Study the role of Obesity and Pancreatic insufficiency as factors of type 2 diabetes

Edrees M. Ameen[a], Hassan Y. Muhammad*[b]
[a]Biology Department , Science college , SalahaddinUniversity, Erbil, Iraq.
[b]Biology Department , Science college , Baghdad University, Baghdad, Iraq.
***Corresponding Author:** Hassan Y. Muhammad

Abstract

Background: Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugarlevels over a prolonged period. It is known that type 2 dibetes mellitus and obesity are bound common metabolic problems. The adipokines are mediators produced by adipose tissue,their action is thought to modify many obesity-related diseases.

Objective: The main aim of the study wasto assess the effect of Pancreatic insufficiency and obesity as factor caused diabetes type 2 by investigate the alteration in insulin productionandAdipokines in male.

Materials and Methods: The study was included 120 male with the ages between(30-60)years, the subjects were divided into four groups , group(I) Control, none-obese group(II) Control, obese group(III) None-Obese diabetic patient, group (IV) Obese diabetic patient, group. The parameters in the present study were included HbA1c%, Fasting Serum insulin , C-peptide, Adipokines (Leptin, Resistin andApelin), Biochemical analysis (Fasting blood sugar).

Results: The results of diabetic (None-obese and obese) groups showed highly significant increase in Fasting blood sugar, HbA1c, Fasting serum insulin and C-peptide concentration when compared with control groups(None-obese and obese). The current data revealed highly increased in Leptin, ResistinandApelin in diabetic groups (None-obese and obese) when compared with control groups..

Conclusions: It was concluded from the present study thatpancreatic insufficiency and obesity are most common factors that lead to diabetes type 2.This results indicates Obesity affected diabetes-2 by direct inhibitory effect of Adipokines on β-cell action and mass.

KEYWORDS: Diabetes-2,Pancreatic insufficiency, Fasting blood sugar, HbA1c, Fasting serum insulin , C-peptide, obesity, Adipokines, Leptin, Resistin, Apelin.

Introduction

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases in which there are high blood sugarlevels over a prolonged period(WHO,2014). Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger,if left untreated, diabetes can cause many complications(WHO,2013).Acute complications include diabetic

ketoacidosis and nonketotichyperosmolar coma(Kitabchi*et. al.*,2009) Serious long-term complications include cardiovascular disease, stroke, kidney failure, foot ulcers anddamage to the eyes(WHO,2013).Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced(David and Dolores,2011)

Hemoglobin A1c (HbA1c), the most abundant minor hemoglobin component in human erythrocytes, (Taggar*et. al*., 2012). Insulin is a peptide hormone produced by beta cells in the pancreas, it regulates the metabolism of carbohydrates and fats by promoting the absorption of glucose from the blood to skeletal muscles and fat tissue and by causing fat to be stored rather than used for energy(Sonksen and Sonksen,2000).Equimolar amounts of C-peptide and insulin are then stored in secretory granules of the pancreatic beta cells and both are eventually released to the portal circulation. Initially, the sole interest in C-peptide was as a marker of insulin secretion and has as such been of great value in furthering the understanding of the pathophysiology of type 1 and type 2 diabetes(Hills and Brunskill ,2008).
It is known that type 2 dibetes mellitus and obesity are bound common metabolic problems.Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health, leading to reduced life expectancy and/or increased health problems(Haslam and James,2005). The adipokines are mediators produced by adipose tissue; their action is thought to modify many obesity-related diseases.Adipose tissue is an endocrine organ that secretes numerous protein hormones, including leptin, Apelin and resistin. These hormones generally influence energy metabolism, which is of great interest to the understanding and treatment of type 2 diabetes and obesity .In obesity, a decreased sensitivity to leptin occurs, resulting in an inability to detect satiety despite high energy stores(Margetic*et. al.,* 2002).Apelin is expressed at the surface of some cell types. It is widely expressed in various organs such as the heart, lung, kidney, liver, adipose tissue, gastrointestinal tract, brain, adrenal glands, endothelium, and human plasma(Audigier,2006).In pancreas, apelin inhibits the insulin secretion induced by glucose(SörhedeWinzell*et. al.*,2005).Resistin is secreted by immune and epithelial cells and adipose tissue,Resistin role in energy metabolism and type 2 diabetes can be derived from studies showing strong correlations between resistin and obesity Resistin is an adipose-derived hormone (similar to a cytokine) whose physiologic role regarding its involvement with obesity and type 2 diabetes mellitus (Lazar,2007).

