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Original Research Paper

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EVALUATION OF SERUM GHRELIN LEVEL IN IMPAIRED GLUCOSE REGULATION

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ABSTRACT

Background: A newly discovered endocrine substance called ghrelin is thought to govern eating habits and energy balance. Recent research has revealed that people with diabetes mellitus type 2 (DM 2) had lower-than-normal ghrelin concentrations. To make the clear ghrelin and insulin resistance, as well as diabetes type 2: a cross-sectional research was planned. **Method & Material:** Present study is a cross-sectional study,was conducted in the Department of Biochemistry,J.L.N. Medical college,Ajmer(Raj.).186 subjects were enrolled in three groups, 62 with DM2,62 prediabetes state and 62 norml subjects of first-degree relatives of diabetic group. After clinical examination, blood samples were taken to measure fasting blood glucose, HbA1c, lipids, insulin, and ghrelin concentrations. **Result:** The average blood levels of Serum ghrelin in all groups (52.6 ± 30.2 pg/ml) were lower than the normal range (120.3± 36.4 pg/ml) (P: 0.006) with no discernible variation between groups (P: 0.1).P articipants showed an inverse connection between ghrelin level and insulin resistance (HOMA-IR). **Conclusion:** Serum ghrelin can be used as biomarker for early dedection of predaibetic and diabetic patient in general population to prevent the morbidty and mortality with is associated with impaired glucose regulation.

KEYWORDS : Ghrelin,Diabetes mellitus, Prediabetes

INTRODUCTION

Diabetes is quickly turning into the pandemic of the twenty-first century.(1) Over the past 30 years, diabetes' position has evolved from that of a moderate illness to one of the leading causes of morbidity and mortality [2]. Prevalence rates for diabetes and pre-diabetes were 10.3 percent and 7.3%, respectively, according to the 2017 ICMR-INDIAB population-based cross-sectional survey [3]. Many alterations in various hormones and cytokines have recently been linked to DM, one of which is a hormone called ghrelin [4].

In the past ten years, ghrelin has been known as a novel endocrine route in the regulation of food behaviour and energy balance. The stomach mucosa is the primary site of production for the 28-amino acid hormone ghrelin [4–7]. Acylated ghrelin, the active form of ghrelin, has some metabolic actions including stimulating appetite, increasing growth hormone secretion, decreasing insulin secretion from the pancreas, reducing energy expenditure by the body, and effects on growth and peripheral metabolism, particularly of fats and carbohydrates [8–11]. Growth hormone receptors (GHS-R) in the hypothalamus-pituitary axis, kidney, small intestine, and placenta influence the actions of ghrelin, which promotes growth hormone production and inhibits the release of calcium and insulin from the pancreas.

Another potential mode of action for ghrelin is the reduction of electrical activity in -cells.High insulin and insulin resistance levels are correlated with low ghrelin levels [12]. In contrast, while levels are decreased in obesity, they increase in anorexia nervosa, cachexia, and malnutrition. In obesity, lower ghrelin levels have been linked to greater insulin levels, and in other studies, lower ghrelin levels after meals have been linked to higher insulin levels following carbohydrate intake.

Recent research has revealed that people with DM2 have lower-than-normal ghrelin concentrations [3]. According to some research, a low ghrelin level might be viewed as a potential risk factor for the development of diabetes mellitus [12]. It is unclear if low ghrelin levels are a risk factor for DM2 and whether they occur first or afterwards [13].Uncertainty exists about the primary or secondary nature of the decreased ghrelin content in DM2. In order to better understand serum ghrelin concentrations with new-onset diabetes mellitus type 2, impaired fasting glucose (IFG) (prediabetes situation), and normoglycemic first-degree relatives of patients with diabetes mellitus type 2, this study was designed. Additionally, we sought to understand the association between insulin resistance and serum ghrelin in the research groups.

MATERIALS AND METHODS:

This study is a cross-sectional study, was conducted in the Department of Biochemistry, J.L.N.Medical college and Associated group of Hospitals, Ajmer(Raj.).124 cases of predaibetes,Diabetes Mellitus attending Medical OPD of J.L.N. Hospital were enrolled in three groups,62 newly diagnosed DM2 subjects (one year or less) were put into the first group.62 with fasting plasma glucose (FPG) concentration between 100 and 126 mg/dl confirmed twice in repeated measurements or those with impaired glucose tolerance (IGT)defined by ADA criteria (Two-hour plasma glucose 140–200 mg/dL during a75 gr oral anhydrous glucose tolerance test)were considered as prediabetes group 2.For control group 62 Offspring of patients with DM2 who were more than twenty years old were invited and checked for their FPG and those who were normoglycemic (FPG <100 mg/dl) were entered in 3rd group.

