Research Article

Relationship between acylated ghrelin and uric acid in prediabetic obese individuals

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Abstract

Objective: To evaluate acylated ghrelin and uric acid in prediabetic subjects and compare it with type 2 diabetics and healthy subjects and to examine the relationship between plasma acylated ghrelin and uric acid concentrations. **Method:** The study was conducted on 100 subjects: 50 subjects of prediabetes (Group - I) and 50 subjects of type 2 diabetes (Group - II). 50 healthy non-diabetic control subjects (Group-III) of same age of either sex were selected. All subjects with BMI > 30 kg/m^2 were considered. Blood samples were analyzed for fasting blood glucose, insulin, HbA1c, uric acid and acylated ghrelin. **Result:** Acylated Ghrelin and uric acid increases in group -I when compared with group -III and decreases in group-II when compared to group –I. The strongest positive association of acylated ghrelin was observed between acylated ghrelin and Homa- β in prediabetic subjects while the strongest association of uric acid was observed between uric acid and acylated ghrelin in prediabetic subjects. **Conclusion:** Although the mean values of both acylated ghrelin and uric acid are high in prediabetic subjects as compared to obese control yet the mechanism related to prediabetes i.e. impaired insulin secretion has strong positive association with acylated ghrelin while it shows negative association with uric acid. Hence, acylated ghrelin and uric acid shows inverse relationship in prediabetic obese individuals.

Keywords: Acylated ghrelin, prediabetes, uric acid.

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INTRODUCTION

Diabetes is a chronic disease which is characterized by hyperglycemia, with disturbances in carbohydrate, fat and protein metabolism which results from defects in insulin secretion and/or insulin action. Over the last decades, there has been a rapid increase in the prevalence of type 2 diabetes in parallel with the obesity epidemic. Nowadays a lot of changes in different hormones and cytokines have been reported in DM and one of them is a hormone called ghrelin[1]. Ghrelin is known as a new endocrine pathway in the control of feeding behavior and energy balance in the last decade. Ghrelin is circulated in two major forms in the blood – acyl ghrelin (AG) and unacyl ghrelin (UAG). Acylated ghrelin has some metabolic actions like stimulating the appetite, increasing the secretion of growth hormone, decreasing insulin secretion from the pancreas, reducing energy consumption by the body and effect on growth and peripheral metabolism especially of fats and carbohydrates[2-5]. Earlier studies suggest that Low ghrelin levels are associated with high levels of insulin and insulin resistance[6]. The plasma ghrelin levels rise in malnutrition, cachexia and anorexia nervosa while the levels are reduced in obesity. Recent studies have shown that ghrelin concentration in subjects with DM2 is lower than normal[1]. Some studies supports the hypothesis that low ghrelin can be considered as a risk factor of diabetes mellitus developing[7]. It is still not clear whether low ghrelin level influences insulin secretion or insulin resistance. Uric acid is the final oxidation product of human purine metabolism and is used clinically as a marker of inflammation and metabolic disease. In

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previous study, we observed higher uric acid levels in subjects with coexisting prehypertension and prediabetes[8] and serum uric acid was positively associated with indices of insulin resistance[9,10]. Our attempt was to evaluate acylated ghrelin and uric acid in prediabetic subjects and compare it with type 2 diabetics and healthy subjects. We also studied association of acylated ghrelin and uric acid with insulin resistance indices. Further, we examined the relationship between plasma acylated ghrelin and uric acid concentrations.

MATERIAL AND METHOD

Subjects

The study has been conducted on 100 subjects of both gender and age group 45 to 60 years attending/admitted in OPD/wards of the Department of General Medicine, Jawaharlal Nehru Medical College and Associated Group of Hospitals, Ajmer (Rajasthan). These were further grouped as: 50 subjects of prediabetes (Group - I) and 50 subjects of type 2 diabetes (Group - II).50 healthy non-diabetic subjects of same age of either sex with BMI > 30 kg/m^2 were selected as control (Group-III). Basic anthropometric measurements including weight, height, BMI, Waist, Hip, Waist/hip ratio were carried out and those with BMI >30 kg/m^2 were considered. Subjects who had history of using oral hypoglycemic agents or insulin, medications that affect blood lipids or insulin levels, supplements and appetite altering drugs, heart failure, liver or renal failure and acute or chronic inflammatory disorders were excluded from the study. Consent from all the subjects was obtained for the study.

