Egypt J Pediatr Allergy Immunol 2011;9(2):63-70.

Original article

Plasma ghrelin level in children with type 1 diabetes mellitus

Background: *Type-1 diabetes mellitus(T1DM) is the commonest endocrine-metabolic disease in childhood. Ghrelin is a 28-amino-acid peptide hormone secreted predominantly by P/D1 cells lining the fundus of the stomach and epsilon cells of the pancreas that stimulates appetite with lesser amounts secreted by other cells. It is not clear which factors are involved in the regulation of ghrelin secretion in children with T1DM.* ***Objective****: This study aimed to estimate the level of pre-prandial plasma ghrelin level in children with T1DM and to clarify the relationship between its level and some parameters that may affect it as BMI, serum glucose and HbA1C levels and the effect of insulin therapy on its level.* ***Methods:*** *This study included 88 children, 66 diabetic children with type-1 diabetes mellitus(22 new-onset diagnosed diabetic children, 22 good glycemic controlled diabetic children on regular insulin therapy and 22 poor glycemic controlled diabetic children on insulin therapy) and 22 healthy controls. Diabetic children selected from the Diabetic Clinic and Inpatient Pediatric department, Minia University hospital from April 2009 to March 2010. Their ages ranged from 4 to 10 years with a mean 8.5 ± 1.53 years. All children were subjected to history taking, clinical examination, anthropometric measurements and laboratory investigations included: Fasting and two hours post-prandial blood glucose, HbA1C , liver and renal function tests and pre-prandial plasma ghrelin level using enzyme linked immunosorbant assay (ELISA).* ***Results:*** *Pre-prandial plasma ghrelin levels were significantly higher in diabetic children than controls(24.4±21.4 & 9.8±3.6 pg/ml respectively, p value=0.002).Both new-onset and poorly controlled diabetic groups were significantly higher in plasma ghrelin levels (37.03±24.2 & 25.1±19.5 pg/ml, p value=0.001 & 0.001 respectively),but no significant difference between good glycemic controlled diabetic group and controls(11.09±9.6 & 9.8±3.6 pg/ml respectively, p value=0.5). Significant negative correlations were found between ghrelin level and weight , weight on centile, BMI and BMI on centile, ,fasting and 2 hours post prandial glucose levels in all diabetic children. No significant difference between males and females as regards ghrelin level was present.* ***Conclusions:*** *Children with T1DM had significantly higher levels of pre-prandial plasma ghrelin level. Its level increased in both of new-onset and poorly controlled diabetic children. Significant negative correlations between pre-prandial ghrelin level and weight, BMI, fasting and 2-hours post-prandial levels were present. Insulin therapy plays an important role in normalizing plasma ghrelin level in good glycemic controlled T1DM children. No significant correlation between ghrelin and HbA1C was present.*

Keywords: T1-DM, Ghrelin, Children, Glucose, Insulin, HbA1C

Laila El-Morsi Aboul-Fotoh, Abd El-Azeem M. El-Mazary, Ahmed M. Emara, Ashraf M. Osman*

Departments of Pediatrics and Clinical Pathology, Faculty of Medicine, Minia University, Egypt.*

Correspondence:
Abdel-Azeem El-Mazary.
Pediatric department, Faculty of Medicine, Minia University, Egypt
E-mail: *abdelazeemhemed @yahoo.com*

INTRODUCTION

Type-1 diabetes mellitus (T1DM) is the commonest endocrine-metabolic disease in childhood[1]. Incidence of T1DM in Egypt in children less than 15 years ranged from 5% to 9.99%[2,3]. Ghrelin is a 28-amino-acid peptide hormone that is secreted predominantly by P/D1 cells lining the fundus of the human stomach and epsilon cells of the pancreas that stimulates appetite[4], with lesser amounts secreted by other cells as in the hypothalamic arcuate nucleus, where it stimulates

the secretion of growth hormone from the anterior pituitary gland[5].

Aim of the study: Measurement of pre-prandial plasma ghrelin level in children with T1DM and to clarify the relationship between its level and some parameters that may affect it as body mass index (BMI), serum glucose and glycosylated hemoglobin (HbA1C) levels and the effect of insulin therapy on its level.