Materials and Methods

Patients Selection

The study was carried out on (120) Iraqi diabetic patients. They were ranged between (30-60) years of ages. Informed consent was obtained from both patients and controls group to fill the study protocol sheet before venipuncture. Those patients were diagnosed according to the level glucose . The clinical examination was performed under supervision of physician specialist in diabetes and endocrinology. and their classification into groups was according to criteria of ADA(American Diabetes Association,2010).

The experimental groups :

This study included 120individuals (male),they are divided into four groups:

1. Group 1 (45) whom have diabetes-2 and Obese(BMI≥30) .
2. Group 2 (45) whom have diabetes-2 and non-Obese(BMI≤25) .
3. Group 3 (15) Control(Non-diabetes)with BMI≥30(Obese).
4. Group 4 (15) Control(Non-diabetes)with BMI≤25(Non-obese).

The study was conducted for a period from April 1st 2013 to August 1th 2014.

Collection and handling of samples

From each subject, 10ml of blood were obtained by venepuncture, using a 10 ml disposable syringe. The blood sample was divided into two aliquots; 2 and 8 ml. The first aliquot was dispensed in tube containing ethylene diaminetetracetic acid (EDTA) (1.5 mg/ml). This blood was processed in less than three hours and was used for HbA1C estimation.The second aliquot was centrifuged at 3000 rpm for ten minutes to collect serum. The serum was stored in Deep freezer at -20 degree celcieus(c^o) until it was assayed.

Measurement of Body Mass Index (BMI):

BMI is calculated for all groups, as weight (kg) divided by height squared (m^2).

$$\textbf{BMI}=\frac{\square\square\square\square\square\square(\square\square)}{\{\square\square\square\square\square\square(\square)\}2}$$

Laboratory investigations:

Plasma glucose was measured by enzymatic colorimetric assay, HbA1c% by NycoCard HbA1c is a boronate affinity assay, c-peptide , insulin, leptin, Resistin and Apelin measured byenzyme-linked immunosorbent assay (ELISA) .

Results:

The results of the current data showed significant differences (P<0.001) in fasting blood sugar concentration between control and patients groups and the highest value of sugar were observed with diabetes type 2 obese . Diabetes groups (obese and non- obese) showed significantly (P<0.001) higher level of glycated hemoglobin, when compare with control groups (obese and none- obese) and the highest value were recorded with diabetes obese group .The results of the study revealed significant increased (P<0.001) in insulin in none-obese and obese diabetes-2 subjects when compared with control groups and there was significant elevation of C-peptide in diabetes groups (obese and none-obese) when compared with others control groups (table 1).

Chi-square analysis showed that 88.9% of diabetes none-obese and 0.0% 0f control groups (none-obese) have abnormal value of HbA1c (p-value= 0.001) (table 2). Also 93.3% of diabetes obese group and 4.0% of control group (obese) have abnormal value of HbA1c ((p-value= 0.001) (table 3). None-significant differences were observed between diabetes groups (obese and none-obese) in abnormal level of HbA1c (table 4).

