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Association of Parameters of Metabolic Syndrome with Plasma Active Ghrelin Levels: A Study from North India

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Abstract: ***Background: Ghrelin is the strongest orexigenic hormone. Ghrelin is a gut-derived peptide hormone. Administered ghrelin has shown an increase in food intake and also stimulate the secretion of the growth hormone in rodents as well as in humans. Objective: The aim of the study was to association of parameters of Metabolic Syndrome with Plasma Active Ghrelin levels. Materials & Methods: A total of 196 patients were enrolled in the study out of them 99 were cases and 97 were controls. Results: A significant negative correlation was found between Fasting plasma active ghrelin levels and systolic blood pressure (r = -0.68, p <0.02), diastolic blood pressure (r = -0.60, p <0.02), obesity (r = -0.65, p <0.01) ,fasting insulin levels (r = -0.52, p <0.01), insulin resistance (r = -0.39, p <0.01).A deeper understanding on this interesting pleiotropic hormone could be anticipated to open new insights into the metabolic pathways causing a predisposition for obesity and its co-morbidities.***

Keywords: Metabolic Syndrome, Ghrelin, insulin resistance, dyslipidemia,

1. Introduction

Ghrelin is the strongest orexigenic hormone. Ghrelin is a gut-derived 28 amino acid1-3 peptide hormone. Ghrelin has been shown to increase food intake and stimulate the secretion of growth hormone in rodents and in humans. This is the strongest[4] orexigenic hormone. It circulates in body in active and inactive forms of which active form is less than the inactive form.[5] Ghrelin has been demonstrated to have effects on glucose metabolism, blood pressure, cardiac function and also on inflammatory processes. Based on these findings ghrelin has been proposed to be involved in the pathophysiology of obesity and its associated co-morbidities, such as type 2 diabetes and atherosclerotic cardiovascular diseases.

Previous studies on ghrelin shows that ghrelin levels are lower in type 2 diabetes patients in comparison to normal patients. Plasma levels of ghrelin in obese patients with type 2 diabetes mellitus were significantly decreased compared with nonobese patients. Decreased plasma levels of active ghrelin are significantly associated with abdominal adiposity, hyper-8 insulinemia and insulin resistance in type 2 diabetic patients.

Ghrelin concentration were negatively associated with fasting insulin, systolic, diastolic BP and the prevalence of type 2[10] diabetes and insulin resistance. Given the association of Insulin Resistance, Hypertension, Dyslipidemia and Obesity with Plasma Active Ghrelin levels in patients of type-II Diabetes Mellitus, we wanted to study whether all these association might show any type of variations in their correlation.

2. Materials and Methods

This Case–Control study was conducted in the Department of Medicine and Pathology, Career Institute of Medical Sciences and Hospital, Lucknow between January 2013 to February 2014. Patients having type 2 diabetes mellitus, attending diabetes clinic and admitted in medical wards were studied after taking their informed consent. A total of 196 patients were enrolled for the study. 99 of these were cases and 97 were controls, (controls were selected from general population not having history of diabetes and hypertension). Those in the diabetic group were divided into four subgroups which included 15 patients having type 2 diabetes only (group 1), 19 patients were having type 2 diabetes and insulin resistance (group 2), 25 patients were having type 2 diabetes, insulin resistance and hypertension (group 3) and last subgroup included 40 patients having type 2 diabetes , insulin resistance, hypertension and obesity (group 4).

In our study population of 99 diabetic patients and 97 11 controls, we measured Fasting blood sugars (FBS), Postpran-11 dial blood sugars (PPBS), lipid profile, weight, height, BMI,125 fasting insulin level was measured by I - radio immunoassay (RIA) technique using RIAK-1 kit (BARC Vashi complex, 12 Turbhe, Mumbai), fasting active Ghrelin was measured by Active Ghrelin ELISA kit (Mitsubishi Chemical Medience Corporation, TOKYO) which measures the active form of Ghrelin based on the principle of 2 site sandwich enzyme 13 linked immuno-sorbent assay (ELISA), insulin resistance by 14 HOMA-IR, HbA1c was measured by using ion exchange 15 resin method. Circulating ghrelin includes active (acylated) and inactive (desacylated) form. In our study we estimated active form of ghrelin. Patients with type-II diabetes mellitus for the last three years in age group 25-70 years with or without treatment. Patients with Type-I diabetes mellitus and Hyperthyroidism patients were excluded from the study.