METHODS

This study included 88 children. Sixty-six children with T1DM using conventional criteria as classic clinical picture (of polyuria, polydipsia and weight loss) and blood sugar>200mg/dl and positive islet cell antibodies(ICA) and positive glutamic acid decarboxylase (GAD) antibodies and 22 apparently healthy children serving as controls. Diabetic children were selected from the Diabetes Clinic and Inpatient Pediatric Department, Minia University Hospital during the period from April 2009 to March 2010.

The study population was grouped into:

Group A: included 22 children with new onset diabetes mellitus that diagnosed according to criteria adapted by the American Diabetes Association 2007[6]. They were 11 males and 11 females, aged from 7 to 10 years with a mean 9.04 ± 1.04 years.

Group B: included 22 children with good glycemic controlled diabetes mellitus (HbA1c less than 8%)[6] on insulin therapy. They were 11 males and 11 females, aged from 7 to 10 years with a mean of 9.2 ± 1.0 years.

Group C: included 22 children with poor glycemic controlled diabetic patients (HbA1c more than 8%)[6] on insulin therapy. They were 13 males and 9 females, aged from 6 to 10 years with a mean of 8.7 ± 1.6 years.

Group D: included 22 apparently normal healthy children with age and sex matched serving as control group. They were 12 males and 10 females, aged from 7 to 10 years with a mean of 9.1 ± 1.1 years. This prospective study was approved by Minia University Council and Faculty of Medicine Council and written consents were taken from children fathers or care takers before study .

Exclusion criteria: Comatose children, children with diabetic ketoacidosis, children with type-II diabetes mellitus and children with transient hyperglycemia with acute concurrent illness.

All children after careful history taking, thorough complete clinical examination underwent the following laboratory investigations: Five mls of venous blood withdrawn under complete aseptic conditions, two ml of them collected onto 500µ aprotinin and EDTA (1.2 mg/ml blood) and centrifuged. The obtained plasma was separated then stored at -50˚C till the time of assay of the quantification plasma levels of active ghrelin. Pre-prandial plasma ghrelin level was assayed using enzyme linked immunosorbant assay (ELISA)[7] which is based on a double-antibody sandwich technique[8]. Another three ml of blood were used for HbA1C measurement using resin column chromatography[9], fasting and two-hour post-prandial blood glucose levels, liver function tests, renal function tests and serum cholesterol and triglyceride using konelab (an automated clinical chemistry).

Statistical analysis:

Values are given as means ± SD, range, or as the number of subjects and proportions. The student t test was used for group comparisons of normally distributed variables, and the Mann-Whitney U test and Wilcoxon signed-rank test were used for comparisons of variables with skewed distribution. The chi square test was used to compare proportions. Correlation coefficients were used to describe associations between variables, and multiple regression analysis was used to detect any relationships between the variables. $P < 0.05$ was considered significant. Analyses were performed using the SPSS software package (SPSS V 8.0 for Windows).

RESULTS

Multiple regression analysis of factors affecting the ghrelin concentration among the studied groups revealed that the most important factors affecting ghrelin concentration were FBG followed by 2h post-prandial glucose, weight, BMI,……., while the least important factor was the sex.

Comparison between the levels of urea, creatinine, ALT, AST and serum albumin between each diabetic group and controls revealed insignificant differences, also comparison between the levels ghrelin levels between males and females in various groups revealed insignificant differences.

Table 1. Comparison between cases and controls as regards demographic, clinical and laboratory data.

