenous ligand of the growth hormone secretagogue (GHS), possessing a strong growth hormone-releasing activity [(7)]. In humans, circulating ghrelin consists of des-acyl ghrelin, acyl ghrelin, and C- ghrelin and Obestatin. While much of the ghrelin found in the blood stream occurs in the non-acylated form, only the acylated form is active and able to bind to GHS-R1a to stimulate GH secretion [(8,9)]. The exact role of ghrelin in the growth process of children is unclear. Its effect in control of GH secretion has not yet been clarified. So, many studies have been conducted in short-stature children, but there is variability in the results [(10)].

2. Subjects and Methods

Patients

Candidates for the study were selected from patients who consulted for GHD group (30 patients) (height $\leq$ –2.5 standard deviation scores (SDS), the

maximum GH level below 7ng/dl in GH provocation test) and ISS group (30 patients) [height ≤ –2.5 SDS, the maximum GH level above 7ng/dl] in the pediatric endocrine clinic at the Endocrinology Unit of Pediatric Department, Faculty of Medicine at Tanta University and Mansoura University. Selection criteria included prepubertal stage, short stature, normal birth weight, delayed bone age. We excluded patients with other identifiable endocrine, systemic, genetic, or psychosocial causes of short stature, skeletal dysplasia and familial short stature. Specifically, the children had normal body proportions and normal CBC, ESR, blood chemistries, plasma electrolytes, renal and liver function tests, total IgA, antiendomysial and anti-tissue transglutaminase antibodies [(11)], urinalysis, stool exam, thyroid function and karyotype (girls) [(12)]. This study was approved by ethical committee of research center in Tanta University Hospital. Informed consent was obtained from at least one parent of eachpatient.

Height was measured 10 times using Stadiometer and the average was used for further analysis. Weight was measured using a manual scale with a 10- gram gradation. Data was transformed into SDS. Bone age was determined by the method of Greulich and Pyle [(13)]. In all participants a fasting blood sample (between 8 and 9 a.m.) was obtained for ghrelin determination.

Control Subjects

These children were part of a cohort of additional ongoing studies for obtaining normal childhood laboratory values at Faculty of Medicine at Tanta University and Mansoura University. They were invited to participate at their local schools through a letter addressed to their parents. All these children underwent a complete physical examination by a pediatric endocrinologist in order to rule out abnormalities in growth and development. Mean height was -0.02 ± 0.35 SDS with a target height –1 SDS. A fasting blood sample (between 8 and 9 a.m.) was obtained for IGF-I and ghrelin determination.

Hormone Assays

Serum insulin-like growth factor I (IGF-1) was measured by a double- antibody sandwich enzyme-linked immunosorbent assay (ELISA). IGF-1 was added to monoclonal antibody enzyme well which is pre-coated with human IGF-1 monoclonal antibody, incubation; then, IGF-1 antibodies labeled with biotin was added, and combined with Streptavidin-horseradish peroxidase (HRP) to form immune complex; then incubation and washing again were carried to remove the uncombined enzyme. Then Chromogen Solution A and B were added, the color of the liquid changed into the blue, and at the effect of acid, the color finally became yellow. The chroma of color and the concentration of the Human Substance IGF-1 of sample were positively correlated. This assay has a sensitivity of 0.153ng/ml,

Total ghrelin levels were measured using ELISA. Ghrelin was added to monoclonal antibody enzyme well which was pre-coated with human ghrelin monoclonal antibody, incubation; then, ghrelin antibodies labeled with biotin was added, and combined with Streptavidin-HRP to form immune complex; then incubation and washing again were carried out to remove the uncombined enzyme. Then Chromogen Solution A and B were added, the color of the liquid changed into the blue, and at the effect of acid, the color finally became yellow. The chroma of color and the concentration of the Human Substance ghrelin of sample were positively correlated. The assay sensitivity was 28.225pg/ml.

Statistical Analysis

The collected data were organized, tabulated and statistically analyzed using the mean, standard deviation, unpaired T test, paired t test, and one way ANOVA (analysis of variance) by SPSS version 22 (Statistical Package for Social Studies). Comparison between patients and controls was performed using unpaired t-test. Comparison of the mean values between the studied groups of patients was done using analysis of variance (F). Pearson's correlation coefficient was used to test association between two variables. The cutoff value for significance was adopted at $p< 0.05$.