Statistical Analysis: Data analysis was done using STRATA 10.1 (College Station, Tx, USA), Two sample t-test used, if data met normally assumption otherwise, Mann Whitney U test is used. Normally all data tested by using Shapiro Wilk's test. Data were presented as mean ±SD, median (IQR) and *p* value. *p* value <0.05 was considered as significant.

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3. Results

Cases and controls were similar in baseline characteristics such as age (51.8±13.1vs 56.3±9.1 years, p=0.087), sex 2 composition, HDL (34.2±14.3vs 38.3±10.7mg/dl, *p*=0.15) and LDL (77.5±35.2 vs 71.1±28.7 mg/dl, *p*=0.18). Fasting blood sugars (230.1±100.7 vs 90.8±10.2 mg/dl, *p*<0.0001) and postprandial blood sugars (317.8±119.2 vs 133.7±20.2 mg/dl, *p* <0.0001) were significantly higher in cases as compared to controls (Tables 1). Mean SBP (148.9±17.7 vs 124.3±12.6mmHg, *p* <0.0001) and mean DBP (109.3±11.9 vs 78.9±7.1mmHg, *p* <0.0001) were significantly higher in cases as compared to controls. Insulin resistance, measured by HOMA-IR, was higher in cases in comparison to controls (11.2±9.6 vs 2.9±0.7, *p* <0.0001).

Fasting plasma active ghrelin levels were significantly lower in cases as compared to controls (10.47±5.4 vs 36.6±6.4 fmol/ml, *p* <0.0001). Plasma active Ghrelin levels are negatively associated with SBP (r = -0.68, *p* <0.02) and DBP (r = -0.60, *p* <0.02) in hypertensive patients. There is also an inverse association between ghrelin and fasting insulin concentrations (r = -0.52, *p* <0.01). Plasma active ghrelin levels were lower in patients with having insulin resistance (HOMA-IR >3.8). Also insulin resistance is negatively correlated to ghrelin (r=-0.39, *p* <0.01). Plasma active ghrelin levels were lower in obese subjects when compared to non obese subjects (5.1±1.6 vs 12.1±1.8 fmol/ml, *p* <0.0001) after adjustment for type 2 diabetes, hypertension and insulin resistance states (r = -0.65,*p* <0.01).

There were significant correlations between the plasma levels of ghrelin and BMI (r = -0.56, *p* <0.01), in type 2 diabetic patients. On multivariate regression analysis, it was found that SBP and BMI were the significant factors contributing to lower levels of ghrelin (Table 2). On comparing different groups among cases it was found that ghrelin levels are decreasing as the number of risk factors increases with high levels in patients having type 2 diabetes only and lowest in the group of patients having type 2 diabetes, hypertension, obesity and insulin resistance.

Out of 99 cases only 15 patients were having triglycerides >250 mg/dl. A negative correlation was found between triglycerides and ghrelin levels (r = -0.3, *p* <0.05) but no inference can be drawn as only 15 patients have high triglyceride values. On multivariate regression analysis of ghrelin, it was found that BMI and SBP were the significant factors contributing to lower levels of ghrelin.

4. Discussion

Cases and controls were similar in baseline characteristics such as age, sex composition, BMI, HDL and LDL. Circulating ghrelin includes active (acylated) and inactive (desacylated) form. In our study we are estimating active form of ghrelin. In our study fasting plasma active ghrelin levels were significantly lower in cases as compared to controls; when ghrelin values of controls were compared with patients having type 2 diabetes only fasting ghrelin values were significantly lower in the later group (36.6±6.4 vs 21.8±0.9 fmol/ml, *p* <0.001). Jang YS *et al* also conducted a study to compare the serum ghrelin concentrations between forty type 2 diabetes mellitus patients and forty normal adults and found that ghrelin levels of the type 2 diabetic group (71.1+/-30.5 ng/L) were significantly lower than the control group (139.7+/-36.9ng/L). This finding was similar to previous study but here we are measuring active ghrelin levels which varies from 1:5 to 1:10 (active: inactive) by different methods. There is no significant difference of BMI between cases and controls. However plasma active ghrelin levels were lower in obese subjects when compared to non obese subjects after adjustment for type 2 diabetes, hypertension and insulin 7 resistance states. Katsuki A *et al* investigated the relationship between the circulating level of active ghrelin and abdominal adiposity, serum levels of insulin or insulin resistance in patients with type 2 diabetes mellitus and measured the plasma levels of the active form of ghrelin in 18 obese and 18 nonobese patients with type 2 diabetes mellitus. Plasma levels of ghrelin in obese patients with type 2 diabetes mellitus were significantly decreased compared with nonobese patients.

