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Comparative study of acylated ghrelin levels in obese diabetes mellitus type 2 and lean diabetes mellitus type 2 female aged 30-45.

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ABSTRACT… Objectives: To critically analyze the concentration of Acylated ghrelin and its comparative level between lean and obese type two Diabetes Mellitus female patients aged Thrity-fourty five. **Study Design:** Cohort study design. **Setting:** Department of Endocrinology, Lahore General Hospital, Lahore and Sheikh Zayad Hospital Lahore. **Period:** 1st March 2019 to 31st August 2019 (6 months). **Material & Methods:** Blood sample of fifty female individuals were taken after twelve hours of fasting which were divided into two groups obese type two diabetes mellitus patients having BMI> twenty eight and lean type two diabetes mellitus patients. For measurement of ghrelin concentrations, blood samples collected in specialized EDTA–aprotinin chilled test tubes were immediately centrifuged at two hundreds rpm and their acidification with 1 mol/L HCl (10% of sample volume) done to adjust pH four to preserve plasma acylated ghrelin. The serum was stored at -20c for hormonal assay of Ghrelin by Elisa kit of Biovender Laboratani of Czek Republic. **Result:** The statistical analysis of Acylated ghrelin concentration was done by using paired t-test and one way ANOVA showing significant difference between the concentration of ghrelin in both obese and lean with reference to BMI having P-value<0.0001. **Conclusions:** The study showed that acylated ghrelin levels were comparative on the lower side in obese type two DM as compared to lean type two DM with significant P-value <0.0001 by using two-tailed P-value.

Key words: Acylated Ghrelin, BMI, Diabetes Mellitus, Glucose, Insulin.

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INTRODUCTION

In modern world obesity and diabetes mellitus are major health issues which affect these days almost every culture and racial civilization specially the underdeveloped countries and putting huge burden on their health care systems.[1] Diabetes mellitus is a group of metabolic diseases presented by chronically high level of glucose because of problem in insulin secretion by beta cells of pancreas and resistance to its action on target tissues especially skeletal muscles, adipocytes and to a lesser extent liver. These defects are usually found at insulin receptors levels, its signal transmission system and effectors enzymes or genes which caused unusual metabolic changes in carbohydrates, lipids, and proteins metabolism.2 Obesity which is due to imbalance in caloric intake and energy expenditure, results in excess lipids store in adipocytes. When fat cells become thoroughly soaked, lipids begin to assemble inside heptocytes and muscles that make them insulin resistant lead to pancreatic cell secretion insufficient and make susceptible to type 2 diabetes mellitus.[3]

In 1999, Masayasu and Kangawa with their colleagues extracted and pruified the gastrointestinal peptide hormone named ghrelin from rat stomach, which act as the endogenic substance for the growth hormone secretagogue receptor (GHSR)1a., This gastric hormone acts on anterior pituitary gland and facilities the release of growth hormon.[4] In 2000, Mark Heiman and Matthias Tschöp found that hormone named ghrelin regulates food intake via acting on brain, glucose metabolism, body weight and adiposity.[5]

Within gastric oxyntic glands appetite stimulant hormone named Ghrelin produced by X/A-like cells into two forms: active form (acylated ghrelin) and inactive form (des-acyl ghrelin). For biological actions of it, its convert after synthesis by adding fatty acid octanyl group preferably C8:or C10, on 3rd N-terminal amino acid position, which is a serine.[6] The protein coding gene, membrane bound O-acyltransferase 4 (MBOAT4), is vital in the activation of ghrelin and located on chromosome 8 and which transcribes an only known post translationally acylate ghrelin enzyme protein called as ghrelin O-acyltransferase (GOAT).[7] Ghrelin binding with seven transmembrane G protein-coupled receptor i.e GHSR1 promotes most of its neurological actions. GHSR1a is localized in feeding center neurons of hypothalamus that secretes food stimulating neuropeptide Y and Agouti related peptide. GHSR1 present in two forms, GHSR1a which is mediating most of metabolic effects and a truncated form GHSR1b.[8] Besides that for appetite amplify actions of ghrelin, it also require the activation of hypothalamic sirtuin-1 (SIRT1) / p53, AMP-activated protein kinase and mammalian target of rapamycin pathways and these ultimately increase food intake.[9,10] The prefect cannabinoids signaling pathway is also required for activation of AMPK signaling pathway for effect of ghrelin on appetite.[11,12] On one side ghrelin increases the activity of these neurons whereas on the other hand it inhibits neurons that express proopiomelanocortin food satiety neuropeptide.[13,14] The importance of this hypothalamic melanocortinergic system is highlighted by fact that in mice lack of Npy and Agrp neuropeptides or their block fails to increases food intake.[15,16]

