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ASSESSMENT OF SERUM GHRELIN AND CYTOKINES PROFILING LEVELS IN TYPE-2 DIABETIC IRAQI PATIENTS

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Abstract

Ghrelin, a peptide hormone is responsible for alteration in energy homeostasis. It also modulates glucose metabolism. Systemic inflammatory markers such as interleukin-6 and interleukin-8 are implicated in the development of diabetes. It has been suggested that levels of ghrelin fluctuates in various physiological conditions like obesity and diabetes mellitus.

The target of the current work was to evaluate serum leptin, ghrelin, interleukin-6 and interleukin-8 levels in type 2 diabetic Iraqi patients.

Forty-five patients who visited to the Medical City Hospital, Baghdad through June, 2019 until November, 2019 with the age of (38-55) years were employed in this study. They were compared with 45 individuals as control group. Fasting glycemic test, lipid profile, serum leptin, ghrelin, interleukin-6 and interleukin-8 were determined in this study.

There was a substantial rise ($p < 0.05$) in serum leptin, interleukin-6 and interleukin-8 in diabetic patients when paralleled with the control group. While, there was a significant decrease ($p= 0.001$) in serum ghrelin in diabetic as compared to the controls (22.18 ± 6.84 *vs*. 32.45± 2.20) ng/ml. Moreover, a considerable elevation in serum leptin, interleukin-6 and interleukin-8 were found in female patients when paralleled with males.

In this study, diabetic patients had higher serum leptin, interleukin-6 and interleukin-8 levels but lower serum ghrelin levels in diabetic patients as paralleled to the controls. This result led to the supposition that the rise in serum leptin level is related with obesity. Also, it suggests that interleukin-6 and interleukin-8 being an inflammatory mediators might be responsible for some underline changes which may donate for the progress of type 2 diabetes.

Key words: Osteoarthritis, Type 2 diabetes mellitus, Leptin, Ghrelin, Interleukin-6, Interleukin-8.

Introduction

Diabetes Mellitus (DM) is a cluster of metabolic diseases manifested by hyperglycemia causing by defeats in insulin secretion/ action or both (Sarkar and Meshram, 2017).

Type 2 DM (T2DM) typically initiates as insulin resistance (IR), a disorder in which the cells do not use insulin well. Reduced insulin secretion and increased IR in T2DM prompts hepatic glucose output resulting in the progress of hyperglycemia (Centers for Disease Control and Prevention, 2017).

It has been reported that adipose tissue releases a number of humoral influences defined as adipokines (Trayhurn *et al.*, 2006). Visceral and subcutaneous adipose tissues produce adipokines (Kershaw and Flier, 2004). It plays a significant role in IR through production of adipose-derived proteins. The adipokine molecules belong to various functional classes comprising endocrine function that are associated with leptin and ghrelin, which are play a main role in a numeral of metabolic roles and control of energy metabolism (Gualillo, 2007).

Leptin, is the most deliberate adipocyte derived hormone with 16 kDa, a product of ob gene primarily produced by white adipose tissue (Zhang *et al.*, 1995). When leptin increases, it leads to IR. Also, leptin triggers lipolysis and suppresses lipogenesis and genetic defect in leptin or its receptor will lead to extreme overeating and massive obesity. So leptin shows an essential pathophysiological connection between T2DM and obesity which is not correctly clear (Osegbe *et al.*, 2016).

Elevation of leptin in DM or obesity makes it an important factor to measure in clinical research studies. For years it has been identified that obesity associated diabetes is related to IR as the central basis for the

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relationship (Michel *et al.*, 2016). Research can also explore leptin therapy in grouping with starving which triggers leptin signaling as a means of regulating obesity and DM (Naylor and Petri, 2016).

Ghrelin is a peptide hormone with growth hormone-releasing activity secreted from stomach A-like cells. It is a novel en-dogenous ligand for growth hormone secretagogue receptor (GHS-R). GHS-R type 1a gene encodes the cognate recep-tor of ghrelin; its full-length sequence contains 366 amino acids encoded by two exons on chromosome 3q25. Ghrelin has been informed to have a positive influence on glucose metabo-lism and insulin sensitivity (Pacifico *et al.*, 2009).

