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Full Length Research Paper

Ghrelin levels in obese children of diabetic parents

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Obesity beginning in childhood often leads to hyperinsulinemic state. Ghrelin, a recently discovered growth hormone (GH) secretagogue with orexigenic effects, is proposed to be a regulator of energy balance. It is thought to be associated with obesity and metabolic syndrome. Increased abdominal fat may lead to the development of metabolic syndrome in children and has been strongly associated with insulin resistance. The aims of this study were to determine fasting ghrelin levels of obese children of diabetic parents and obese children of non-diabetic parents and to investigate possible correlations between ghrelin hormones with insulin levels. This was a cross sectional study. Eighty obese children ranging from 5 to 18 years of age were recruited from the Pediatric Clinic of Shalamar Hospital, Lahore. These children were divided in two groups. Group A consists of forty obese children of diabetic parents and Group B having forty obese children of non-diabetic parents. A significant increase in the mean level of insulin, insulin resistance, triglycerides and fasting blood glucose were observed with concomitant significant decrease in the mean level of ghrelin. Fasting ghrelin levels were negatively correlated with insulin, homeostatic model assessment-insulin resistance (HOMA-IR), body mass index (BMI) and waist circumference. The results suggest that the down regulation of ghrelin secretion may be a consequence of higher insulin resistance associated with visceral fat accumulation. Ghrelin might prove to be a useful tool to identify obese children at risk for developing insulin resistance, diabetes or metabolic syndrome.

Key words: Ghrelin, obesity, insulin resistance, metabolic syndrome.

INTRODUCTION

Childhood obesity acquires its importance as a predictor of obesity in adult life. In Pakistan, where over 43% of the population comprises of children (<15 years of age), it has been observed that children are adopting more and more unhealthy eating habits and physical activities. Khuwaja et al. (2003) found that majority (58%) of the school children had at least one modifiable risk factor for cardiovascular diseases like physical inactivity, unhealthy dietary habits, overweight and obesity. The factor which gained importance regarding obesity worldwide is the ghrelin hormone. Ghrelin is a peptide hormone with activity in modulating feeding behavior and energy balance. Ghrelin is a peptide principally secreted from oxyntic cells in the stomach. It consists of 28 amino acids. Lower levels of ghrelin were found in obese individuals (Tschop et al., 2001) with the exception of patients with Prader-Willi syndrome (Haqq et al., 2003) and were found raised in anorexia nervosa (Otto et al., 2001). It is thought to play a role in causing obesity and was initially discovered as an endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (Kojima et al., 1999).

Bacha and Arslanian (2005) reported an inverse relationship between fasting ghrelin level and fasting insulin

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in childhood obesity. Recent literature suggests that in addition to food intake and energy balance, ghrelin also controls glucose metabolism (Poykko et al., 2003). Furthermore, current evidence suggests that ghrelin could contribute to the metabolic syndrome. It has been shown that ghrelin concentrations are reduced in different diseased conditions including obesity, type 2 diabetes and other conditions with metabolic disturbances (Østergård et al., 2003). The relationship between ghrelin secretion to insulin sensitivity and insulin secretion has been under research till present time. High levels of insulin and glucose due to insulin resistance are believed to be the origin of metabolic syndrome and type 2 diabetes (Boden et al., 2005). Clinical studies are insufficient to reveal whether ghrelin level is a physiological response or a causative factor for the insulin resistance. The aim of this study was to determine the ghrelin levels in obese children having parents with type 2 diabetes mellitus.

MATERIALS AND METHODS

In this cross sectional study, 80 obese children ranging from 5 to 18 years were included in the study. 40 obese children had diabetic parents and 40 obese children had non-diabetic parents. Ethical approval was obtained from the Ethical Committee of University of Health Sciences, Lahore. A written consent was obtained from all the parents of children included in this study. All study cases were recruited from Pediatric Clinic of Shalamar Hospital, Lahore. The children enrolled in this study were classified according to their body mass index (BMI) which was ≥95th percentile. Their anthropometric measurements mainly waist and hip circumference measurements were taken. All the obese children were screened on the basis of medical history and physical examination. Ghrelin, insulin, blood glucose, and lipid profile were then performed on their blood samples. Insulin resistance was calculated by using homeostatic model assessment (HOMA) model. The obese children of diabetic parents were labeled as group A, and obese children of non-diabetic parents were labeled as group B.