The glucose regulation role of ghrelin is supported by fact that if GOAT is inhibited pharmacologically it would prevent fall in blood glucose level during utmost spell of calorie restriction, improves the glucose level control and increase release of insulin amount.[17,18] Ghrelin secretion increases in undulating fashion before taking meal in fasting and decreases after feeding.[19,5] This acute increase in ghrelin level is recommending that it might act as a sign for meal anticipation concerned with meal timing instead of starting eating.[20] When ghrelin administrated both centrally and peripherally in rats, it initiates intake of food, decreases vitality causing increase in body weight.[21,13,22,5,23] Similar increase in craving and intake of food after intravenously administration of ghrelin in human seems.[23] Ghrelin release from gastric tissues crossing the blood-brain barrier reaches brain and initiates appetite by central and peripheral pathways.[24,25] Whereas centrally synthesized ghrelin activates arcuate nucleus, paraventricular nucleus, dorsomedial region, central nucleus of amygdala, and the nucleus of solitary tract in the hypothalamus and resultant in food intake.[26,27,24]

Inhibition of insulin secretion by ghrelin is mediated through Gαi-dependent GHSR1a signaling of the beta-cells and it also involves interaction with the somatostatin receptor subtype-5 (SST5).[17,28] Despite the fact that inhibitory response among ghrelin and insulin recommends the converse connection between the two however relying upon test conditions, ghrelin at low level may inhibitory affect insulin discharge and a stimulatory impact at high level.[29,30,31,32,33] Various clinical investigations recommended the opposite relation between ghrelin and insulin in glucose level regulation that when ghrelin administer the plasma level of glucose increases and insulin level decreases.[29] This relationship is additionally emphasized in restorative examinations between ghrelin, impaired glucose resilience and insulin resistance The blocking role of ghrelin on insulin secretion is additionally upheld in clinical preliminaries where a single intravenous dose of ghrelin elevated the glucose plasma levels in lean and obese patients follow by fall in fasting insulin levels.[19,34,5] Hence in the light of pervious notion it was observed in clinical study that plasma ghrelin concentration as predictor for type 2 diabetes was decreased in the healthy offspring of these diseases' subjects. Whereas there were compensatory high insulin secretions because of insulin resistance which significantly reduced ghrelin concentrations in type 2 diabetes patients. Also, in various model trials significant difference was observed even by considering age, sex and BMI of subjects that patients with diabetes type 2 have lower fasting total ghrelin plasma level as compared to subjects

without diabetes type 2.[34]

MATERIAL & METHODS

Sampling was done from the endocrinology outdoor patient department of the General hospital and Sheikh Zayad hospital Lahore after the Informed consent from the Obese and lean female patients having Diabetes Mellitus type two which included their basic information plus their height weight and waist measurements for BMI. The both groups had equal numbers i.e twenty five in each groups. The inclusion criteria was Obese newly diagnosed type two DM female BMI >twenty eight and Lean newly diagnosed type two DM Female. Whereas the exclusion criteria were any gastrointestinal disease or cachectic states such as cancer, thyroid disease, liver disease, or infection, patients with renal impairment, patients of DM more than one year or using any Diet or Medicine interfere with carbohydrates metabolism.

Blood sample of fifty female individuals were taken after twelve hours of fasting which were divided into two groups obese type two diabetes mellitus patients having BMI> twenty eight and lean type two diabetes mellitus patients. For measurement of ghrelin concentrations, blood samples collected in specialized EDTA–aprotinin chilled test tubes were immediately centrifuged at two hundreds rpm and their acidification with 1 mol/L HCl (10% of sample volume) done to adjust pH four to preserve plasma acylated ghrelin. The serum was stored at -20c for hormonal assay of Ghrelin. Eliza kit for the test of Acylated ghrelin was brought from Biovender Co from Czek Republic and specialized test tubes brought from Fsicher Scientific Co from UK.