Analysis of data with Chi-square showed that obese diabetes subjects have significantly higher prevalence of abnormal value of C-peptide 46.7% comparing with other three groups (control none-obese 0.0%, table 5), (control obese 0.0%, table 6) and (diabetes none- obese 24.4%, table 7).The present study showed that the mean fasting blood sugar

and HbA1c was significantly higher in diabetic patients when compared with control groups, these results are in agreement with the results of (Kharroubi*et al.*,2014;Danaei*et al*. 2013; and Guo*et al.* 2014), who found that the mean fasting blood sugar and HbA1c was higher in diabetic patients than control group. This fact may be explained by that not respond to insulin which produce from pancreatic beta cells in T2DM patients that leads to accumulation of glucose in the blood. When blood glucose enters the erythrocytes, it glycates the amino terminals of hemoglobin. The fraction of glycatedHb, normally about 5%, is proportionate to blood glucose concentration. Since the life of human erythrocyte is nearly 90-120 days, so the level of glycated hemoglobin reflects the mean blood glucose concentration over the preceding 12-16 weeks. Measurement of HbA1c therefore provides valuable information for evaluation of diabetes mellitus (Murray *et al*., 2012).
Glycated hemoglobin is a form of hemoglobin that is measured primarily to predict the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous months prior to the measurement (Larsen *et al*., 1990). Thus the present study data reported that blood sugar was positively correlated with HbA1c. This result is in agreement with the result of Ikekpeazu*et al*. (2011) found that fasting blood sugar (FBS) and HbA1c correlated positively, as the FBS increases, the HbA1c also increases .

In present study the level of insulin in diabetes groups were significantly higher when compared to the control groups. These results are in the line with the finding of (Sheena *et al*., 2011; Yuan *et al.,* 2015). Where insulin resistance is countered by an increase in pancreatic β-cell mass and function , the β-cell succumbs to rising insulin resistance via various mechanisms (including glucotoxicity , lipotoxicity and endoplasmic reticulum stress) and hyperglycaemia ensues.Increased circulating glucose levels stimulate the β-Cells function by sensing and secreting of insulin in appropriate amount (Meglasson and Matschinsky, 1986)

In contrast (Xiaohua*et al*., 2015), observed that the mean of serum insulin in diabetic patients was lower which due to β-cell insufficiency which proven valuable results for predicting an individual's risk for developing diabetes. Excess body weight and adiposity are directly associated with a reduction in insulin sensitivity, which is generally compensated by a further stimulation of pancreatic insulin secretion to prevent an increase in blood glucose levels.

The results also demonstrated that mean C-Peptide were significantlyhigher in diabetic patients than in the controls. Our results agreed with the results of (Sacks *et al.*, 2011 ;Jones and Hattersley, 2013). The physiology of C-peptide makes it appropriate for assessing insulin secretion. Insulin is produced in the pancreatic β-cells by enzymatic cleavage of the prohormone precursor proinsulin to produce insulin and C-peptide in equimolar amounts. C-peptide has negligible extraction by the liver and constant peripheral clearance. Its half-life is longer than insulin (20–30 vs. 3–5 min) and it therefore circulates at concentrations approximately five times higher in the systemic circulation.Patients with insulin resistance or type 2 diabetes are more likely to, but will not always, have high levels of C-peptide due to an over production of insulin.C-peptide is commonly used in preference to insulin measurement when assessing β-cell function in

clinical practice.Insulin produced by the pancreas is extensively (approximately 50%) first-pass metabolized by the liver, both the extent of first-pass metabolism and peripheral clearance of insulin is variable, therefore peripheral insulin levels may not accurately reflect portal insulin secretion. Even in non-insulin-treated patients, peripheral C-peptide levels more accurately reflect portal insulin secretion than measurement of peripheral insulin.

