ISSN: 0067-2904

Alterations of Obestatin, Cardiac Markers and Lipid Profile Levels in Type 2 Diabetes Mellitus

Hazhar M. Balaky[*1], Ismail S. Kakey[2]
[1]Department of Chemistry/ Faculty of Science/ Soran University/ Kurdistan Region- IRAQ
[2]Department of Biology/Faculty of Science and Health/ Koya University / Koya KOY45, Kurdistan Region – F.R. Iraq

Received: 19/7/2020 Accepted: 29/9/2020

Abstract

The current investigation aimed to test the alterations of the levels of obestatin hormone, lipid profile and cardiac function markers in relation to hyperglycemia in patients with non-insulin diabetes mellitus. The study included 118 diabetic subjects (56 males, 62 females) and 60 healthy non-diabetic subjects (30 males, 30 females). Diabetic and healthy subjects were age-matched. Serum levels of obestatin, lipid profile markers including total cholesterol (STC), triglycerides (STG), low and high density lipoproteins (LDL-C and HDL-C), as well as cardiac function markers including, creatine kinase and lactate dehydrogenase enzymes were determined in all subjects. The findings revealed a remarkable decrease in the level of serum obestatin in both diabetic males and females with both age ranges (40-59 and 60-80 years). On the other hand, the results showed that serum STC, LDL-C, and STG levels were statistically significantly elevated, while that for HDL-C was significantly decreased in diabetic males and females with both age ranges. Concerning the cardiac markers, the results found out that the levels of CK-MB and LDH were significantly increased in type 2 diabetic males and females with both age ranges. The results suggested that a low concentration of obestatin is a significant risk factor for type 2 DM, with a key role for this hormone in the pathogenesis. Accordingly, altered levels of obestatin could be used as an important indicator for type 2 DM. The current study also suggests a direct relationship between lipid profiles, except for HDL, the decreased obestatin level, the increased cardiac function markers, and hyperglycemic status in type 2 DM.

Keywords: (Type 2 Diabetes Mellitus, Lipids profile, obestatin, Cardiac markers)

التغيرات في مستويات الاوبستاتين و صور الدهون و بعض مؤشرات وظائف القلب في مرضى السكري من النوع الثاني

هژار محمد حمدامين[1]* ، اسماعيل صالح كاكيى[2]
[1]قسم الكيمياء، فاكلتى العلوم ، جامعة سوران، كوردستان– العراق
[2]قسم علوم البيولوجية، فاكلتى العلوم و الصحة، جامعة كويه، كوردستان ، العراق

الخلاصة

استهدفت الدراسة الحالية إلى بحث التغييرات في مستويات **الاوبستاتين** و صور الدهون وبعض مؤشرات وظائف القلب في ما يتعلق بفرط سكر الدم في داء السكري الغير المعتمد علئ الأنسولين. اشتملت هذه

*Email: hazharbalaky86@yahoo.com

الدراسة على 118 مريضا من المصابين بالسكري (56 ذكور ، 62 إناث) وكما شملت أيضًا 60 شخصا سليما (30 ذكورًا ، 30 إناثًا) من نفس الفئات العمرية اعدت كمجموعه ضابطه. قيست مستويات الاوبستاتين و صور الدهون شاملا الكوليسترول الكلي (TC) والدهون الثلاثية (TG) والبروتينات الدهنية منخفضة الكثافة وعالية الكثافة (LDL– و HDL–C) ومستويات مؤشرات وظائف القلب المتضمنة الكرياتين كاينيز (CK–MB) وإنزيم لاكتات ديهيدروجينيز د (LDH) في مصل دم المصابين والاصحاء .

أوضحت النتائج انخفاضا معنويا في مستوى الاوبستاتين في كلا الجنسين ضمن المجموعتين العمريتين (40–59) و (60–80) عامًا، كما اظهرت النتائج ارتفاعا معنويا في مستويات STC و –LDL C و STG في المصل ، وانخفاضا معنويا في مستوى HDL–C في الذكور والإناث المصابين بمرض السكري في كلتا المجموعتين العمريتين (40–59) و (60–80) عامًا . بخصوص مؤشرات وظائف القلب ، فقد أظهرت النتائج زياده معنويه في مستوى CK–MB و LDH في المصابين بالسكري من النوع الثاني من الذكور والإناث المصابين من كلتا الفئتين العمريتين (40–59) و (60–80) سنة. يستنتج من هذه الدراسة وجود علاقة ثنائية الاتجاه لصور الدهون بارتفاع مستويات صور الدهون عدا HDL ، مع انخفاض مستوى الاوبستاتين و زيادة معنويه لمؤشرات وظائف القلب في المصابين بداء السكري غير المعتمد على الأنسولين (NIDDM).