Variables		Cases (n=66)	Control (n=22)	p-value
Age (years)	Range Mean± SD	6-10 9±1.2	7-10 9.1±1.1	0.6
Sex No (%)	Male Female	35(53%) 31(47%)	12(54.5%) 10(45.5%)	0.5
Weight on centile	Range Mean± SD	2-80 32.6±21.1	5-90 50.6±16.9	0.001**
Height on centile	Range Mean± SD	4-80 44.6 ±17.8	20-60 45±8.7	0.8
BMI on centile	Range Mean± SD	2-85 18.9±17.7	10-80 42.5±22.1	0.001**
FBG (mg/dl)	Range Mean± SD	75-360 195.2±79.8	74-106 87.9±9.7	0.001**
2 hours post prandial glucose (mg/dl)	Range Mean± SD	137-460 275.8±84.05	132-190 165.5±16.4	0.001**
HbA1c %	Range Mean± SD	3-18 8.6±3.3	1.9-7.8 4.4±1.6	0.001**
Ghrelin (ng/dl)	Range Mean± SD	1-86 24.4±21.4	5.2-19 9.8±3.6	0.002**
Total cholesterol (mg/dl)	Range Mean± SD	124-294 171.3±28.4	97-239 145.09±33.06	0.001**
Triglycerides (mg/dl)	Range Mean± SD	63-253 151.5±42.4	52-217 119.5±40.8	0.001**

*=significant **= highly significant.

Table 2. Comparison between cases subgroups as regards demographic, clinical and laboratory data.

Variables		New onset DM-1	Good glycemic control	Poor glycemic control	P value
Age (years)	Range Mean± SD	7-10 9.04±1.04	7-10 9.2±1.006	6-10 8.7±1.6	0.5
Sex No (%)	Male Female	11 (50%) 11 (50%)	11 (50%) 11 (50%)	13 (59.1%) 9 (40.9%)	0.7
Weight (kg)	Range Mean± SD	16-30 22.7±3.9	22-35 27.6±3.2	16-34 22.1±5.8	0.001**
Weight on centile	Range Mean± SD	2-45 15.5±11.9	10-80 47.2±15.5	2-75 22.3±21.3	0.001**
Height (Cm)	Range Mean± SD	117-142 131.09±7.5	126-144 133.2±4.6	97-139 126.09±6.9	0.4
Height on centile	Range Mean± SD	4-70 42.4±20.2	30-70 46.5±9.3	5-80 44.9±19	0.5
BMI (Kg/M²)	Range Mean± SD	9.2-15.4 13.1±1.9	12.7-18.9 15.4±1.6	11-12.5 11.6±0.4	0.001**
BMI on centile	Range Mean± SD	2-35 8.5±9.9	5-85 36.8±22.9	2-15 9.3±3.01	0.001**
FBG (mg/dl)	Range Mean± SD	185-320 246.1±35.06	75-124 93.04±17.4	190-360 246.5±41.9	0.001**
2 hour post prandial glucose (mg/dl)	Range Mean± SD	255-460 339.5±49.6	137-200 170.8±16.1	259-411 317.1±39.3	0.001**
HbA1c %	Range Mean± SD	6.1-14.1 9.4±2.4	3-7.7 5.2±1.2	8.4-18 11.2±2.7	0.001**
Total cholesterol (mg/dl)	Range Mean± SD	124-294 177.8±39.2	143-160 151.1±4.6	148-225 184.09±14.9	0.001**
Triglycerides (mg/dl)	Range Mean± SD	83-253 160±39.03	63-240 121.5±39.3	95-247 173±49.4	0.001**

*=significant **= highly significant.

Table 3. Comparison between cases subgroups as regards demographic, clinical and laboratory data.

Variables		Control	New onset DM-1	Good glycemic control	Poor glycemic control	P1 value	P2 value	P3 value
Age (years)	Range Mean± SD	7-10 9.1±1.1	7-10 9.04±1.04	7-10 9.2±1.00	6-10 8.7±1.6	0.7	0.8	0.4
Sex No (%)	Male Female	12(54.5%) 10(45.5%)	11 (50%) 11 (50%)	11 (50%) 11 (50%)	13 (59.1%) 9 (40.9%)	0.5	0.5	0.6
Weight (kg)	Range Mean± SD	21-35 28.2±4.4	16-30 22.7±3.9	22-35 27.6±3.2	16-34 22.1±5.8	0.001**	0.7	0.001**
Weight on centile	Range Mean± SD	5-90 50.6±16.9	2-45 15.5±11.9	10-80 47.2±15.5	2-75 22.3±21.3	0.001**	0.4	0.001**
Height (Cm)	Range Mean± SD	117-141 131±6.2	117-142 131.0±7.5	126-144 133.2±4.6	97-139 126.09±6.9	0.9	0.4	0.4
Height on centile	Range Mean± SD	20-60 45±8.7	4-70 42.4±20.2	30-70 46.5±9.3	5-80 44.9±19	0.5	0.6	0.9
BMI (Kg/M^2)	Range Mean± SD	14.1-17.5 16.1±1.2	9.2-15.4 13.1±1.9	12.7-18.9 15.4±1.6	11-12.5 11.6±0.4	0.001**	0.8	0.001**
BMI on centile	Range Mean± SD	10-80 42.5±22.1	2-35 8.5±9.9	5-85 36.8±22.9	2-15 9.3±3.01	0.001**	0.3	0.001**