The results showed significant differences ($P<0.001$) in serum leptin level between all groups and the high level of them were recorded with diabetes obese group (22.868 ng/ml) when compare with other three groups (4.691, 10.676 and 9.701ng/ml respectively) .Significant differences ($P<0.001$) were appeared in serum resistin between different studied groups. Control none-obese group showed lower value of resistin when compare with control obese, patients none- obese and patients obese groups and higher level of resistin were recorded with patients obese group .Significant variation in the level of serum apelin were recorded between obese groups when compare with none-obese groups. Highly significant elevation ($P<0.001$) in both obese control and obese diabetes type-2 groups were observed compared with none-obese diabetes-2 and none-obese control groups (table 8).
Data of Chi-square observed significant high prevalence of abnormal level of serum leptin in diabetes none-obese group (85.7%) when compare with control none-obese group (14.3%) (table9). None-significant differences were appeared in abnormal level of serum leptin between diabetes obese group with control obese group (table 10) and diabetes none-obese group (table 11).Analysis of the data with Chi-square showed that patient's none-obese group has higher prevalence of abnormal resistin (46.7%) when compare with control none-obese group (0.0%) (table 12). On the other hand, the higher prevalence of abnormal resistin were recorded with patients obese group (93.3%), and this value are higher when compare with control obese group (12.0%) (table 13) and patients none-obese group (46.7%) (table14).

Obesity and diabetes, together called as diabesity, is the ever growing metabolic disorder of industrialized and developing countries.There is strong evidence that adipocytes play a critical role in metabolism through the secretion of hormones and cytokines that alter whole-body energy homeostasis (Fruhbeck*et al*., 2001).

The results of current study showed that the mean serum leptin was significantly higher in obese diabetic group when compared to non-obese diabetic and control groups. These results are in agreement with the results of (Seuk Moon *et al*., 2013; Coimbra *et al*., 2014).Adipose tissue is an active endocrine organ that secretes several inflammatory cytokines, namely, adipokines, which interfere with insulin sensitivity, with glucose and lipid metabolism, and with the inflammatory process. The pathophysiologic link between obesity and type 2 diabetes is not entirely understood, but adipokines seem to play an important role.Leptin may also directly regulate glucose homeostasis independently of its effects on adiposity; leptin regulates glycemia at least in part via the CNS, but it may also directly regulate the physiology of pancreatic β-cells and peripheral insulin-sensitive tissues.Several lines of evidence support the hypothesis that in addition to leptin, the pancreatic hormone insulin also acts in the brain as an "adiposity negative feedback signal". Both hormones circulate at levels that vary in proportion to body fat stores and

both enter the CNS in proportion to their plasma level, where they act on their respective receptors expressed in key brain areas that control energy balance, glucose metabolism and autonomic function.

The results also demonstrated that mean serum resistinwassignificant increased in obese control, none-obese diabetes-2 and obese diabetes-2 groups compared with none-obese control group. These results are in agreement with the presenation of (Menzaghi*et al.*, 2013; Moreno *et al.*, 2015). Resistin down-regulated insulin receptor expression levels (necessary for maintenance of β-cell mass) in clonal β-cell and hence decreased cell viability. Resistin actually induced insulin resistance in pancreatic islets causing a subsequent reduction in glucose-stimulated insulin secretion (GSIS).

The results of current study showed that highly significant elevations in serum apelin were observed in both obese control and obese diabetes type-2 groups compared with none-obese diabetes-2 and none-obese control groups. These results agreed with the data of (Ringström*et al.*, 2010; Bertrand *et al*., 2015). Apelin is widespread expression including in adipose tissue and therefore functions as an adipokine with effects on feeding behavior and glucose utilization. The apelin receptor, the APJ receptor, is expressed in islets and apelin activation of its receptor inhibits insulin secretion .Recent evidence suggests that apelin is itself expressed in pancreatic islets, particularly in β- and α-cell, raising the possibility of autocrine/paracrine effects.

Significant BMI-independent correlations between reduced apelin levels and improved insulin sensitivity were found (Ringström*et al.*, 2010). Thus, it could be hypothesized that the increased plasma apelin observed in type 2 diabetic patients, is, as in type 1 diabetes, a compensatory mechanism devoted to directly decrease insulin resistance since apelin exerts different metabolic actions itself. When insulin resistance is decreased, this may lead to decreased apelin levels. It has thus been proposed that lower apelin serum concentrations in healthy lean individuals may be a consequence rather than a cause of normal insulin sensitivity (Krist*et al.*, 2013).