3. Results

Demographic data of patients and controls are shown in table 1.

Table 2 showed that there was significantly higher level of fasting ghrelin in GHD in compared to ISS. There was also significant higher level of fasting ghrelin in both GHD and ISS compared to control. There was significantly lower level of IGF-1 in GHD compared to ISS and controls. IGF-1 was not significantly different in both ISS and controls.

There was significant lower of ghrelin in pubertal group in compared to prepubertal group (t: 2.245 p value: 0.029).

Figure 1 showed significant negative correlations between Ghrelin with IGF-1, figure 2 showed significant negative correlations between Ghrelin with BMI, figure 3 showed significant negative correlations between Ghrelin with height and figure 4showed significant negative correlations between Ghrelin with age in all GHD and ISS patients.

Table (1): Demographic data of patients and controls

		Groups			ANOVA	TUKEY'S Test		
		GHD group (N=30)	ISS group (N=30)	Controls (N=30)	P-value	G & I	G & C	I & C
Age (Years)	Mean ±SD	7.32 ± 1.56	7.55 ± 1.65	7.65 ±1.59	0.719			
HT/SDS	Mean ±SD	-3.02 ± 0.42	-2.84 ± 0.61	-0.02 ± 0.35	<0.001*	0.646	<0.001*	<0.001*
WT/SDS	Mean ±SD	-2.63 ± 0.41	-2.36 ± 0.45	0.71 ± 0.60	<0.001*	0.100	<0.001*	<0.001*
BMI/SDS	Mean ±SD	-0.68 ± 1.92	-0.59 ± 1.87	-0.10 ± 1.06	0.359			
US/LS ratio	Mean ±SD	1.07 ± 0.06	1.07 ± 0.06	1.06 ± 0.06	0.825			
Bone age (Years)	Mean ±SD	4.97 ± 1.55	5.20 ± 1.62	7.64 ± 1.59	<0.001*	0.838	<0.001*	<0.001*
Birth weight (Kg)	Mean ±SD	3.06 ± 0.35	3.03 ± 0.35	3.07 ± 0.34	0.918			

Table (2): Ghrelin and IGF-1 in patients and controls

		Groups			ANOVA	TUKEY'S Test		
		GHD group (N=30)	ISS group (N=30)	Controls (N=30)	P-value	G & I	G & C	I & C
Ghrelin (pg/ml)	Mean ±SD	1855.07 ± 276.68	1593.80 ± 443.37	878.90 ± 175.62	<0.001*	0.006*	<0.001*	<0.001*
IGF-1 (ng/ml)	Mean ±SD	46.35 ± 22.71	149.72± 42.35	170.73 ± 61.38	<0.001*	<0.001*	<0.001*	0.242

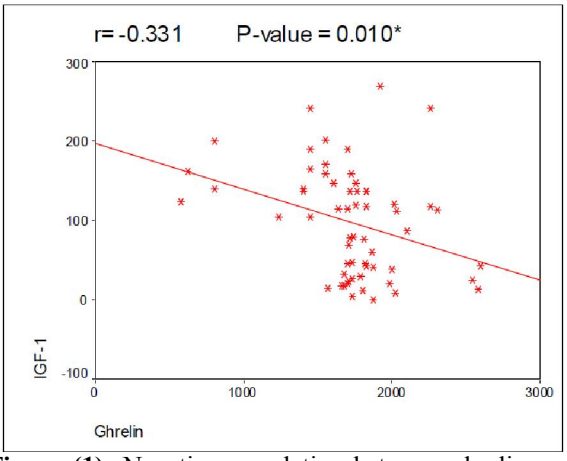

Figure (1): Negative correlation between ghrelin and IGF-1 in all GHD and ISS patients.