*=significant **= highly significant.

P1 Comparison between new onset DM-1 and control groups.

P2 Comparison between good glycemic control and control groups.

P3 Comparison between poor glycemic control and control groups.

Table 4. Comparison between cases subgroups as regards demographic, clinical and laboratory data.

Variables		Control	New onset DM-1	Good glycemic control	Poor glycemic control	P1 value	P2 value	P3 value
FBG (mg/dl)	Range Mean± SD	185-320 246.1±35.1	185-320 246.1±35.1	75-124 93.04±17.4	190-360 246.5±41.9	0.001**	0.2	0.001**
2 h-post prandial glucose (mg/dl)	Male Female	255-460 339.5±49.6	255-460 339.5±49.6	137-200 170.8±16.1	259-411 317.1±39.3	0.001**	0.2	0.001**
HbA1c %	Range Mean± SD	6.1-14.1 9.4±2.4	6.1-14.1 9.4±2.4	3-7.7 5.2±1.2	8.4-18 11.2±2.7	0.001**	0.07	0.001**
Total cholesterol (mg/dl)	Range Mean± SD	124-294 177.8±39.2	124-294 177.8±39.2	143-160 151.1±4.6	148-225 184.09±14.9	0.005**	0.3	0.001**
Triglycerides (mg/dl)	Range Mean± SD	83-253 160±39.03	83-253 160±39.03	63-240 121.5±39.3	95-247 173±49.4	0.001**	0.8	0.001**
Ghrelin (pg/ml)	Range Mean± SD	5.2-19 9.8±3.6	8.6-86 37.03±24.2	1-42 11.09±9.6	8-74 25.1±19.5	0.001**	0.5	0.001**

*=significant **= highly significant.

P1 Comparison between new onset DM-1 and control groups.

P2 Comparison between good glycemic control and control groups.

P3 Comparison between poor glycemic control and control groups.

Table 5. Correlation between ghrelin concentration and some demographic, clinical and laboratory data and in cases.

Variables	Ghrelin concentration r (P)		
	New onset DM-1	Good glycemic control	Poor glycemic control
Age (years)	0.12 (0.5)	0.05 (0.8)	0.09(0.6)
Weight (Kg)	-0.51(0.009**)	-0.44 (0.03*)	-0.57(0.003**)
Weight on centile	-0.47 (0.02*)	-0.46 (0.03*)	-0.53(0.008**)
Height (Cm)	-0.03 (0.8)	0.23 (0.2)	-0.23(0.2)
Height on centile	0.17 (0.4)	0.23 (0.2)	0.12 (0.6)
BMI	-0.47 (0.02*)	-0.45 (0.03*)	-0.58(0.004**)
BMI on centile	-0.48 (0.02*)	-0.41 (0.04*)	-0.47(0.02*)
FBG (mg/dl)	-0.70 (0.001**)	-0.46 (0.02*)	-0.60(0.004**)
2 hour post prandial glucose (mg/dl)	-0.46 (0.02*)	-0.46 (0.03*)	-0.57 (0.005**)
HbA1c %	0.24 (0.2)	0.20 (0.3)	0.30 (0.4)
Total cholesterol (mg/dl)	-0.11 (0.6)	-0.09 (0.6)	0.02(0.9)
Triglycerides (mg/dl)	-0.22(0.3)	0.24 (0.1)	0.12 (0.5)
Urea (mg/dl)	0.24 (0.2)	-0.13 (0.5)	0.06(0.7)
Creatinine (mg/dl)	0.23 (0.2)	-0.09 (0.6)	0.09(0.6)
ALT (u/l)	0.17(0.4)	0.24(0.2)	0.15(0.4)
AST (u/l)	0.21(0.3)	0.02(0.9)	-0.05(0.8)
Serum albumin (g/dl)	-0.19(0.3)	-0.24(0.1)	-0.24(0.1)