Measurements

Standard OGTT was performed for all the study subjects: 75 grams of oral glucose load was given and blood samples were collected after 2 hours for plasma glucose measurement. Fasting blood sample was subjected to fasting plasma glucose, glycosylated hemoglobin (HBA1C) and serum uric acid on fully automated analyzer (Randox Imola) using Randox kits. Diagnosis of prediabetes was made according to the criteria recommended by the American Diabetes Association standards 2014[11]. Fasting serum insulin was estimated using enzyme-linked immunosorbent assay (ELISA) technique. Following indexes derived from fasting blood samples for assessment of insulin sensitivity/resistance were calculated:

- Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) was calculated using the formula glucose x insulin / 405[12]
- Homeostasis Model Assessment for Beta cell function (HOMA-β) was calculated using the formula 360 x insulin/ (glucose - 63)% [12]

To acquire accurate data on acylated ghrelin concentrations, a standard procedure for the collection of blood samples was used including collection of blood samples with EDTA–aprotinin, keep them chilled and centrifuge as soon as possible, within 30 min after the collection; and acidification with 1 mol/L HCl (10% of sample volume) added to the plasma sample for adjustment to pH 4 to preserve plasma acylated ghrelin. Plasma acylated-ghrelin was determined using the Elisa kit by SPI-BIO. These tests were done with long immunological reaction method (incubation 4 to 20 hours) to achieve maximum sensitivity of 0.3 pg/ml for acylated ghrelin.

Statistical analyses

Data were recorded in a predesigned proforma and managed in an excel spread sheet. Data were reported as mean ± SD (standard deviation). All p values were based on a two-sided test of statistical significance done by Graph pad software (Quick calcs).Significance was accepted at the level of $p < 0.05$.

RESULTS

Table 1 depicts the anthropometric parameters of prediabetics, newly diagnosed type 2 diabetics and obese (nondiabetic) control, subjects. The biochemical parameters viz., plasma glucose (mg/dl), serum insulin (μIU/ml), serum uric acid (mg/dl) and plasma acylated ghrelin (pg/ml) levels in prediabetics [Group -I] and obese (nondiabetic) control [Group- III] are summarized in Table 2. Fasting plasma glucose, Homa –IR and Homa –β was significantly higher in group –I but serum insulin did not significantly differ. The analysis showed that in group – I, Uric acid (5.22 ± 0.96 vs 6.06 ± 0.86 mg/dl, $p<0.0001$) and Acylated ghrelin (39.64 ± 8.24 vs 44.86 ± 11.57 pg/ml, $p = 0.0108$) levels were significantly higher as compared to group – III. As shown in Table 3, newly diagnosed type 2 diabetics (Group -II) had significantly higher fasting plasma glucose, serum insulin, Homa –IR and Homa –β when compared with group –I ($p< 0.0001$).Serum Uric acid and plasma acylated ghrelin was lower in group-II than in group-I, but the difference was statistically significant for plasma acylated ghrelin ($p< 0.0001$) and non significant for serum uric acid ($p = 0.1068$).The mean values of Glucose, insulin, homa –IR and homa- β shows sturdy increase in all 3 groups while both acylated ghrelin and uric acid increases in group -I when compared with group -III and decreases in group-II when compared to group –I.(Fig.1)

Pearson correlation coefficient (r) analysis was used to determine the correlation of serum uric acid and plasma acylated ghrelin levels each with plasma glucose, serum insulin, Homa –IR and Homa- β in all subjects. Correlation analysis between acylated ghrelin and other