This study was approved by institutional ethical committee. All the participants were informed about the aims of study and written consent were obtained from all of them.

Inclusion Criteria for study group

Age group between 20-50 year of both sex diagnosed as DM2,predaibetec and healthy individuals.

Exclusion Criteria for study group

- Patient with history of using oral hypoglycemic agents or insulin.

- Patient with history of medications that affect blood lipids or insulin levels, supplements and appetite altering drugs.
- Case of Heart failure
- Hepatic and Renal Failure cases
- Acute and Chronic Inflammatory dieases .

Venus Blood samples were taken after at least 12 hours of fasting in all the participants. Plasma glucose was measured by the glucose-peroxidase colorimetric enzymatic method with a sensitivity of 5 mg/dl. HbA1c Serum Cholesterol and Triglyceride and High density lipoprotein cholesterol (HDL-C) levels were measured with AU-680 fully autoanalyeser, fasting serum Insulin level were measured with electro chemilu minescence immunoassay (ECLIA) using commertial kits (Roche, German), with sensitivity of 0.75 μiu/ml (normal range:0.7-25 μiu/ml). To calculate insulin resistance, HOMA-IR was used based on the formula of glucose × insulin/405 and values higher than 2.1 was considered insulin resistance[14].

A standard approach for the collection of blood samples was employed, including collection of blood samples, to get precise data on ghrelin concentrations. Place a full blood sample in a centrifuge for 20 minutes at a speed of around 1000 g for 2 hours at room temperature or overnight at 2 to 8 °C. Take the supernatant and do the test right away.

Serum ghrelin was determined using the Elisa kit from Wuhan Fine Biotech Co., Ltd,china.These tests were done with long immunological reaction method (incubation 4 to 20 hours) to achieve maximum sensitivity of 0.3 pg / ml for serum ghrelin. Intra assay and inter assay CV of the kit to measure acylated ghrelin was <8% and <10% respectively.

Statistical Analysis

All data were analyzed with SPSS-13 version. Descriptive statistics such as mean,median and standard deviation were used to describe the statistics. ANOVA was used to compare the groups for quantitative and chi-square test was used for qualitative variables. To assess the relationship between the variables simple and multivariate regression analysis were used.

RESULTS

186 eligible patients were assigned in 3 groups as follows: 62 subjects with diabetes mellitus type 2 in 1st group, 62 patients with IFG or IGT in 2nd group, and 62 participants with normoglycemic status in 3rd group.The mean age of the first group was 48.7 ± 12.6 years and in the second group was 52.6 ±13.2 years. Although no significant difference was found between diabetic and prediabetic patients for their age, normoglycemic subjects in the third group were significantly younger(31.4 ± 10.3, p < 0.0001). There were no significant differences within three groups by sex.Clinical and laboratory characteristics of the participants have been illustrated in Table 1.

Table -1 Anthroprometric Parameters Of DM2,Prediabetic And Control Healthy Subjects

	Group1 (Dm2) [N: 62]	Group2 (prediabetic) N: 62]	Group 3(control) [N: 62]	P-value
Age (years)	48.7 ± 12.6	52.6 ± 13.2	31.4 ± 10.3	0.00011
Weight (kg)	78.2 ± 18.2	73.7 ± 13.2	77.3 ± 14.1	0.521
BMI(kg/m2)	29.2 ± 5.4	27.8 ± 4.5	28.8 ± 4.3	0.548

As illustrated in Table 2, triglyceride (TG) concentration in the diabetic group was higher than two other (p < 0.0001). Although HDL-c concentrations were lower in diabetic and prediabetic subjects compared with normal individuals (p < 0.0001), the mean levels of LDL-c had no significant differences among the three groups. The prevalence of hypercholesterolemia in diabetic group was significantly higher than the pre-diabetic and also normoglycemic subjects (p: 0.008).

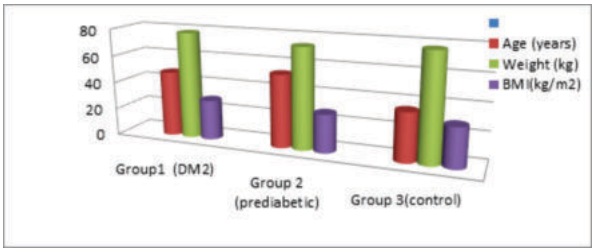
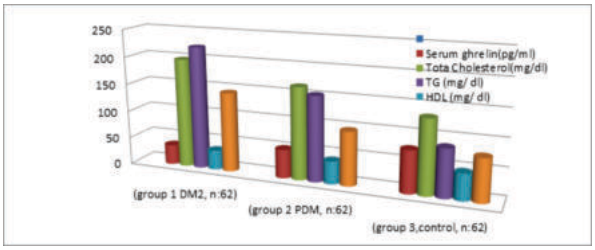