DISCUSSION

Ghrelin, a hormone that is produced mainly by the stomach, was identified originally as the endogenous ligand of the growth hormone secretagogue (GHS) receptor[10,11]. Ghrelin might also be synthesized in other organs, where it might have autocrine and/or paracrine effects. GHS receptors are present in tissues other than the hypothalamus and pituitary, which indicates that ghrelin has other effects in addition to stimulating the release of growth hormone[12].

Rindi et al., 2002[13] reported that there is a new cell type in the human pancreas that produces ghrelin. The pancreas also expresses GHS-R[14]; hence, in addition to stimulating GH secretion[15], controlling food intake, fat utilization, and fat synthesis[16-18], ghrelin may play an important role in glucose and insulin metabolism.

In the present study, both of weight, weight on centile, BMI and BMI on centile were significantly lower in newly diagnosed and poorly controlled diabetic children than controls. On the contrary, we found an increase in the weight and BMI of the good glycemic group to be almost near that of controls. In spite of orixigenic effect of ghrelin which increases food intake and leads to increase of BMI, this is not apparent in newly diagnosed and poorly controlled diabetic children which may be referred to the catabolic effect of insulin deficiency and the important role of insulin itself for ghrelin actions.

These results were is in agreement with Martos-Moreno et al,2006[19] who demonstrated that diabetic children with T1DM had decreased BMI at the time of diagnosis and normalized after 1 month of insulin therapy. Also this is in agreement with other studies[20,21] who reported a significant increase in weight and BMI in diabetic patients after insulin therapy. This was associated with a significant decrease in glucose levels and HbA1c.

As anticipated, our results showed that fasting blood glucose, 2 hours post-prandial glucose, HbA1c, serum cholesterol and triglycerides levels were significantly higher in newly-diagnosed and poorly glycemic diabetic children when compared with controls, but there was no statistically significant difference between good glycemic controlled diabetic children and controls.

The combination of insulin deficiency and elevated levels of the counter-regulatory hormones are responsible for promoting glycogenolysis, gluconeogenesis, while decreasing glucose utilization, glucose clearance, also accelerated lipolysis and impaired lipid synthesis, with resulting increased plasma concentrations of total lipids, cholesterol, triglycerides, and free fatty acids[22].

In the present study, the results showed a significant higher level of ghrelin in children with newly-diagnosed diabetes mellitus than controls. This is in agreement with other authors who reported that in diabetic patients, the mean plasma ghrelin level was significantly higher at the time of

diagnosis with significant reduction of its level after 3 month of insulin therapy[21].

Soriano-Guillen et al., 2004[20] on the contrary found that in diabetic patients, the mean plasma ghrelin levels were significantly lower than controls at the time of diagnosis. They hypothesized that the high glucose levels found at the onset of diabetes inhibit ghrelin secretion. As ghrelin has been shown to stimulate hyperglycemia[24,25], hence in situations of elevated glucose concentrations the organism may respond by decreasing ghrelin levels so that it does not contribute to a further increase in glucose concentrations, so it may be a defensive mechanism against hyperglycemia.

The difference between Soriano-Guillen et al., 2004[20] and our results may be attributed to higher BMI of diabetic children in their study in comparison to our patients, and since the inverse association between plasma ghrelin and both of weight and BMI in children is well established[23] this may contribute to decreased plasma ghrelin level in their study.

The hyperglycemic effect of ghrelin might result from the endocrine effects of ghrelin as well as from its direct effects on hepatocytes in which it modulates glycogen synthesis and gluconeogenesis, ghrelin stimulates hepatic glucose production[24], inhibits insulin secretion both in vitro and in most human and animal studies[25] and stimulates secretion of the counter-regulatory hormones, including GH, cortisol, adrenaline[26] and possibly glucagon[27]. Thus increased plasma ghrelin may lead to hyperglycemia by the previous mechanisms. But it is not clear whether the inappropriate ghrelin responses independently contribute to hyperglycemia of the disease or are merely a consequence of insufficient insulin secretion from the islet[28].