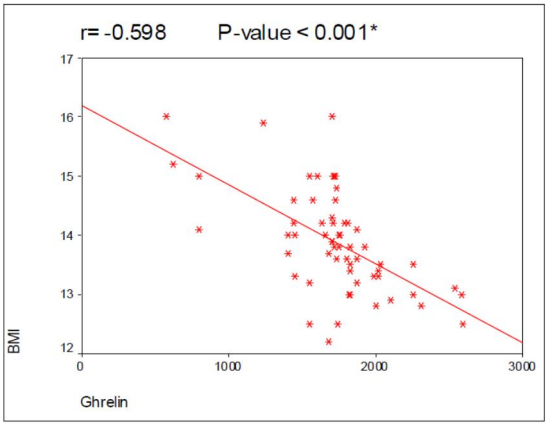

Figure (2): Negative correlation between ghrelin and BMI in all GHD and ISS patients.

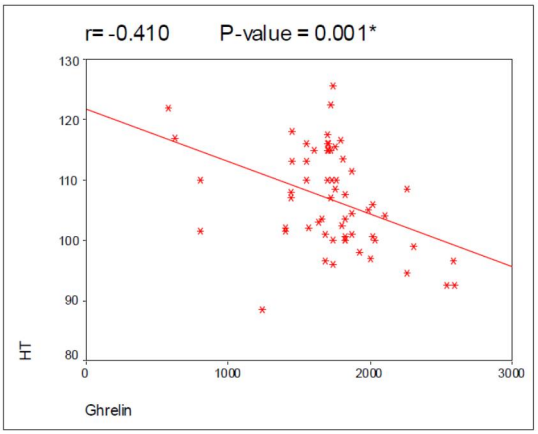

Figure (3): Negative correlation between ghrelin and HT in all GHD and ISS patients.

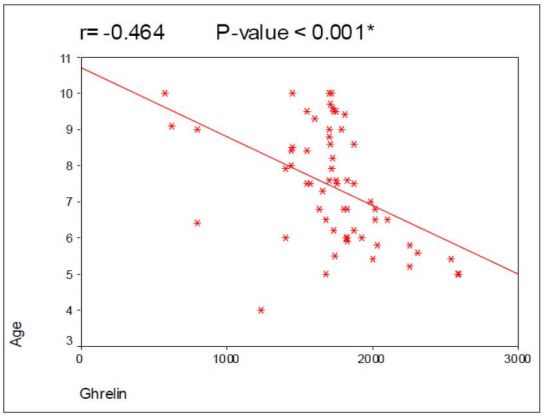

Figure (4): Negative correlation between ghrelin and age in all GHD and ISS patients.

4. Discussion

According to our study, IGF-1 was significantly low in GHD children in comparison to both ISS group and controls and no significant deference between ISS and controls.

Our data are matched with **Azar et al., (2015)** who revealed that IGF-1 was also not significantly different between control with 25 prepubertal and pubertal ISS [14].

Stawerska et al., (2017) showed low IGF-1 in GHD compared to controls. GHD patients were 26 children aged 5.7–15.3 yrs. [7].

In contrary with our study**, Prodam et al., (2012) and Barrett et al., (2014)** observed that IGF-1 was not significantly different between prepubertal GHD and prepubertal growth hormone sufficiency (GHS) groups. The former study had 10 GHD aged 10.7 ± 0.9 years and 10 GHS children aged 10.3 ± 0.6 years during an arginine (ARG) infusion test. The other one studied 11 GHD and 56 GHS prepubertal children of mean age 10.21±2.56 years during provocation test with arginine and clonidine**.** The variations of the data may be explained by that the use of arginine and clonidine as the primary GH secretagogues are generally regarded as weak secretagogues and the effect recorded after continuous infusion of ARG is weaker than acute bolus or oral loads. The variations of the data may also be attributed to the mean of age of patients which was older and small group size [15,16].

According to our study, fasting ghrelin level was significant higher in GHD children in comparison to ISS and controls. Its level in ISS was significant high in comparison to controls.

Our data are also matched with **Stawerska et al., (2012)** who observed that Ghrelin concentrations in GHD was significantly higher than in ISS and in Controls. Ghrelin level in ISS is higher than in controls without significant difference. This study included 147 children aged 3.7–16.8 years with short stature. One possible explanation is the presence of some oligosymptomatic GI tract disease (i.e., helicobacter infection), whose prevalence in children with ISS is higher than that in normal population. Oligosymptomatic GI tract diseases stimulate ghrelin secretion [8].