Study had been conducted after the approval of synopsis by the technical review committee of UOL. The experimental protocol was approved by the Research Ethical Committee of University of Lahore.

RESULT

The statistical analysis of Acylated ghrelin concentration was confirmed on absorbance 405 by using paired t-test and one-way ANOYA. In the Figure-1 show the standard curve of ghrelin concentration at 405 absorbance and R2 value near to 1 whereas the valued concentration of ghrelin has shown in Table-I. The concentration of ghrelin in obese and lean type 2 diabetes mellitus with comparison to BMI had been shown in the Table-II&III respectively.

Using paired t-test on the concentration of acylated ghrelin between obese and lean type 2 diabetes mellitus groups had shown that P-value<0.0001 which shows significant difference between the concentration of ghrelin both obese and lean with reference to BMI as shown in Figure-2.

Also, when paired t-test was applied on the BMI of the Lean and obese type 2 diabetes mellitus significant difference P-value<0.0001 that less than <0.5 observed as shown in Figure-3.

The association between age, BMI and concentration of ghrelin in lean Type 2 DM was shown significant p-value 0.0001 when one-way ANOVA was applied in Figure-4.

In the Figure-5, the comparison between age, BMI and concentration of ghrelin in obese type 2 DM had shown significant P-value < 0.00001 when one-way ANOVA is applied.

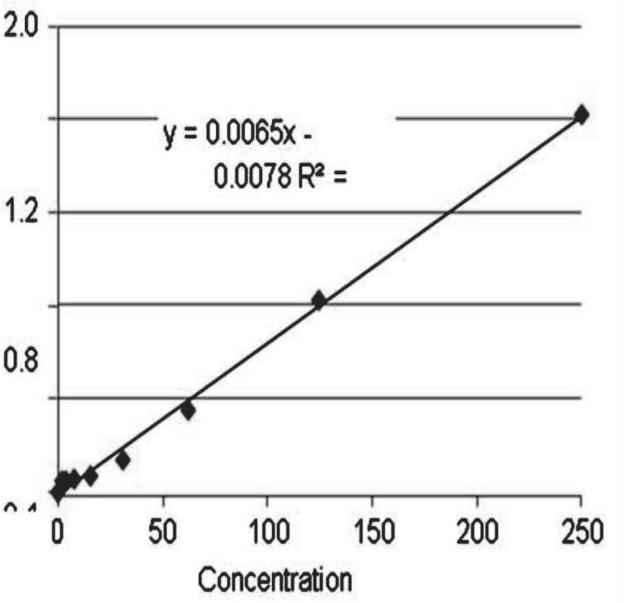

Figure-1

Sample	Sample_ abs	Abs-Blank	conc	Conc. (pg/mL)
1	0.265	0.041	7.51	15.02
2	0.272	0.048	8.58	17.17
3	0.27	0.046	8.28	16.55
4	0.26	0.036	6.74	13.48
5	0.266	0.042	7.66	15.32
6	0.264	0.040	7.35	14.71
7	0.268	0.044	7.97	15.94
8	0.274	0.050	8.89	17.78
9	0.261	0.037	6.89	13.78
10	0.282	0.058	10.12	20.25
11	0.263	0.039	7.20	14.40
12	0.262	0.038	7.05	14.09
13	0.254	0.030	5.82	11.63
14	0.274	0.050	8.89	17.78
15	0.272	0.048	8.58	17.17
16	0.278	0.054	9.51	19.02
17	0.276	0.052	9.20	18.40
18	0.275	0.051	9.05	18.09
19	0.271	0.047	8.43	16.86
20	0.263	0.039	7.20	14.40
21	0.246	0.022	4.58	9.17
22	0.262	0.038	7.05	14.09
23	0.266	0.042	7.66	15.32
24	0.271	0.047	8.43	16.86
25	0.26	0.036	6.74	13.48
26	0.266	0.042	7.66	15.32
27	0.265	0.041	7.51	15.02
28	0.26	0.036	6.74	13.48
29	0.252	0.028	5.51	11.02
30	0.253	0.029	5.66	11.32
31	0.265	0.041	7.51	15.02
32	0.281	0.057	9.97	19.94
33	0.282	0.058	10.12	20.25
34	0.265	0.041	7.51	15.02
35	0.262	0.038	7.05	14.09
36	0.252	0.028	5.51	11.02
37	0.248	0.024	4.89	9.78
38	0.258	0.034	6.43	12.86
39	0.293	0.069	11.82	23.63
40	0.273	0.049	8.74	17.48
41	0.259	0.035	6.58	13.17
42	0.26	0.036	6.74	13.48
43	0.265	0.041	7.51	15.02
44	0.262	0.038	7.05	14.09
45	0.273	0.049	8.74	17.48
46	0.25	0.026	5.20	10.40
47	0.252	0.028	5.51	11.02
48	0.249	0.025	5.05	10.09
49	0.259	0.035	6.58	13.17
50	0.263	0.039	7.20	14.40