In the present study, there was a significantly higher plasma level of ghrelin in poor glycemic controlled diabetic (uncontrolled) children than controls; this reflects the important role of insulin therapy on both of ghrelin and glucose levels and the relation between them.

As uncontrolled diabetes is characterized by profound insulinopenia, severe hyperglycemia, hyperphagia[29], and if this hyperphagia does not keep pace with the glycosuria, loss of body fat arise with clinical weight loss and diminished subcutaneous fat stores[22].

The elevated plasma ghrelin level in poor glycemic controlled (uncontrolled) diabetic patients in our study may be due to the negative association between systemic ghrelin and insulin levels[30], and the negative association between plasma ghrelin and both of weight and BMI in children[23].

Our results showed that there were no statistically significant differences regarding plasma ghrelin levels between good glycemic controlled diabetic (controlled) children and controls. These results are in agreement with another study[21] which reported a significant reduction in the mean plasma ghrelin levels in diabetic patients after 3 months of insulin therapy in comparison with its higher level at time of diagnosis, Also this is in agreement with other reports found that mean plasma ghrelin levels decreased after insulin therapy in diabetic patients[20].

Proper insulin supplementation improves insulin deficiency and also improves the glycemic state nearly to a normal level in this group and this plays a great role in reduction of the plasma ghrelin level[21], also increased BMI in those patients may share in this reduction.

Saad et al., 2002[31] reported that insulin could mediate the effect of chronic energy balance on plasma ghrelin. The lower insulin level as in lean subjects leads to increased ghrelin concentration, so changes in insulinemia could also underlie the decrease in plasma ghrelin and weight gain.

Ashraf et al., 2007[21] reported that exogenous and endogenous insulin has been shown to have a negative association with ghrelin while maintaining euglycemia. This suggests that insulin treatment or an improved metabolic profile may conceivably play a role in reducing plasma ghrelin, thus mitigating the hyperphagia which often accompanies poorly controlled diabetes. Our results infer that improved glycemic control after insulin therapy in diabetic children plays an important role in pre-prandial ghrelin regulation.

Significant negative correlations were present between plasma ghrelin levels and weight, weight on centile, BMI, and BMI on centile in all diabetic children. These results were in agreement with other studies[20,21].

Celi et al., 2005[30] reported a negative association between systemic ghrelin and insulin levels, this may explain the decline in plasma ghrelin level in children with good glycemic control.

In this study a significant negative correlations was present between plasma ghrelin level and both of fasting and 2-hours post-prandial blood glucose levels in all diabetic groups so the overall picture indicates a functional link between ghrelin, the insulin secretion and glucose metabolism, these results are in accordance with other studies[20,21,32].

However, the physiological and pathological importance of this link remains mostly unclear .

We found no significant correlations between plasma ghrelin concentrations and either of triglycerides or total cholesterol levels in all diabetic groups. However this contradicts with the a study reported that pre-prandial plasma ghrelin levels were negatively correlated with serum triglycerides and positively correlated with hyper density-lipoprotein (HDL) cholesterol in boys not in girls with no explanation[33]. Moreover, other studies found that serum triglycerides decrease and HDL cholesterol increase significantly with increasing plasma ghrelin levels in a study conducted in middle-aged adults not in children[34].

Children with diabetes mellitus in all groups, showed no correlation between plasma ghrelin level and HA1c. This result was in agreement with Soriano-Guillen et al, 2004 and Ashraf et al, 2007[20,21] who reported the same results.

In the present study, multiple regression analysis of factors affecting pre-prandial plasma ghrelin level revealed that the most important and significant factors affecting ghrelin levels were fasting blood glucose, 2-hours post-prandial glucose, weight and BMI consequently supporting the strong relationship between ghrelin level and the previous parameters, while the least important factors were HbA1C, triglyceride and sex. It is possible that plasma glucose concentrations are stronger regulators of plasma ghrelin than HbA1c, which is a substitute marker of chronic glycemia in T1DM patients.