Reference

American Diabetes Association.(2010).Diagnosis and Classification of Diabetes Mellitus.*Diabetes Care*. (33):S62-S69.

Audigier, Y. (2006). Apelin Receptor. UCSD-Nature Molecule Pages.*Nature Publishing Group*. doi:10.1038/mp.a000304.01.

Bertrand,C.; Valet,P. and Castan-Laurell,I.(2015). Apelin and energy metabolism. *Front Physiol*. 6: 115.

Coimbra, S.; Proença, J.; Santos-Silva, A and João-Neuparth M. (2014).Adiponectin, Leptin, and Chemerin in Elderly Patients with Type 2 Diabetes Mellitus: A Close Linkage with Obesity and Length of the Disease.*Biomed Res Int.* 701915.

Danaei, G.;Finucane, M.M.; Lu, Y.;Singh, G.M.;Cowan, M.J.; Paciorek, C.J.; Lin, J.K.; Farzadfar, F.; Khang ,Y.H.; Stevens, G.A.; Rao, M.; Ali, M.K.; Riley, L.M,.; Robinson,

C.A. and Ezzati, M.(2013).National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet*. 2:378(9785):31-40.

David, G. and Gardner, D. (2011). Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical. pp. Chapter 17.

Fruhbeck, G.; Gomez-Ambrosi, J.; Muruzabal, F.J.; Burrell, M.A.(2001). The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *Am J PhysiolEndocrinolMetab*. 280(6):E827–847.

Guo, F.; Moellering, D.R. and Garvey W.T.(2014).Use of HbA1c for diagnoses of diabetes and prediabetes: comparison with diagnoses based on fasting and 2-hr glucose values and effects of gender, race, and age. *MetabsyndrReatdisord*.12(5):258-268.

Haslam, D.W. and James, W.P. (2005). "Obesity". *Lancet*. 366 (9492): 1197–1209.

Ikekpeazu,E.J.; Neboh,E.E.; Ejezie,F.E.; Ibegbu,M.D. and Ike,I.E. (2011). Oxidative Stress and Glycaemic Control in Type 2 Diabetic Patients in Enugu, South-East Nigeria .*Ann. Med. Health. Sci. Res*. 1(1): 123–128.

Jones, A.G. and Hattersley, A.T.(2013). The clinical utility of C-peptide measurement in the care of patients with diabetes.*Diabetic Medicine* .30(7):803-817.

Kharroubi, A.T.; Darwish, H.M.; Abu Al-Halaweh, A.I. and Khammash, U.M.(2014). Evaluation of glycated hemoglobin (HbA1c) from diagnosing type 2 Diabetes and prediabetes among Palestinian Arab population.*Plos one*. 5;9(2):e88123 .

Kitabchi,A.E.;Umpierrez,G.E.;Miles,J.M.and Fisher, J.N. (2009). Hyperglycemic crises in adult patients with diabetes. . *Diabetes Care. 32* (7): 1335–1343.

Krist, J.; Wieder, K.; Kloting, N.; Oberbach, A.; Kralisch, S.; Wiesner, T., Schön, M.R.; Gärtner, D.; Dietrich, A.; Shang, E.; Lohmann, T.; Drebler, M,.; Fasshauer, M.; Stumvoll, M. and Blüher, M. (2013). Effects of weight loss and exercise on apelin serum concentrations and adipose tissue expression in human obesity.*Obes.Facts*. 6: 57–69.

Larsen, M.L.; Hørder, M. and Mogensen, E.F. (1990)."Effect of long-term monitoring of glycosylated haemoglobin levels in insulin-dependent diabetes mellitus".*N. Engl. J. Med*. 323 (15): 1021–1025.

Lazar, M.A. (2007). Resistin- and Obesity-associated metabolic diseases. *Horm.Metab. Res*. 39 (10): 710–716.