There were significant correlations between the plasma levels of ghrelin and BMI in type 2 diabetic patients; decreased plasma levels of active ghrelin are significantly associated with abdominal adiposity, hyperinsulinemia and insulin resistance in type 2 diabetic patients. 8 Matthias Tschop *et al* measured body composition (by dual X-ray absorption) as well as fasting plasma ghrelin concentrations by radioimmunoassay in 15 Caucasians and 15 Pima Indians. Fasting plasma ghrelin was negatively correlated with percent body fat (r = –0.45; *p*=0.01). Plasma ghrelin concentration was decreased in obese Caucasians as compared with lean Caucasians (*p* < 0.01). Fasting plasma ghrelin concentrations were negatively correlated with body weight (r= -0.50, *p* < 0.01), BMI (r = -0.50, *p* < 0.01). In our study our findings are in accordance to the above mentioned studies related to observed parameters. In line with the above mentioned studies our study also demonstrated adiposity to be an important determinant of ghrelin concentrations. Therefore, the lower ghrelin concentration in type 2 diabetic subjects could be due to their higher adiposity. However, the difference remained after adjustment for insulin resistance, hypertension, type 2 diabetes. Ghrelin stimulates food intake, and reduced levels might be teleologically aimed at preventing the increase in body mass.

Insulin resistance was measured by HOMA-IR method and values were higher in cases in comparison to controls. We found that plasma active ghrelin levels to be lower in patients with having insulin resistance (HOMA-IR>3.8) and these findings were independent of type 2 diabetes status. Also insulin resistance is negatively correlated to ghrelin. Paulo [9] Marzullo *et al* analyzed plasma active and serum total ghrelin levels in 20 obese and 20 lean subjects. In obese patients, plasma active (180 ± 18 vs. 411 ± 57 pg/ml; *p* < 0.001) and serum total ghrelin levels (3650 ± 408 vs. 5263 ± 643 pg/ml; *p* < 0.05) were significantly lower when compared with lean subjects. There was a significant correlation between active and total ghrelin (r = 0.62; *p* < 0.001), and between total ghrelin and insulin (r = -0.53; *p* < 0.001) or insulin resistance using the homeostatis model of

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assessment- insulin resistance (r = -0.49; $p < 0.001$) approach.

Taking in consideration the choice of method to measure insulin resistance in a population based study, out of HOMAIR and glucose clamp technique, HOMA-IR has been extensively applied as a test to measure insulin resistance in such situations. The figure of insulin resistance obtained with this method has a relatively low reproducibility, which reflects day-to-day variability in fasting glucose and insulin, as well as analytical uncertainty. This is mainly the case for insulin levels, and a change of 1 µU/ml insulin may determine a change of up to 20% HOMA-IR. Despite this, the method proved to correlate closely with quantitative, functional tests such as the glucose clamp technique.

Mean systolic and diastolic blood pressure were significantly higher in our cases as compared to controls with a negative correlation plasma active Ghrelin levels negatively correlating with blood pressure (systolic as well as diastolic) in 10 hypertensive patients. Seppo M Poyokko *et al* also found similar negative correlations of ghrelin with hypertension. The effect of hypertension on plasma active ghrelin levels remained after adjustment for type 2 diabetes and insulin resistance (11.72±1.68 vs 15.22±4.05 fmol/ml, p <0.02). In experimental models, ghrelin exerts beneficial hemodynamic effects by decreasing the mean arterial pressure and increasing the cardiac output. The vasodilatory effects of ghrelin in vitro suggest that the mechanism is independent of the GH/IGF-1/nitric oxide axis. These findings suggest that ghrelin might have a role in the regulation of BP, especially in the hypertensive state. The low plasma active ghrelin levels in hypertensive patients were independent of diabetes and insulin resistance.