In conclusion, pre-prandial plasma ghrelin level increased in newly diagnosed and poorly controlled children with T1DM . Significant negative correlations between Pre-prandial ghrelin level and weight , BMI , fasting and 2-hours post-prandial levels were present. Insulin therapy and improvement of blood glucose levels play an important role in normalizing plasma ghrelin level in good glycemic controlled T1DM children. No relationship between ghrelin and HbA1C was present.

AKNOWLDEGEMENT

We express our great thanks to Dr. Basma Abdul-Moez, Assistant professor of pediatrics and head of endocrine unit , and to Ahmed Mohamed Emarah, resident in pediatric department, Minia University Hospital for their kind support for completing this work, also great thanks to Dr Eptisam Ammar, Lecturer in Community Medicine for her support for finishing the statistics of this work.

REFERENCES

1. **Haller MJ, Atkinson MA, Schatz D.** Type 1 diabetes mellitus: etiology, presentation, and management. Pediatr Clin North Am 2005; 52(6):1553-78.
2. **Salem M, Tulba K, Faris R.** An epidemiological study of insulin dependent diabetes mellitus in Egyptian students. Egypt J Community Med 1990; 6(1): 183-93.
3. **Abdullah MA.** Epidemiology of type I diabetes mellitus among Arab children. Saudi Med J 2005; 26(6):911-7.
4. **Inui A, Asakawa A, Bowers CY, Mantovani G, Laviano A, Meguid MM, et al.** Ghrelin, appetite, and gastric motility: the emerging role of the stomach as an endocrine organ. FASEB J 2004; 18(3):439-56.
5. **Mondal MS, Date Y, Yamaguchi H, Toshinai K, Tsuruta T, Kangawa K et al.** Identification of ghrelin and its receptor in neurons of the rat arcuate nucleus. Regul Pept 2005;126(1-2):55-9.
6. American Diabetes Association. Standards of medical care in diabetes.2007. Diabetes Care 2007; 30 (Suppl 1): S4-S41.
7. **Porstmann T, Kiessig ST.** Enzyme immunoassay techniques. An overview. J Immunol Methods 1992; 150(1-2):5-21.
8. **Pradelles P, Grassi J, Chabardes D, Guiso N.** Enzyme immunoassays of adenosine cyclic 3',5'-monophosphate and guanosine cyclic 3',5'-monophosphate using acetylcholinesterase. Anal Chem 1989; 61(5):447-53.
9. **Nathan DM, Singer DE, Hurxthal K, Goodson JD.** The clinical information value of the glycosylated hemoglobin assay. N Engl J Med 1984; 310(6):341-6.
10. **Zou CC, Huang K, Liang L, Zhao ZY.** Polymorphisms of the ghrelin/obestatin gene and ghrelin levels in Chinese children with short stature. Clin Endocrinol (Oxf) 2008; 69(1):99-104.
11. **Leite-Moreira AF, Soares JB.** Physiological, pathological and potential therapeutic roles of ghrelin. Drug Discov Today 2007; 12(7-8):276-88.
12. **Kojima M, Kangawa K.** Ghrelin: structure and function. Physiol Rev 2005; 85(2):495-522.
13. **Rindi G, Necchi V, Savio A, Torsello A, Zoli M, Locatelli V, et al.** Characterisation of gastric ghrelin cells in man and other mammals: studies in adult and fetal tissues. Histochem Cell Biol 2002; 117(6):511-9.
14. **Gnanapavan S, Kola B, Bustin SA, Morris DG, McGee P, Fairclough P, et al.** The tissue distribution of the mRNA of ghrelin and subtypes of its receptor, GHS-R, in humans. J Clin Endocrinol Metab 2002; 87(6):2988.

15. **Arvat E, Maccario M, Di Vito L, Broglio F, Benso A, Gottero C, et al.** Endocrine activities of ghrelin, a natural growth hormone secretagogue (GHS), in humans: comparison and interactions with hexarelin, a nonnatural peptidyl GHS, and GH-releasing hormone. Clin Endocrinol Metab 2001; 86(3):1169-74.