Table 2 Comparison of Lipid Profile and Serum Ghrelin in three study groups

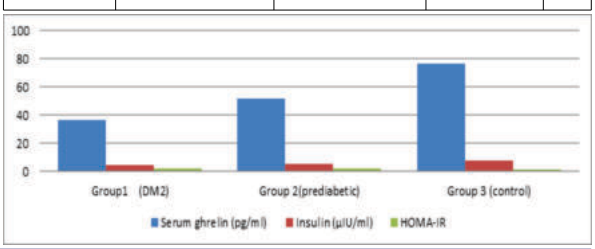
Lab.variable	Mean ±SD median (group 1 DM2, n:62)	Mean ±SD median (group 2 PDM, n:62)	Mean ± SD median (group 3 control, n:62)	P - value
Serum ghrelin (pg/ml)	37.2 ± 31.2 36.1	49.1 ± 13.4 51.9	71.5 ± 46.1 76.3	0.1
Tota Cholesterol (mg/dl)	186.3 ±31.4 198	161.6 ± 45.6 166	138.6 ±20.1 133	0.1
TG (mg/dl)	215.9 ±91.7 222	153.4 ± 82 153.5	86 ± 44.5 86	<0.0001
HDL (mg/dl)	35.2 ± 6.4 32	40.1 ± 7.9 41	47.72 ± 3.9 47.5	<0.0001
LDL (mg/dl)	141.4 ± 35.5 144	94.2 ±38.7 97.5	71.6 ± 17.2 77	0.2



There was an inverse relationship between acylated ghrelin and insulin levels .Serum ghrelin concentrations in all three groups(52.6 ± 30.2 pg/ml) were lower than normal values (120 ± 36.4 *pg/ml*) (P: 0.006).

Table 3. Comparison of Serum Ghrelin and serum insulin in study groups

Lab.variable	Group1(DM2) [N: 62] Mean ±SD median	Group 2(prediabetic) [N: 62] Mean ±SD median	Group 3 (control) [N: 62] Mean ±SD median	P
Serum ghrelin (pg/ml)	37.2 ± 33.2 36.1	49.1 ± 10.5 51.9	71.5 ± 40.2 76.3	0.1
Insulin (μIU/ml)	5.3 ± 7.5 4.3	6.5 ± 5.3 5.1	7.6 ± 8.6 7.5	0.18
HOMA-IR	2.1 ± 2.5 2.3	1.7 ± 1.4 1.6	1.4 ± 1.6 1.5	0.4



BMI: Body mass index, TG: triglycerid.CHOL: total cholestrole, LDL: low density lipoprotein.

P value <0.05 is considered statistically significant

DISCUSION:

This study shown that serum ghrelin levels are lower than normal in all of the participants, including those with overt DM, those in the early stages of prediabetes, and first-degree relatives of those with DM who are not diabetic. However, there was no discernible change in the mean blood concentrations of acylated ghrelin across the three research groups.

Seppo et al. [13] discovered that ghrelin levels are negatively connected to blood glucose levels and that individuals with type 2 DM have lower ghrelin levels.Also shown an independent relationship between low ghrelin levels and insulin concentrations, as well as the prevalence of type 2 diabetes and insulin resistance. They raised the question of whether decreased ghrelin levels in type 2 DM are a main or secondary cause of the condition.

Our investigation shown that serum ghrelin concentrations, even in insulin-resistant, normoglycemic individuals (group 3), are markedly lower than the typical values previously described for the general population. This problem likely demonstrates that acylated ghrelin levels may start to drop before hyperglycemia manifests itself. However, this finding may be limited since our study lacked a normal control group without insulin resistance.

Poyoko et al. [19] discovered an inverse correlation between Ghrelin and IGF-1 levels and insulin resistance in type 2 diabetes in patients in their middle years. Additionally, we discovered that all participants' levels of serum ghrelin and insulin resistance were negatively correlated.

Obesity and diabetes lower ghrelin levels, and changes in the ghrelin precursor molecule have been related to insulin resistance. Ghrelin may aid in the prevention of diabetes-related illness since it appears to have antioxidant and antiapoptotic effects on endothelial and neuronal tissue. Further research is necessary to determine the function of ghrelin in the onset of diabetes and its potential therapeutic value for some of the complications associated with the disease

CONCLUSION

Leval of Serum Ghrelin was found to be decreases prior to the onset of hyperglycemia i.e predaibetic,DM2, also can be used as biomarker for early deduction of prediabetic and DM2 patient in general population to prevent the morbidty and mortality with is associated with impaired glucose regulation. Based on laboratory investigations and symptoms, cases of DM2,prediabetic need to be monitored and treated individually.

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