There is also an inverse association between ghrelin and fasting insulin concentrations. These findings support the view that low ghrelin could have a causative role in the development of type 2 diabetes. Low ghrelin concentrations have been associated previously with insulin resistance in subjects with polycystic ovary syndrome and in obese children and adolescents. The negative correlation between fasting ghrelin and insulin has also been reported earlier. Our findings are in accordance with these results, showing that ghrelin level is negatively associated with insulin concentrations and type 2 diabetes.

Based on current knowledge on the interplay between plasma insulin, glucose, and ghrelin, it is difficult to explain the mechanism behind the association between low ghrelin concentrations and type 2 diabetes. In adults, ghrelin administration induces hyperglycemia and decreases plasma insulin concentrations. Conversely, hyperglycemia and insulin decrease plasma ghrelin levels. Similarly, a negative correlation between ghrelin and insulin concentrations has been reported in children and adolescents. Taken together, these data suggest that ghrelin is closely associated with glucose metabolism in adults as well as in children and adolescents.

Our study has certain limitations; the small sample size could limit the power of the analyses. However, the similar results seen by other investigators support our findings. No functional measurements of insulin response were investigated, since such measurements would have required the use of invasive and expensive techniques such as clamps. Instead, HOMA-IR evaluation provides high sensitivity and specificity for measuring insulin resistance, especially among adolescents.

In conclusion, Ghrelin is the strongest orexigenic hormone. Ghrelin circulates in body in active (acylated) and inactive (desacylated) forms of which active form is less than the inactive form. Previous studies on ghrelin shows that ghrelin levels are lower in type 2 diabetes patients in comparison to normal patients. Plasma levels of ghrelin in obese patients with type 2 diabetes mellitus were significantly decreased compared with nonobese patients and decreased plasma levels of active ghrelin are significantly associated with abdominal adiposity, hyperinsulinemia and insulin resistance in type 2 diabetic patients. Similar findings were shown as were observed in previous studies. Our study demonstrated a significant negative correlation between fasting plasma active ghrelin levels and systolic and diastolic blood pressure.

Fasting plasma active ghrelin levels were also negatively associated with obesity (BMI>25) significantly. Significant negative association of fasting plasma active ghrelin levels was shown with fasting insulin levels and insulin resistance as with other studies plasma ghrelin levels were negatively associated with diabetes mellitus.

A deeper understanding on this interesting pleiotropic hormone could be anticipated to open new insights into the metabolic pathways causing a predisposition for obesity and its co-morbidities. Further studies are necessary to reveal the exact mechanism of ghrelin physiology and possible phenotypic roles for changes in ghrelin levels such as those seen with insulin resistant states. Further studies are also needed for exploring the potential clinical applications for ghrelin and other ghrelin mimetics.

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Table 1: Comparison of Blood variables between cases and controls

Parameters	Cases (n=99)	Controls (n=97)	p-value
Age	51.8±13.1	56.3±9.1	0.087
Fasting Blood sugar	230.1±100.7	90.8±10.2	<0.0001*
Postprandial Blood sugar	317.8±119.2	133.7±20.2	<0.0001*
HbA1c	7.98±2.2	5.69±1.30	<0.0001*
Systolic BP	148.9±17.7	124.3±12.6	<0.0001*
Diastolic BP	109.3±11.9	78.9±7.1	<0.0001*
Total Cholesterol	163.7±45.3	123.7±38.2	0.0001*
TG	191.5±106.9	123.7±43.4	0.001*
HDL	34.2±14.3	38.3±10.7	0.15
LDL	77.5±35.2	71.1±28.7	0.18
VLDL	37.2±16.1	24.2±9.1	0.016*
INSULIN	20.6±12.3	12.7±4.7	<0.0001*
HOMA-IR	11.2±9.6	2.9±0.7	<0.0001*
GHRELIN	10.47±5.4	36.6±6.4	<0.0001*

*Statistically significant

Table 2: Correlation of Ghrelin with Variables in Cases

Variable	'r"	P-value
FBS	-0.07	0.50
PPBS	-0.05	0.70
HbA1c	0.17	0.50
TC	-0.10	0.70
TG	-0.29	<0.05*
HDL	0.10	0.30
LDL	-0.13	0.30
VLDL	-0.22	0.08
INSULIN	-0.52	<0.01*
HOMA-IR	-0.39	<0.01*
SBP	-0.68	<0.02*
DBP	-0.60	<0.02*
BMI	-0.65	<0.01*

**Statistically Significant*

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