Height (cm) was measured using wall-mounted stadiometer and weight (kg) was measured using a weighing balance to calculate BMI (as an expression of obesity). All the subjects were lightly clothed and without shoes. BMI was calculated by the following formula:

$$BMI = \text{Weight (kg)}/\text{Height (m}^2\text{)}$$

The waist circumference was measured at a level midway between the lowest rib and the iliac crest, in the standing position, using a non elastic flexible tape and recorded to nearest centimeters and the hip circumference at the level of the greater trochanters (Han et al., 1995) with the legs close together.

Laboratory investigations

After 10 to 12 h fasting, samples were drawn from all subjects in the study. All the samples were centrifuged (10 min at 3000 rpm) and sera were separated. Ghrelin, insulin, and blood glucose was performed on the same day. Serum for lipid profile was stored at -20°C until assayed. Fasting blood glucose level was performed using GOD-pap method.

The quantitative determination of serum ghrelin was conducted by enzyme-linked immunosorbent assay (ELISA) technique, using commercially available reagent kit (IBL Ghrelin Sandwich by SPI BIO) with ELISA having Catalogue number A05106. Insulin was assessed using chemiluminescence method. Insulin resistance (IR) was assessed using the HOMA-IR according to the formula of fasting insulin (μIU/ml) × fasting glucose (mmol/L)/22.5 (Matthews et al., 1985).

Statistical analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), Version 16 (SPSS Inc., Chicago, IL, USA).

For each variable, mean, standard deviation (SD), and ranges were calculated. The categorical variables were expressed in frequencies. Results are given as means ± SD. Two sample t-test was applied and P values for comparison of serum ghrelin, insulin, blood glucose, lipid profiles levels, BMI, and waist circumference (abdominal obesity), and insulin resistance were determined in healthy obese children of diabetic parents and obese children of non-diabetic parents. The significance of difference between the two groups was also tested. For correlation between serum ghrelin and insulin resistance in obese children of type 2 diabetic parents and obese children of non-diabetic parents, r-values were determined by Pearson's correlation test. A p value <0.05 was taken as statistically significant.

RESULTS

Demographic data is presented in Table 1. There were 20 females (50%) and 20 males (50%) in both groups. serum ghrelin levels were observed to be lower in group A as compared to group B which was statistically significant ($p<0.0001$). However, there was no statistically significant gender difference regarding serum ghrelin levels found between the two groups ($p=0.937$). Mean serum ghrelin levels in males of both groups were 8.7±6.7 pg/ml and in females of both groups were 8.8±7.0 pg/ml. The lipid profile values were on the higher side in group A as compared to group B. The differences of cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) between the two groups were statistically significant ($p<0.001$). BMI had a strong positive correlation with serum insulin concentration ($r=0.252$, $p=0.024$) and insulin resistance ($r=0.241$, $p=0.031$). BMI had a significant negative correlation with ghrelin levels ($r=-0.366$, $p=0.001$). BMI positively correlated with waist circumference ($r=0.769$, $p<0.001$). There was significant negative correlation between serum ghrelin concentrations and insulin levels ($r=-0.254$, $p=0.023$). There was also significant negative correlation between serum ghrelin levels and insulin resistance ($r=-0.300$, $p=0.007$) as shown in Figure 1. There was also a significant negative correlation between ghrelin levels and age of patients ($r=-0.278$, $p=0.013$). There was also a negative correlation between ghrelin levels and waist circumference ($r=-0.255$, $p=0.022$). It was noteworthy that there was no correlation between the fasting serum ghrelin and fasting blood glucose concentrations in these groups.

Waist circumference positively correlated with BMI,

Table 1. Baseline characteristics of obese subjects in groups A and B.

Parameter	Group A (Mean±SD) Obese (diabetic parents) (n=40)	Group B (Mean±SD) Obese (non-diabetic parents) (n=40)	p-Value
Age (years)	12.4±3.5	11.9±2.6	0.438
Height (cm)	150±14.6	149±15.1	0.837
BMI (kg/m^2)	29.9±4.0	28.1±4.4	0.065
Weight (kg)	69±19.6	65±19.1	0.328
Waist circumference (cm)	91.6±12	91.1±11.9	0.878
Serum ghrelin (pg/ml)	4.7±1.7	12.8±7.6	0.000*
Fasting blood glucose (mg/dl)	105.2±8.6	101±12.7	0.079
Fasting insulin (IU/L)	30.5±15.0	22.4±11.4	0.009*
Insulin resistance (HOMA-IR)	7.8±4.1	5.0±2.1	0.000*

*Significant at p<0.05.

Table 2. Correlation of ghrelin with risk parameters of metabolic syndrome.