Table-I. Concentration of ghrelin in all patients

Serial No	BMI	Ghrelin Conc
01.	30	14.85
02.	31	13.46
03.	45	13.94
04.	38	13.64
05.	42	13.94
06.	45	13.94
07.	26	11.82
08.	35	14.55
09.	26	10
10.	29	13.94
11.	29	14.85
12.	35	13.64
13.	36	14.24
14.	33	13.03
15.	44	15.15
16.	42	14.55
17.	34	11.52
18.	38	13.33
19.	40	14.24
20.	28	11.52
21.	36	14.24
22.	34	11.52
23.	28	10.61
24.	34	14.4
25.	26	11.21
AVERAGE	34.56	13.2852

Table-II. BMI and ghrelin concentration in obese type 2 DM Patients

Serial no	BMI	Ghrelin Conc
1.	24	15.45
2.	20	16.67
3.	21	16.06
4.	25	16.97
5.	23	15.45
6.	24	17.27
7.	24	16.36
8.	25	17.88
9.	23	18.79
10.	23	18.18
11.	24	16.06
12.	23	17.56
13.	22	16.06
14.	19	26.97
15.	24	19.39
16.	25	18.44
17.	25	18.18
18.	24	19.91
19.	20	20
20.	22	21.97
21.	23	20.79
22.	22	20.5
23.	21	23.44
24.	20	20.21
25.	19	30.21
AVERAGE	22.6	19.1508

Table-III. BMI and ghrelin concentration in lean type 2 DM patients

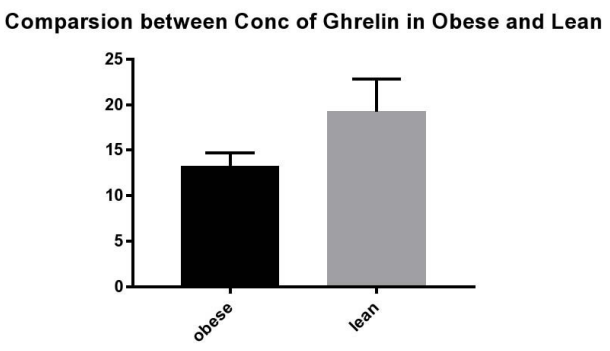

Figure-2

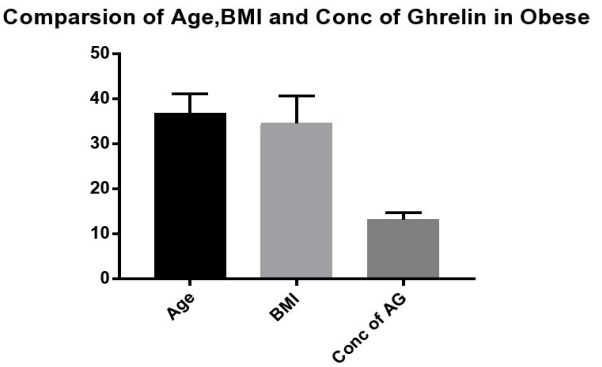

Figure-4.