Margetic, S.; Gazzola, C.; Pegg, G. and Hill, R. (2002).Leptin: a review of its peripheral actions and interactions. *Int. J. Obes. Relat.Metab.Disord*. 26 (11): 1407–1433.

Meglasson, M.D. and Matschinsky, F.M(1986). Pancreatic islet glucose metabolism and regulation of insulin secretion.*DiabetesMetab Rev*. 2:163–214.

Menzaghi, C.; Bacci, S.; Salvemini, L.; Mendonca, C.; Palladino, G.;Fontana, A.; De Bonis, C.; Marucci, A.; Goheen, E.; Prudente, S.; Morini, E,.; Rizza, S.; Kanagaki, A.; Fini, G.; Mangiacotti, D.; Federici, M.; De Cosmo, S.; Pellegrini, F.;Doria, A. and Trischitta, V.(2013). Serum resistin, cardiovascular disease and all-cause mortality in patients with type 2 diabetes.*PLoS One*. 3;8(6):e64729.

Moreno,L.; Salvemini,L.; Mendonca,C.; Copetti,M.; De Bonis,C.; De Cosmo,S.; Doria,A.; Trischitta,A and Menzaghi1,C.(2015). Serum Resistin and Glomerular Filtration Rate in Patients with Type 2 Diabetes.*PLoS One*. 10(3): e0119529.

Murray,R.K.;Bender,D.A.;Botham,K.M.;Kennelly,P.J.;Rodwell,V.W. and Weil, P.A.(2012).Harpers illustrated biochemistry.29th edition. McGraw-Hill Companies.PP: 55.

Ringström, C.; Nitert, M.D.; Bennet,H. ;Fex, M.; Valet, P.; Rehhfeld, J.F.; Friis-Hansen, L. and Wierup, N.(2010).Apelin is a noval islet peptide.*Regulatory peptides*.162:44-51.

Sacks, D.B.; Arnold, M.; Bakris, G.L.; Bruns. D.E.; Horvath, A.R. and Kirkman, M.S.(2011) .Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clin Chem*. 57:e1–e47.

Seuk-Moon,H.; Dalamaga,M.; Yong-Kim,S.; Polyzos,S.A.; Petter -Hamnvik,O.; Magkos,F.; Paruthi,J. and Christos, S.(2013). Leptin's Role in Lipodystrophic and Nonlipodystrophic Insulin-Resistant and Diabetic Individuals.*Endocr Rev*. 34(3): 377–412.

Sheena, K.; Reinhold, V.; Ravi,R.; Julia, A. ;Ying, Q.; Hertzel, C. ,Gerstein, M.; Bruce, A.; Perkins, M.; Stewart, B. ;Harris, M.; Bernard, Z.; and Anthony, J. (2011). Association of Vitamin D With Insulin Resistance and β-Cell Dysfunction in Subjects at Risk for Type 2 Diabetes.*Diabetes Care*. 33(6): 1379–1381.

Sonksen, P. and Sonksen, J. (2000). Insulin: understanding its action in health and disease.*Br J Anaesth***.** 85 (1): 69–79.

SörhedeWinzell, M.; Magnusson, C. and Ahrén, B. (2005). The apj receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice.*Regul. Pept*. 131 (1–3): 12–7.

Taggar ,J.S.; Coleman, T.; Lewis, S. and Szatkowski, L.(2012).The impact of the Quality and Outcomes Framework (QOF) on the recording of smoking targets in primary care medical records . *BMC Public Health*. 12(1):329.

World Health Organization.(2013). "Diabetes Fact sheet N°312".Retrieved25 March 2014.

World Health Organization.(2014). "About diabetes".Retrieved 4 April 2014.

Xiaohua, L.; Dalin, S.; and Sean, X .(2015). Link between type 2 diabetes and Alzheimer's disease: from epidemiology to mechanism and treatment.*ClinInterv Aging.* 10: 549–560.