Parameter	Serum ghrelin levels (r value)	p value
BMI	-0.366	0.001*
Waist circumference (>90th percentile)	-0.255	0.022*
Fasting insulin	-0.254	0.023*
HOMA-IR values	-0.300	0.007*
Fasting glucose	-0.167	0.138

*****Means statistically significant.

insulin levels (r=0.387, p=0.014), and insulin resistance (r=0.398, p=0.011) and this correlation was statistically significant. Insulin resistance also positively correlated with the triglycerides (r=0.332, p=0.036). A significant increase in the mean level of ghrelin, insulin, insulin resistance, cholesterol and triglycerides was observed in the group of children having parents with type 2 diabetes. Concomitantly, significant decrease in the mean level of ghrelin and insulin levels upon comparing the two groups was found. Ghrelin was found to correlate negatively with insulin and insulin resistance showing that ghrelin and insulin are inversely related. Also, ghrelin concentrations were lower in both obese groups and a negative relationship with BMI was found (Table 2). Only healthy obese subjects of diabetic parents were included and were compared with obese children of non-diabetic parents.

DISCUSSION

Nowadays, obesity and diabetes are considered as the twin epidemic and so are the dangers of childhood obesity and its hazardous consequences. The incidence of childhood obesity has tripled over the past three decades. Nearly 16% of children and adolescents between the ages of 6 and 19 years are currently overweight and an additional 15% are considered at risk for becoming overweight (Han et al., 2010).

The aim of the present study was to determine serum ghrelin levels in obese children of type 2 diabetic and non-diabetic parents. We also determined the correlation of ghrelin with insulin levels. This cross sectional study in 80 obese children described the significant correlation of serum ghrelin concentrations with insulin, insulin resistance and BMI.

The ghrelin levels in our study had negative correlation with age (r=-0.278, p=0.013) (Figure 2). In our study, the children with increasing age had lower ghrelin levels. These results are in accordance with Ikezaki et al. (2002) who reported that ghrelin levels are more in prepubertal age, as compared to pubertal age. Circulating ghrelin concentrations progressively increased during the first 2 years of life and then decreased during late childhood and adolescence. Secondly, ghrelin is a growth hormone secretagogue, and growth velocity decreases with rising age and puberty, so ghrelin levels decreases with increasing age (Kelishadi et al., 2008).

We found out that BMI was negatively correlated with ghrelin levels in these obese children (r=-0.366, p=0.001). This finding is consistent with the study by Zou et al. (2008),