biochemical parameters (Table 4) showed that acylated ghrelin is significantly positively correlated in group-III while significantly negatively correlated in group – I and II with glucose and homa-IR. Acylated ghrelin is significantly negatively correlated in group-III while significantly positively correlated in group – I and II with Homa- β and serum insulin. The strongest positive association of acylated ghrelin among insulin, insulin indices and uric acid was observed between acylated ghrelin and Homa- β in prediabetic subjects. Positive statistically significant correlations between uric acid and glucose, insulin and homa-IR were noted in group –III and I while negative significant in group-II. Homa –β showed an inverse relationship being significantly negative in group –I and III while positively significant in group-II. (Table 5) Unlike trend of correlations in the 3 groups, analysis of association between acylated ghrelin and uric acid revealed positive statistically significant correlation in group-III (r = + 0.38), negative statistically significant correlation in group-I(r = - 0.20) and positive statistically significant correlation in group-II(r = + 0.21) (Table 5). The strongest association of uric acid among insulin, insulin indices and acylated ghrelin was observed between uric acid and acylated ghrelin in prediabetic subjects. Although the mean values of both acylated ghrelin and uric acid are high in prediabetic subjects still they show an inverse association.

Table 1: Anthropometric variables of prediabetic (Group-I), type 2 diabetic (Group-II) and obese control groups (Group-III)

Parameters	Group-i (mean ± sd)	Group-ii (mean ± sd)	P^1	Group-iii (mean ± sd)	P^2
Bmi	33.31 ± 5.38	35.28 ± 6.71	0.150	32.34 ± 4.62	0.360
Waist	105.31 ± 12.08	110.38 ± 12.83	0.077	100.74 ± 11.72	0.071
Hip	113.74 ± 10.00	117.38 ± 12.91	0.236	113.95 ± 9.03	0.857
W/h	0.93 ± 0.06	0.94 ± 0.08	0.498	0.88 ± 0.07	0.003

p^1 for pairwise comparison Group-I vs Group-II
p^2 for pairwise comparison Group-I vs Group-III

Table 2: Biochemical characteristics of obese control (Group-III) and prediabetic (Group- I)

Parameters	Group-iii (mean ± sd)	Group-i (mean ± sd)	P- value
Glucose	86.19 ± 8.57	113.16 ± 7.08	< 0.0001
Insulin	11.34 ± 3.10	12.35 ± 2.60	0.0807
Homa-ir	2.46 ± 0.87	3.44 ± 0.75	< 0.0001
Homa-β	190.53 ± 54.53	91.07 ± 24.38	< 0.0001
Uric acid	5.22 ± 0.96	6.06 ± 0.86	< 0.0001
Acylated ghrelin	39.64 ± 8.24	44.86 ± 11.57	0.0108

p value <0.0001,highly significant (HS); p value <0.01,significant (S); p value >0.05,non significant (NS)

Table 3: Biochemical characteristics of prediabetic (Group- I) and type 2 DM (Group-II)

Parameters	Group-i (mean ± sd)	Group-i (mean ± sd)	P- value
Glucose	113.16 ± 7.08	176.89 ± 28.40	< 0.0001
Insulin	12.35 ± 2.60	15.03 ± 3.35	< 0.0001
Homa-ir	3.44 ± 0.75	6.75 ± 2.43	< 0.0001
Homa-β	91.07 ± 24.38	48.07 ± 6.55	< 0.0001
Uric acid	6.06 ± 0.86	5.78 ± 0.86	0.1068
Acylated ghrelin	44.86 ± 11.57	34.25 ± 9.30	< 0.0001

p value <0.0001, highly significant (HS); p value >0.05,non significant (NS)

Table 4: Correlation of acylated ghrelin with biochemical variables in all subjects

Parameters	Group-iii	Group-i	Group- ii
Glucose	+0.60*	-0.86*	-0.58*
Insulin	+0.58*	+0.18*	-0.57*
Homa-ir	+0.59*	-0.07*	-0.62*
Homa-β	-0.42*	+0.59*	0.02*

*Correlation is significant at 0.0001 level (2-tailed)

Table 5: Correlation of uric acid with biochemical variables in all subjects

+Parameters	Group-iii	Group-i	Group- ii
Glucose	+0.75*	+0.25*	-0.46*
Insulin	+0.72*	+0.02*	-0.34*
Homa-ir	+0.74*	+0.09*	-0.45

Homa-β	-0.46*	-0.12*	+0.23*
Acylated ghrelin	+0.38*	-0.20*	+0.21*

*Correlation is significant at 0.0001 level (2-tailed)