Iñiguez et al., (2011) revealed that 41 prepubertal ISS patients exhibited a higher level of ghrelin compared to controls. This study had 10 patients with ISS who had ghrelin levels greater than +2 SDS compared to controls [17].

In contrary with our study**, Azar et al., (2015)** found that total ghrelin (TG) levels were not significantly different between the control and 25 children and adolescent ISS children. This study included wide range of age which was not included in our study [14].

Prodam et al., (2012) observed that no significant difference between 10 GHD aged 10.7 ± 0.9 years and 10 GHS prepubertal children aged 10.3±0.6 years groups in basal and during arginine test total ghrelin. The study included older mean of age, small sample size and time of measuring ghrelin basal and during arginine test which were different from our study. The use of arginine as the primary GH secretagogues is also generally regarded as weak secretagogues which can also affect the results [15].

According to our study, Ghrelin showed a significant negative correlation with IGF-1 in whole patients.

Our study is in agreement with **Cambrian et al., (2006)** who reported that Ghrelin was negatively correlated with IGF-1in short stature patients (CDGP and FSS) with controls. **Camurdan et al., (2006)** was conducted on 17 children with CDGP aged 6.5-15years, 19 children with FSS aged 7-15.2 years and 11 controls aged 6.8-14.8 years. The negative correlation between ghrelin and IGF-1 may be related to the stimulatory effects of low IGF-1 on ghrelin [18].

In contrary with our study, **Iñiguez et al., (2011)** observed a positive correlation between IGF-I SDS and ghrelin level in females of controls and of 41 prepubertal ISS patients. When females of two groups were studied separately, this correlation was maintained only for ISS females. The study chose females only and this may cause the variation of data [17].

According to our study, Ghrelin showed negative correlation with age, height, and BMI in whole patients. Our study is in agreement with **Stawerska et al., (2017)** revealed that ghrelin was negatively correlated with height SDS in 116 prepubertal and pubertal children ISS [19].

Stawerska et al., (2012) demonstrated that a significant negative correlation was observed between ghrelin concentration and CA, and BMI SDS for HA. No significant correlation was found between ghrelin concentration and BMI SDS for CA being an evidence for the fact that higher ghrelin concentrations are observed in slimmer children, regardless of their chronological age during the clonidine stimulating test or during the glucagon stimulating test in 15 GHD, 116 ISS pubertal and prepubertal children and 19 controls [8].

In contrary with our study**, Azar et al. (2015)** found that there were not significant correlations between acylated and total ghrelin and auxological parameters in 25 prepubertal and pubertal ISS group and 31 controls. Type of measured ghrelin may be the cause of difference [14].

According to our study, ghrelin was significantly lower in pubertal group compared to prepubertal group.

Our study is in agreement with **Stawerska et al., (2012)** who demonstrated that a comparison of ghrelin concentrations in older and younger children showed statistically higher ghrelin levels in younger than in older children in GHD, NSD, ISS and controls. the younger group: CA below 11 years, without pubertal features, BA below 9 years in boys and below 8 years in girls; the older group: CA more than 11 years and/or with pubertal features and/or BA more than 9 years in boys and more than 8 years in girls [(8)].

There are some limitations to our study. First, our study was a cross-sectional study. Second, the number of subjects in our study was relatively small. Third, we measured the total circulating ghrelin, including acyl and des acyl ghrelin, but not the active form of ghrelin.

Conclusions

From this study, we concluded that:

The presence of features increased ghrelin secretion in children of idiopathic short stature so ghrelin may play role in pathogenesis of ISS.