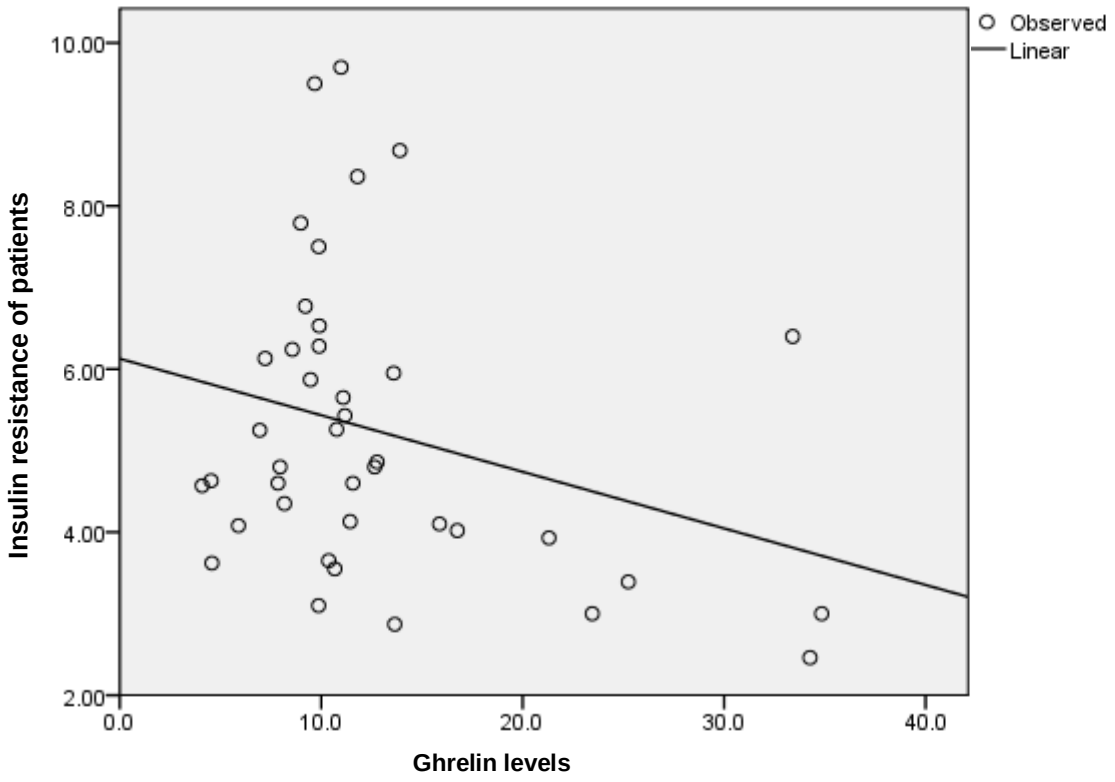

Figure 1. Correlation of serum ghrelin and insulin resistance in obese children.

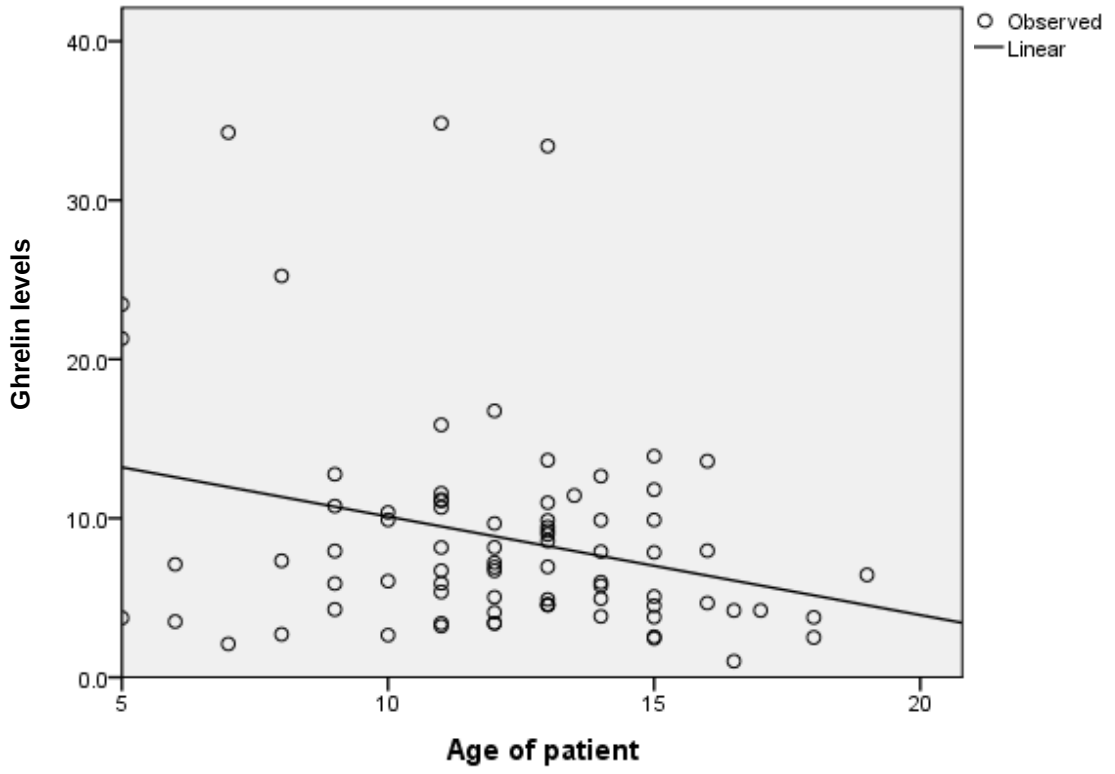

Figure 2. Correlation of serum ghrelin and age of obese children.

showing that ghrelin concentrations are lower in obese children and has a negative correlation with BMI in these obese children. As ghrelin secretion induces the accumulation of lipids in visceral fatty tissue, located in the abdominal zone and thus leads to increase in obesity and BMI. It has been shown in research that with increasing obesity, ghrelin levels decrease and with the loss of weight, ghrelin levels increase (Erdmann et al., 2005; Zou et al., 2009).