Substantial amounts of interleukin-6 (IL-6) and other markers and mediators of inflammation which can improve IR directly in adipocytes, muscle and hepatic cells leading to systemic disorder of insulin sensitivity and impaired glucose balance (Schmidt *et al.*, 1999).

Interleuken-8 is a pro-inflammatory polypeptide belonging to the CXC chemokine superfamily, categorized by the existence of 2 cysteine residues isolated by an intervening amino acid in the first 3 positions and is secreted by various cell types, comprising adipocytes, T-lymphocytes, monocytes/macrophages, epidermal and endothelial cell. Similarly, it has chemoattractant and mitogenic influences on neutrophils in addition to on T-cells, vascular endothelial cells, vascular smooth muscle cells and monocytes (Marino *et al.*, 2015).

The current work was undertaken to measure serum leptin, ghrelin, IL-6 and IL-8 among Iraqi patients with T2DM.

Materials and Methods

Forty-five patients who visited to the Medical City Hospital, Baghdad through June, 2019 until November, 2019 with the age of (38-55) years were included in this study. Patients who had liver, renal, heart diseases or taken taking a steroid medicine were excluded from this work. All diabetic patients reserved anti-diabetic drugs. They were compared with 45 individuals as control group.

Body mass index (BMI) was deliberate as weight in kilograms divided by height in meters squared. Bloods were obtained from all subjects following fasting after 8-10 hours. Laboratory examinations were down, which encompassed fasting serum glucose (FSG), glycated hemoglobin (HbA1c), lipid profile including: serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL) and low density lipoprotein cholesterol (LDL-C). They were determined by using respective kits (Roche Diagnostics) following manufacturer's protocols in the medical laboratories. Serum leptin, ghrelin, IL-6 and IL-8 were measured using Enzyme Linked Immuno Sorbent Assay (ELISA) technique.

Statistical Analysis

The data were done by SPSS (Statistical Package of Social Science) program, t-test was used to estimate the differences between variant groups. A p value of ≤ 0.05 is deliberated to be substantial.

Results

Clinical and biochemical characteristics of the study groups are illustrated in table 1. The BMI, FSG, HbA1c, TC, TG, LDL-C and VLDL were significantly increased ($p \leq 0.05$) in patients when equated with the control group, while there was a substantial reduction ($p= 0.001$) in serum HDL-C when paralleled with the control group.

Table 2, shows the clinical and biochemical characteristics of patients group. There was a substantial rise in BMI, FSG, HbA1c, TC, TG and LDL-C, while there was a substantial reduction in serum HDL-C in female patients when paralleled with males group.

Table 3, shows the hormonal and cytokines profile of patients and controls. There was a substantial rise ($p < 0.05$) in serum leptin, IL-6 and IL-8 in diabetic patients as paralleled to the controls. While, there was a substantial reduction in serum ghrelin when paralleled with the controls. Additionally, there was a substantial rise ($p < 0.05$) in serum leptin, IL-6 and IL-8 in females patients as compared to males, table 4. There was a decrease in serum ghrelin levels in females patients as paralleled to males, but it was not substantial.

Table 1: Clinical and biochemical characteristics of patients and control groups.

p-value	Means ± SD		Parameters
	Control (n=45)	Patients (n=45)	
-	(22/23)	(22/23)	Gender (Male/Female)
0.05	40.05±2.31	49.20±6.11	Age (Years)
0.01	22.20±2.50	32.30±4.10	BMI (Kg/m^2)
0.001	90.72±3.50	185.50±5.84	FSG (mg/dl)
0.05	4.23±1.06	9.34±3.15	HbA1c (%)
0.0001	125.0±4.50	250.0±10.50	TC (mg/dl)
0.001	87.10±3.20	181.95±9.48	TG (mg/dl)
0.01	62.12±7.52	47.52±5.10	HDL-C (mg/dl)
0.0001	45.46±3.66	166.10±3.51	LDL-C (mg/dl)
0.0001	17.42±0.64	36.39±1.89	VLDL (mg/dl)
-	-	35/10	Family history for DM (Yes/No)
-	-	8.0±7.5	Duration of DM (Years)

Table 2: Clinical and biochemical characteristics of patients.