References

1. Nath P, Kumar J, Rahman S and Rai M. Short Stature: Evaluation and Management. In: Muruganathan A, Geetha T, Kamath S, Munjal Y, Shah S, Joshi S, Banerjee S, Kumar A, Avudaiappan K, Palaniappen V, Nadkar M, Punja M, Gupta S, Mathur G, Viswanathan V, Moses I (Eds). Medicine Update 2013. 1st edition. The Association of Physicians of India. 2013; Ch.137:626-631.
2. Whatmore A, Hall C, Jones J, Westwood M and Clayton P. Ghrelin concentrations in healthy children and adolescents. *Clin Endocrinol (Oxf)* 2003; 59(5):649-654.
3. Hawkes C and Grimberg A. Measuring growth hormone and insulin-like growth factor-I in infants: what is normal? *Pediatr Endocrinol Rev* 2013;11(2):126-146.
4. Graber E and Rapaport R. Growth and growth disorders in children and adolescents. *Pediatr Ann*2012;41(4):1-9.
5. Haghshenas Z, Sotoudeh K, Karamifar H, Karamizadeh Z and Amirhakimi G. The role of insulin like growth factor (IGF)-1 and IGF-binding protein-3 in diagnosis of Growth Hormone Deficiency in short stature children. *Indian J Pediatr* 2009;76(7):699-703.
6. Ismail NA, Metwaly NS, El-Moguy FA, Hafez MH, Abd El Dayem SM and Farid TM. Growth response of Egyptian children with idiopathic short stature during four years of growth hormone therapy. *Indian J Hum Genet* 2011; 17(3):218–225.
7. Stawerska R, Smyczyoska J, Hilczer Mand Lewioski A. Relationship between IGF-I Concentration and Metabolic Profile in Children with Growth Hormone Deficiency: The Influence of Children's Nutritional State as well as the Ghrelin, Leptin, Adiponectin, and Resistin Serum Concentrations. *Int J Endocrinol* 2017; 2017:1-9.
8. Stawerska R, Smyczyoska J, Czkwianianc E, Hilczer M and Lewioski A. High concentration of ghrelin in children with growth hormone deficiency and neurosecretory dysfunction. *Neuro Endocrinol Lett* 2012;33(3):331-339.
9. Stengel A and Taché Y. Ghrelin – A Pleiotropic Hormone Secreted from Endocrine X/A-Like Cells of the Stomach. *Front Neurosis* 2012; 6:24.
10. Şen T, Şimşek D, Darcan S, and Çoke M. Ghrelin Levels in Children with Constitutional Delay of Growth and Puberty. *J Clin Res Pediatr Endocrinol* 2010; 2(3): 117– 121.
11. Dale JC, Homburger HA, Masoner DE and Murray JA. Update on celiac disease: New standards and new tests. *Communique* 2008;33:1-9.
12. Lawce HJ and Brown MG. Chromosome stains. In: Arsham MS, Barch MJ and Lawce HJ (Eds). The ACT Cytogenetics Laboratory Manual 4th edition. The Association of Cytogenetic Technologists. Raven Press. New York. 2017; Ch.2-5:58.
13. Gilsanz V and Ratib O. Hand Bone Age. A Digital Atlas of Skeletal Maturity. 2nd edition. Springer-Verlag. Berlin. Heidelberg.2012:1-95.
14. Azar M, Latifi M, Ilbeygi D, Larijani B and Nourbakhsh M. Total and acylated Ghrelin levels in children and adolescents with idiopathic short stature and poor appetite. *Acta Endocrinologica (Buc)* 2015;9(3):401-406.
15. Prodam F, Genoni G, Bellone S, Longhi S, Agarla V, Bona G and Radetti G. Effect of Arginine Infusion on Ghrelin Secretion in Growth Hormone- Sufficient and GH- Deficient Children. *Int J Endocrinol Metab* 2012;10(2):470-474.
16. Barrett J, Maranda L and Nwosu B. The relationship between subnormal peak-stimulated growth hormone levels and auxological characteristics in obese children. *Front Endocrinol (Lausanne)* 2014;5:35.
17. Iñiguez G, Román R, a Ronald Youlton R, Cassorla F and Mericq V. Ghrelin Plasma Levels in Patients with Idiopathic Short Stature. *Horm Res Paediatr* 2011; 75(2):94–100.
18. Camurdan M, Bideci A, Demirel F and Cinaz P. Serum ghrelin, IGF-I and IGFBP-3 levels in children with normal variant short stature. *Endocr J* 2006; 53(4):479- 84.
19. Stawerska R, Czkwianianc E, Smyczyn´ska J, Hilczer M and Lewin´ski A. Nutritional Status in Short Stature Children Is Related to Both Ghrelin and Insulin-like Growth Factor I Concentrations. *J Pediatr Gastroenterol Nutr* 2017;64(5):812-817.

2/25/2020