This study had lower serum ghrelin levels in both groups; however, it was significantly lower in the obese children of diabetic parents as compared to the obese children of non-diabetic parents ($p<0.0001$). Our study comprised 80 obese children with BMI more than 95th percentile, all equally obese, with central obesity (increased waist circumference). It is likely that genetic factors may be the cause of lower ghrelin levels in children of diabetic parents as compared to those having non-diabetic parents. Another factor which contributes to the down-regulation of ghrelin levels in obese subjects seems to be a consequence of elevated insulin levels, because fasting ghrelin levels were inversely correlated with fasting levels of insulin (Murphy et al., 2006). Notably, insulin could play a pivotal role in regulating body weight through its down-regulating effects on plasma ghrelin concen-trations. In the present study, serum insulin levels and HOMA-IR increased significantly ($p<0.0001$) in the obese children of diabetic parents as compared to the obese children of non-diabetic parents. These results are in agreement with study conducted by Van Guilder et al. (2008) who mentioned that insulin resistance and hyper-insulinemia are the hallmarks of obesity, and individuals with truncal obesity exhibit the greatest degree of insulin resistance and hyper-insulinemia. In our study, genetic component is possibly involved in causing raised insulin resistance in group A.

We also found a significant negative correlation between serum insulin levels and serum ghrelin levels in the obese subjects including both groups ($r=-0.254$, $p=0.023$). The negative relationship between fasting ghrelin concentration and obesity might be explained by an inhibitory effect of insulin on ghrelin, since a higher insulin resistance is associated with visceral fat accumulation. Our findings are also in agreement with those of other studies conducted in children by Amina et al. (2011) suspecting that insulin resistance may play an important role in the release of ghrelin.

The present study showed that obesity (BMI) had a strong relation with insulin resistance ($r=0.241$, $p=0.031$), which is in accordance with the studies by Wang et al. (2001) who observed that obesity had central role in the development of insulin resistance. The selected subjects in our present study were healthy, not suffering from diabetes mellitus, cardiovascular disease, but still had high insulin resistance, which seems to be entirely related to obesity. Insulin resistance is a well recognized risk factor for the development of type 2 diabetes mellitus and cardiovascular disease (Mokdad et al., 2003). These children also had elevated triglycerides, elevated insulin levels, more of insulin resistance and low HDL levels. Work done by Hirschler et al. (2005) showed that in obese youth with similar BMI, insulin sensitivity is lower in those with high visceral adipose tissue and high waist circumference.

This study highlighted that there was a significant increase in fasting blood glucose levels in both groups of obese children. Approximately, 63% of children of diabetic parents and 37% of children of non-diabetic parents had impaired fasting glucose, that is, >100 mg/dl (5.6 mmol/L) according to International Diabetes Federation (IDF) definition of metabolic syndrome in children (IDF, 2007). The findings were in agreement with Alberti et al. (2006) claiming obesity as the major risk factor for the development of type 2 diabetes. This moderate hyperglycemia therefore draws attention to the preclinical sign of disturbed glucose metabolism and insulin resistance. We also found no significant correlation between serum ghrelin levels and levels of fasting blood glucose in these groups. Similar results were reported in the studies conducted by Ikezaki et al. (2002) and Amal et al. (2012) who found no correlation between ghrelin and fasting glucose in obese children.

The findings of this study show that ghrelin is negatively correlated with insulin and insulin resistance. In adolescents, ghrelin is negatively correlated with insulin resistance, independent of obesity. This study also highlights that low ghrelin concentrations are associated with tendency to develop insulin resistance syndrome and its components. This can be explained by all the obese children having lower ghrelin levels and having higher BMI. Obesity influences all features of the metabolic syndrome and genetic component may play a role in causing lower ghrelin levels in obese children of type 2 diabetic parents more as compared to obese children of non-diabetic parents. This study has shown that they are more prone to develop diabetes and metabolic syndrome later in life.

Conclusion

This study will help to establish the use of ghrelin as one of the early markers to predict the onset of insulin resistance and metabolic syndrome in these children. Obese children of type 2 diabetic parents should be investigated once they start getting obese to prevent them from entering into metabolic syndrome. It also emphasize that ghrelin may be useful in risk assessment of obese children with family history of diabetes. Its levels can be used as a screening tool for developing diabetes mellitus, cardiovascular disease and it may prove an important marker for the diagnosis of metabolic syndrome. Secondarily, screenings for obese children with diabetic parents should include testing for ghrelin and insulin resistance.

Early intervention by physicians should be encouraged to improve their quality of life.

Limitations of the study were that small sample size was used in this study due to budget constraints. Only healthy obese subjects of diabetic parents were included and were compared with obese children of non-diabetic parents. We could not include non obese group in our study due to financial constraints. So, another study may be planned to further explore the relationship between serum ghrelin, insulin resistance (MS) and obesity in obese and non obese groups.

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