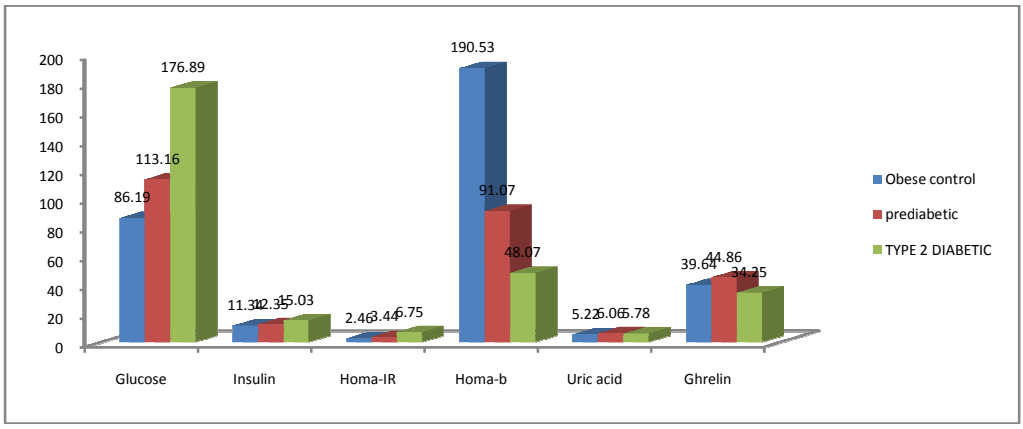

Figure 1: Comparison of biochemical variables in all subjects

DISCUSSION

Our study demonstrates that keeping no significant difference in BMI among the three groups, acylated ghrelin secretion is already altered in prediabetic conditions and the majority of subjects with prediabetes eventually will develop type 2 DM with further alterations in ghrelin metabolism although the acylated ghrelin levels in all the subjects were lower than the normal values. According to previous studies, the onset of decline in acylated ghrelin is prior to the onset of hyperglycemia.[13] Edmann *et al.*[1] studied the effect of weight on ghrelin levels and found that ghrelin levels were lower in obese diabetics compared to normal obese individuals which were in agreement to our study results. Our study also found that ghrelin is inversely related to blood glucose and ghrelin levels are lower in patients with type 2 DM which was similar to study done by Seppo *et al*[7]. We also suggested that acylated ghrelin is strongly influenced by insulin secretion (homa- β) than insulin resistance (homa -IR) in prediabetics. Recent studies have also reported the same.[14] Studies have shown that uric acid is significantly elevated in prediabetic stages and low in diabetes, and rises again after the development of renal insufficiency which was similar to our observation.[15] A biological mechanism underlying the bell-shaped relation between blood glucose levels and serum uric acid levels is thought to be due to the uricosuric effect of glycosuria, which occurs when the blood glucose level is greater than 180 mg/dl.[16] Higher insulin levels are known to reduce renal excretion of urate. Insulin may enhance renal urate reabsorption via stimulation of the urate-anion exchanger URAT1 and/or the sodium-dependent anion cotransporter in brush border membranes of the renal proximal tubule.[17] Correlation of uric acid with biochemical variables cannot rule out obesity and diabetes except acylated ghrelin. Sudhindra Rao M *et al*[18] have also concluded uric acid as potential biomarker independent of insulin indices. We for the first time demonstrated the association between acylated ghrelin and uric acid in obese subjects. This association clearly demarcates obese and prediabetes and the transition of prediabetes to diabetes. It might also predict the different stages of hyperglycemic states.

CONCLUSION

Higher plasma acylated ghrelin and serum uric acid were associated with greater risk of prediabetes suggesting deterioration of glucose metabolism at an early stage irrespective of the subjects being obese. The association between acylated ghrelin and uric acid might produce an insight to the therapeutic challenges for prediabetic and diabetic patients. This holds further more importance as uric acid has now been considered as an independent marker for diabetes associated NAFLD patients. Longitudinal studies with large sample size would help us to clarify the exact mechanism linking acylated ghrelin and uric acid.

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