16. **Tschöp M, Smiley DL, Heiman ML.** Ghrelin induces adiposity in rodents. Nature 2000; 407(6806):908-13.

17. **Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al.** A role for ghrelin in the central regulation of feeding. Nature 2001; 409(6817):194-8.

18. **Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al.** Ghrelin enhances appetite and increases food intake in humans. J Clin Endocrinol Metab 2001; 86(12):5992.

19. **Martos-Moreno GA, Barrios V, Soriano-Guillén L, Argente J.** Relationship between adiponectin levels, acylated ghrelin levels, and short-term body mass index changes in children with diabetes mellitus type 1 at diagnosis and after insulin therapy. Eur J Endocrinol 2006; 155(5):757-61.

20. **Soriano-Guillén L, Barrios V, Lechuga-Sancho A, Chowen JA, Argente J.** Response of circulating ghrelin levels to insulin therapy in children with newly diagnosed type 1 diabetes mellitus. Pediatr Res 2004; 55(5):830-5.

21. **Ashraf A, Mick G, Meleth S, Wang X, McCormick K.** Insulin treatment reduces pre-prandial plasma ghrelin concentrations in children with type 1 diabetes. Med Sci Monit 2007; 13(12):CR533-7.

22. **Alemzadeh R, Wyatt T.** Diabetes mellitus in children: Nelson Textbook of Pediatrics. Behrman RE, Kliegman RM, Jenson HB (eds); 2007, 18th ed., WB Saunders Co, Philadelphia. Pp: 2404-31.

23. **Haqq AM, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, et al.** Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. J Clin Endocrinol Metab 2003; 88(1):174-8.

24. **Murata M, Okimura Y, Iida K, Matsumoto M, Sowa H, Kaji H, et al.** Ghrelin modulates the downstream molecules of insulin signaling in hepatoma cells. J Biol Chem 2002; 277(7):5667-74.

25. **Broglio F, Gottero C, Arvat E, Ghigo E.** Endocrine and non-endocrine actions of ghrelin. Horm Res 2003; 59(3):109-17.

26. **Malagón MM, Luque RM, Ruiz-Guerrero E, Rodríguez-Pacheco F, García-Navarro S, Casanueva FF, et al.** Intracellular signaling mechanisms mediating ghrelin-stimulated growth hormone release in somatotropes. Endocrinology 2003; 144(12):5372-80.

27. **Salehi A, Dornonville de la Cour C, Håkanson R, Lundquist I.** Effects of ghrelin on insulin and glucagon secretion: a study of isolated pancreatic islets and intact mice. Regul Pept 2004; 118(3):143-50.

28. **Chao S, Sussel L.** Ghrelin, insulin, islet development and islet cell types. Curr Opin Endocrinol 2004; 11: 104-9.

29. **Gelling RW, Overduin J, Morrison CD, Morton GJ, Frayo RS, Cummings DE, et al.** Effect of uncontrolled diabetes on plasma ghrelin concentrations and ghrelin-induced feeding. Endocrinology 2004; 145(10):4575-82.

30. **Celi F, Bini V, Papi F, Santilli E, Ferretti A, Mencacci M, et al.** Circulating acylated and total ghrelin and galanin in children with insulin-treated type 1 diabetes: relationship to insulin therapy, metabolic control and pubertal development. Clin Endocrinol (Oxf) 2005; 63(2):139-45.

31. **Saad MF, Bernaba B, Hwu CM, Jinagouda S, Fahmi S, Kogosov E, et al.** Insulin regulates plasma ghrelin concentration. Clin Endocrinol Metab 2002; 87(8):3997-4000.

32. **Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, et al.** Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. Clin Endocrinol Metab 2002; 87(1):240-4.

33. **Park HS, Lee KU, Kim YS, Park CY.** Relationships between fasting plasma ghrelin levels and metabolic parameters in children and adolescents. Metabolism 2005; 54(7):925-9.

34. **Pöykkö SM, Kellokoski E, Hörkkö S, Kauma H, Kesäniemi YA, Ukkola O.** Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes 2003; 52(10):2546-53.