Yuan, G.; Xinchi, Wu.; Qi, F.; Yanyun, L.; Tao, Y.; and Wei, T.(2015).The Relationship between Serum 25-Hydroxy Vitamin D and Insulin Sensitivity and β-Cell Function in Newly Diagnosed Type 2 Diabetes. *Diabetes Res*. 636891.

Table (1): The value of fasting blood sugar,HbA1c%, Insulin and C-peptide between control and diabetes patients (means ± standard errors).

Groups	Fasting blood sugar (mg /dL)	HbA1c (%)	Insulin (µI.U./ml)	C-peptide (ng/ml)
Group1: Control(none-diabetes, none-obese)	76.450 ± 1.212[a]	4.513 ± 0.143[a]	4.250 ± 0.635[a]	0.701 ± 0.073[a]
Group 2: Control(none-diabetes, obese)	84.600 ± 1.244 [a]	4.974 ± 0.177 [a]	8.240 ± 1.677 [a]	1.206 ± 0.218 [a]
Group3: Diabetes type 2, none-obese	164.900 ± 7.804[b]	7.365 ± 0.214[b]	17.813 ± 1.14[b]	2.564 ± 0.287[b]
Group4: Diabetes type 2, obese	204.633 ± 12.179[c]	8.358 ± 0.375[c]	21.380 ± 1.17[b]	3.651 ± 0.280[c]
p-value	0.001	0.001	0.001	0.001

p- value ≤ 0.05 considered significant.
Post Hoc Duncan- test: no differences between groups with the same letter

Table (2): Chi-square analysis for comparison of HbA1c between group of (none-diabetes, none-obese) and (diabetes type 2, none- obese).

	Control (none-diabetes, none- obese)	Diabetes type 2, none-obese
With abnormal value (>6.0%)	0.0% (0)	88.9% (40)
With normal value (<6.0%)	100.0% (25)	11.1% (5)

p- value = 0.001

Fisher chi-square test = 51.852

Table (3): Chi-square analysis for comparison of HbA1c between group of(none-diabetes, obese) and (diabetes type 2, obese).

	Control (none-diabetes, obese)	**Diabetes type 2, obese**
With abnormal value (>6.0%)	4.0% (1)	93.3% (42)
With normal value (<6.0%)	96.0% (24)	6.7% (3)

p-value = 0.001
Fisher chi-square test =54.131

Table (4): Chi-square analysis for comparison of HbA1c between group of (diabetes type 2, none- obese) and (diabetes type 2, obese).

	Diabetes type 2, none-obese	**Diabetes type 2, obese**
With abnormal value (>6.0%)	88.9% (40)	93.3% (42)
With normal value (<6.0%)	11.1% (5)	6.7% (3)

p- value = 0.459 (N.S.)
Fisher chi-square test = 0.549

Table (5): Chi-square analysis for comparison of C-peptide between group of (none-diabetes, none- obese) and (diabetes type 2, none- obese).

	Control (none-diabetes, none-obese)	**Diabetes type 2, none- obese**
With abnormal value (>3.2ng/ml)	0.0% (0)	24.4% (11)
With normal value (0.5-3.2 ng/ml)	100.0% (25)	75.6% (34)

p- value = 0.006
Fisher chi-square test= 7.250

Table (6): Chi-square analysis for comparison of C-peptide between group of (none-diabetes, obese) and (diabetes type 2, obese).

	Control (none-diabetes, obese)	**Diabetes type 2, obese**
With abnormal value (>3.2ng/ml)	0.0% (0)	46.7% (21)
With normal value (0.5-3.2 ng/ml)	100.0% (25)	53.3% (24)

p- value = 0.001
Fisher chi-square test = 16.667

Table (7): Chi-square analysis for comparison of C-peptide between group of(diabetes type 2, none- obese) and (diabetes type 2, obese).