p-value	Means ± SD		Parameters
	Male (n=22)	Female (n=23)	
0.120	50.10±2.21	48.30±5.12	Age (Years)
0.045	28.20±2.18	35.52±5.90	BMI (Kg/m^2)
0.045	170.80±6.50	200.0± 5.18	FSG (mg/dl)
0.05	8.0±1.8	10.0±2.50	HbA1c (%)
0.045	178.13±12.0	320.18± 10.15	TC (mg/dl)
0.01	170.21± 6.80	192.63±12.50	TAG (mg/dl)
0.045	50.82± 3.28	42.80±5.66	HDL-C (mg/dl)
0.045	93.27± 9.14	238.85±6.60	LDL-C (mg/dl)
0.06	34.04± 1.25	38.53±2.60	VLDL (mg/dl)

Table 3: Hormonal and cytokines profile of patients and controls.

p-value	Means ± SD		Parameters
	Control (n=22)	Patients (n=30)	
0.001	15.50±6.0	25.45±10.20	Leptin (ng/ml)
0.001	32.45± 2.20	22.18 ± 6.84	Ghrelin (ng/ml)
0.01	1.35±0.44	2.38±10.50	IL-6 (ρg/ml)
0.0001	120.65±5.30	210.45±10.18	IL-8 (ρg/ml)

Table 4: Hormonal and cytokines comparisons between males and females patients.

p-value	Means ± SD		Parameters
	Male (n=22)	Female (n=23)	
0.0001	20.50±3.0	30.75±5.20	Leptin (ng/ml)
0.06	23.0± 3.45	22.0 ± 5.60	Ghrelin
0.01	5.25±1.63	15.81±3.50	IL-6 (ρg/ml)
0.0001	178.62±3.45	240.75±9.23	IL-8 (ρg/ml)

Discussion

The mean age of type 2 diabetic patients was 49.20±6.11 years agrees with the fact that T2DM commonly progresses after age 40 years (Olokoba *et al.*, 2012). Family history as a risk factor for DM is in covenant with preceding data (Wagner *et al.*, 2013; Chiwanga *et al.*, 2016). The calculated BMI was found to be 22.10±2.10 Kg/m^2 in normal controls and 32.10±4.02 Kg/m^2 in type 2 diabetic patients. The BMI was found to be highly significantly raised in diabetic patients as paralleled to controls. Statistical analysis showed extremely significant raised BMI when comparison was made between females and males patients, which is in accordance with earlier study (Kumar *et al.*, 2015). Fasting glucose levels were found to be considerably increased in diabetic patients as paralleled to normal healthy subjects. Levels of HbA1c were detected to be statistically substantially greater in type 2 diabetic patients (9.34%) as paralleled to normal healthy individuals (4.23%).

Impaired insulin secretion and increased IR in T2DM induces hepatic glucose output resulting in the development of hyperglycemia. In addition, lipolysis in adipose tissue is stimulated leading to raised circulating levels of free fatty acids (FFA) (Rui, 2014). With reverence to serum lipid profile, serum TC, TG and LDL-C levels were considerably greater in diabetic patients when equated to controls whereas serum HDL-C level was considerably reduced in diabetics. These findings are in parallel with that confirmed in previous literature (Meshram *et al.*, 2016). The unusual levels of serum lipids in diabetics is owed mostly to elevate in the mobilization of FFA from fat depots, since insulin suppresses the hormone sensitive lipase. Higher serum FA are converted into TG, phospholipids and cholesterol in liver which may be discharged into blood (Yassin *et al.*, 2017). Another study inconsistence to present data which was conducted no substantial variance between male and female patients (Nogaroto *et al.*, 2015).

Diverse outcomes obtained in the current work about lipid profile might be due to alterations in patient's characteristics and degree of obesity and IR, metabolic variation of other hormones, probable effects of intermediate metabolism and duration of the disease. Changes in the sample size, geographical difference and duration of the disease encompassed in the revisions may be other probable causes which are accountable of this variation.

Obesity is a superior fraction of body fat in relation to lean mass which is major adequate to harmfully affect health. The disease can be affected by lifestyle or hereditary factors. Obesity is hereditary mainly due to genetic influences. Mutation of the gene which causes obesity, results in improved food intake, raised insulin and substantial obesity in T2DM (Sorensen *et al.*, 1996).