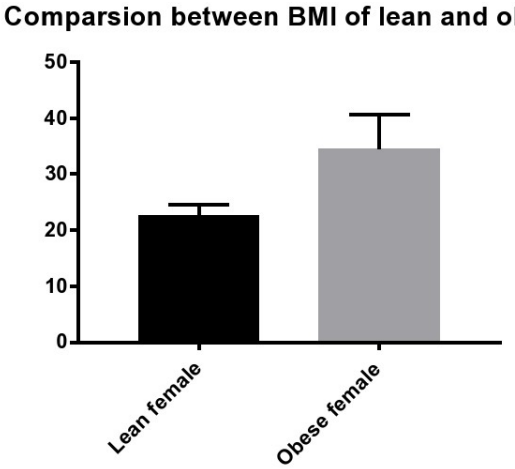

Figure-3.

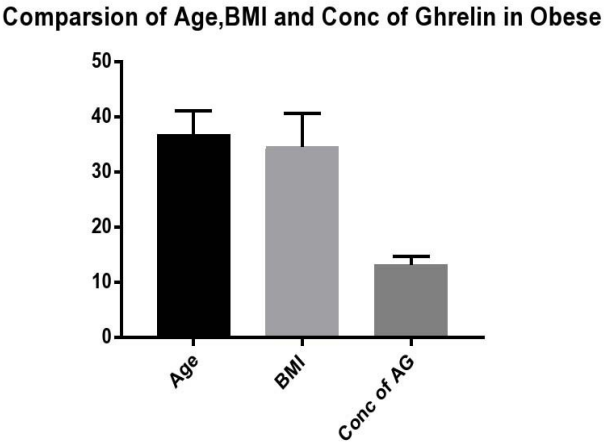

Figure-5.

DISCUSSION

The study was designed to testify the hypothesis that acylated ghrelin level was lower in obese diabetes mellitus type 2 as compared to lean type 2 Diabetes Mellitus. During the research acylated ghrelin levels in obsess found to be on the lower side as compared to lean patients with significant P-value <0.0001 which shown significant difference by using two-tailed P-value. In line above mention theory, their study had also demonstrated that acylated ghrelin levels in subjects of their earlier stages of diabetes was lower as compared to develop full spectrum of the diseases also first degree relatives of these patients who had normal glucose level, had lower levels of active ghrelin as compared to normal people. Whereas acylated ghrelin levels were more associated with body mass index and abdominal girth rather than with high glucose level.[35] BMI, abdominal circumference and levels of ghrelin had inverse relation each other as reported in their study.[36] Edrmann with his colleagues found that subjects with normal glucose level but obese had high ghrelin concentration as compared to those subjects which were obese but high glucose level.[37] Their study found that levels of ghrelin were on lower side in patients with full spectrum of diabetes mellitus and these levels were inversely proportional to the glucose blood level.[38]

They discovered ghrelin amount in subjects having type 2diabetes lower than those of subjects without type 2diabetes. Hence that could be lower because of their higher adiposity.[39] They showed that low plasma ghrelin levels were associated with type 2 diabetes mellitus and insulin resistance. Ghrelin levels were correlated with insulin resilience markers such as body weight, fasting blood insulin level and the HOMA-IR index

in healthy controls. Although the sample size was small healthy peoples have high active ghrelin amount as compared to diabetes type 2 subjects after correction for age differences between the study groups. A similar relation was observed and reported among Finnish participants in the Oulu Project Elucidating Risk of Atherosclerosis study.[40]

Likewise they discovered that fasting levels of ghrelin in normal subjects and patients with anorexia nervosa, straightforward stoutness, or type 2 diabetes mellitus associated contrarily with BMI inside each groups.[41]

CONCLUSION

The study showed that acylated ghrelin levels were comparative on the lower side in obese type two DM as compared to lean type two DM with significant P-value <0.0001 by using two-tailed P-value.

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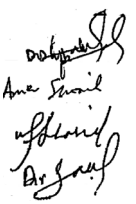
1	Zaheer Iqbal Sheikh	1st Author	
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