	Diabetes type-2, none- obese	**Diabetes type 2, obese**
With abnormal value (>3.2ng/ml)	24.4% (11)	46.7% (21)
With normal value (0.5-3.2 ng/ml)	75.6% (34)	53.3% (24)

p- value = 0.047
Fisher chi-square test = 4.849

Table (8): The value of Leptin, Resistin and Apelin between control and diabetes patients (means ± standard errors).

Groups	**Leptin (ng /ml)**	**Resistin(ng/ml)**	**Apelin(ng /ml)**
Group1: Control(none-diabetes, none-obese)	4.691 ± 0.984^{a}	9.460 ± 1.067^{a}	1.45 ± 0.13^{a}
Group 2: Control(none-diabetes, obese)	10.676 ± 0.961^{b}	22.170 ± 1.743^{c}	2.71 ± 0.14^{b}
Group3: Diabetes type 2, none-	9.701 ± 0.528^{b}	15.319 ± 0.906^{b}	1.91 ± 0.12^{a}

obese			
Group4:Diabetes type 2, obese	22.868 ± 1.162[c]	28.793 ± 1.339[d]	2.95 ± 0.22[b]
p-value	0.001	0.001	0.001

p- value ≤ 0.05 considered significant.
Post Hoc Duncan- test: no differences between groups with the same letter

Table (9): Chi-square analysis for comparison of Leptin between group of (none-diabetes, none-obese) and (diabetes type 2, none- obese).

	Control (none-diabetes, none-obese)	**Diabetes type 2, none- obese**
With abnormal value (>5.6 ng/ml)	14.3% (7)	85.7% (42)
With normal value (2.0-5.6 ng/ml)	85.7% (18)	41.3% (3)

p- value = 0.006
Fisher chi-square test=7.250

Table (10): Chi-square analysis for comparison of Leptin between group of (none-diabetes, obese) and (diabetes type 2, obese).

	Control (none-diabetes, obese)	**Diabetes type 2, obese**
With abnormal value (>5.6 ng/ml)	93.3% (42)	97.8% (44)
With normal value (2.0-5.6 ng/ml)	6.7% (3)	2.2% (1)

p- value = 0.679 (N.S.)
Fisher chi-square test = 0.183

Table (11): Chi-square analysis for comparison of Leptin between group of (diabetes type 2, none- obese) and (diabetes type 2, obese).

	Diabetes type-2, none- obese	**Diabetes type 2, obese**
With abnormal value (>5.6 ng/ml)	85.7% (24)	97.8% (42)
With normal value (2.0-5.6 ng/ml)	4.0% (1)	2.2% (3)

p- value = 0.616 (N.S.)
Fisher chi-square test = 1.047

Table (12): Chi-square analysis for comparison of Resistin between group of(none-diabetes, none- obese) and (diabetes type 2, none- obese).

	Control (none-diabetes, none-obese)	**Diabetes type 2, none- obese**
With abnormal value (>16.3ng/ml)	0.0% (0)	46.7% (21)
With normal value (4.6-16.3 ng/ml)	100.0% (25)	53.3% (24)

p- value = 0.001
Fisher chi-square test = 16.667

Table (13): Chi-square analysis for comparison of Resistin between group of (none-diabetes, obese) and (diabetes type 2, obese).

	Control (none-diabetes, obese)	**Diabetes type 2, obese**
With abnormal value (>16.3 ng/ml)	12.0% (3)	93.3% (42)
With normal value (4.6-16.3 ng/ml)	88.0% (22)	6.7% (3)

p- value = 0.00
Fisher chi-square test = 46.306

Table (14): Chi-square analysis for comparison of Resistin between group of(diabetes type 2, none- obese) and (diabetes type 2, obese).

	Diabetes type-2, none-obese	**Diabetes type 2, obese**
With abnormal value (>16.3 ng/ml)	46.7% (21)	93.3% (42)
With normal value (4.6-16.3 ng/ml)	53.3% (24)	6.7% (3)

p- value = 0.001
Fisher chi-square test = 23.333