It has been reported that lack of leptin in mice has been originate to cause severe obesity because of increased food intake and reduced energy expenditure. This has correspondingly been confirmed in humans. The leptin deficient also progress hyperinsulinemia, frequently times leading to DM. These alterations can be inverted through the administration of the leptin (Farooqi *et al.*, 2002). This adipocyte-specific protein delivered the first relations to the body's system regulating body weight. Even however leptin usually rises with adiposity, it was confirmed that at each BMI value there is inconsistency in serum leptin level. This proposes that there are alterations in its secretion rate from fat (Myers *et al.*, 2010).

Leptin is released from adipocytes in response to improved fat content by aggregate the fat mobilization from the adipose tissue, fatty acid oxidation by the liver and by reducing the appetite (Park and Ahima, 2015). In the present work, as predictable, it establish considerably

greater leptin concentration in diabetic patients, in comparison with healthy subjects. Diabetic patients had higher BMI beside higher leptin levels. This result was in agreement with the study showed by Abdul Hadi and Sultan, 2016 who also informed considerably high leptin concentrations in obese subjects (Abdul Hadi and Sultan, 2016).

A most probable clarification for this increase will be as follows. As the fat content is improved due to low level of hormone sensitive lipase in the obese subjects, leptin level will rise and this rise will still not be able to raise the fat mobilization, due to insufficient amount of hormone sensitive lipase, which is already completely triggered. Thus the fat content will continue to rise with parallel rise in serum leptin concentration (Gupta and Mukherjee, 2018), which is in accordance with the current study.

Moreover, Ghrelin is well-identified to modify insulin secretion and, hence, is observed as in IR sign (Tschop *et al.*, 2001). Diabetic patients have lesser ghrelin levels also, ghrelin had a remarkable negative relationship with the HOMA-IR index regardless of other limitations of IR, which is in accordance with the study of Gruzdeva *et al*., 2014. (Gruzdeva *et al.*, 2014).

Ghrelin well confirmed glycemic effects propose that pharmacological inhibition of ghrelin action might be as helpful effects in T2DM treatment. The relation between insulin and ghrelin is also proposed by the truth that both hormones reveal a reciprocal correlation over the day with insulin levels being high when ghrelin concentrations are decreased and vice versa. Also, epidemiological trials support the converse association between ghrelin and indexes of impaired glucose tolerance and IR (Collden *et al.*, 2017).

Furthermore, diabetes drug, which increases tissue sensitivity to insulin, is associated with lower ghrelin levels. The current study revealed that serum ghrelin concentrations were considerably reduced in diabetic patients, which is in agreement with former study (Jawed *et al.*, 2017). Hence, the current study propose that in DM, the reduction of ghrelin secretion may moreover be owed to the variation in the adipokine system attended by the incapacity of insulin-secreting pancreatic cells, interrupt lipid metabolism and IR appearance. This hypothesis is maintained by the outcomes of investigational revisions that establish the aggressive connection between serum ghrelin level and high levels of FFA can block ghrelin secretion in addition to the data of a relationship study viewing the negative connection between serum ghrelin and insulin in MI patients with and without DM, which is in accordance with the preceding data (Sullivan *et al.*, 2019).

In the present study, it found that diabetic patients showed considerably higher levels of IL-6 than did the controls ($p \leq 0.05$). Consistent with earlier reports, higher serum IL-6 levels were related with age, smoking, adiposity levels, dyslipidemia, or hypertensive status (Endrighi *et al.*, 2016; Ahmed, 2015).

In this study, also it found that females had greater leptin, IL-6 and IL-8 levels than males, this is may be due to higher body fat and BMI in female. In normal physiological state, macrophages, endothelial and epithelial cells produce IL-8 in response to infection or tissue injury, where one of the functions of IL-8 is to induce chemotaxis of granulocytes and primarily neutrophils, to the affected site. Also, IL-8 triggers the angiogenic response (Long *et al.*, 2016).

Conclusions

In this study, it was reported that diabetic patients had higher serum leptin, IL-6 and IL-8 while but serum ghrelin levels when compared with controls. This result led to the supposition that the rise in serum leptin concentration is related with obesity and IR may be contrariwise related with serum ghrelin concentrations among Iraqi diabetic patients. Also, it suggests that lL-6 and IL-8 being an inflammatory mediators might be responsible for some underline changes which may donate for the progress of T2DM.

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Conflict of Interest: There was no conflict of interest for the study.

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