Introduction

Type 2 diabetes is a disorder of carbohydrate metabolism which is considered as the most common secondary cause of dyslipidemia. In various research works in humans, reduction in the levels of obestatin was linked with diabetes mellitus and impaired glucose tolerance. The insulin-sensitivity homeostasis model assessment (HOMA) of insulin resistance suggested a key role for obestatin in controlling the body mass index [1].

It was also reported that the decline in the expression of adhesion molecule-1 and the increase in the binding of oxidized low-density lipoprotein are attributed to the effect of obestatin on endothelial cells [2]. This findings proposed that obestatin may also have a major role in controlling hypertension [3]. Several studies revealed that obestatin promotes the survival of β-cells of pancreace and accelerates the expression of their main controlling genes for insulin biosynthesis [4]. It inhibits glucose-induced insulin secretion [5] and prevents lipolysis. It acts similarly to insulin by reducing insulin resistance and reducing adipocyte, inflammation that occurs in tissue with high rate of metabolism. These roles support the proof of a novel role for obestatin in fatty cell function and metabolism of sugar, which proposes a possible curative indication for the dysfunctions of the metabolic pathways and insulin resistance [6].

The etiology of dyslipidemia in type 2 diabetes is mainly attributed to the deficiency in the activity of lipoprotein lipase, a key enzyme in the metabolism of lipoprotein, as a consequence of resistance or deficiency in insulin production [7]. Low activity of lipoprotein lipase contributes to a decline in the levels of the good cholesterol (HDL) with increasing concentrations of both triglycerides and low-density lipoprotein (LDL) in type 2 DM [8]. However, severe hypercholesterolemia is not frequently found in diabetic patients in comparison to high concentrations of TG and low-density lipoprotein which are very common in T2DM [9]. Furthermore, diabetes mellitus is considered as an independent predictor for heart disease [10]. In view of the potential impact of cardiovascular disease (CVD) on the health of diabetic patients, the medical approach to the people with diabetes must goal to regulate all CVD predictors. Some of those include increasing body fat, lack of physical activity, lipid abnormalities, and high blood pressure, which are persistent in individuals with DM; typically those suffering from type 2 DM [11]. The incidence of type 2 DM in patients with cardiovascular disease is up to 50% in a large number of countries. The impact of rigorous management of diabetes mellitus on CVD morbidity and mortality has been reported controversially with different findings, which attracted special attention in the current management of diabetes [12].

Methodology

Participants and Sample collection

The current research is a case control study which included 118 patients (56 males and 62 females) whose age ranged between 40-80 years. The patients were diagnosed as having type 2 DM and

attended Laila Qasim Diabetic Center in Erbil Governorate. The diabetic patients were classified into four groups on the bases of age and gender: female (40-59 years), female (60-80 years), male (40-59 years), male (60-80 years). The control group was comprised of sixty age and sex matched normal non diabetics. Specimens were collected from the study subjects (patients and healthy individuals) after 10-12 hours of fasting. Blood samples were preserved in Gold-top serum separator tubes (SST) and Ethylenediaminetetraacetic acid (EDTA) tubes before the centrifugation process. The specimens were centrifuged at 3500 rpm for 10 min and the resulting serum samples were separated and stored in Eppendorf tubes. After completion the processes of sample collection, all samples were dissolved in order to be ready for biochemical analyses.

Biochemical assays

Serum glucose concentration was determined by the enzymatic colorimetric method by using BIOLABS kit (France). Also, the level of HbA1c (Hemoglobin A1c) in whole blood was determined by the colorimetric fluorescence Immunoassay (FIA) using Boditech Med kit. Serum obestatin was determined by sandwich enzyme-linked immunosorbent assay (ELISA) using the Human Obestatin ELISA kit manufactured by SunLong Biotech, China. Furthermore, serum levels of CK, LDH STC, LDL, HDL and STG were measured by the enzymatic colorimetric method using BIOLABS kit (France).

Statistical Analysis

SPSS version 21 and GraphPad prism version 8 computer programs were used for the statistical analysis of data. Statistical test results and Bar graphs were expressed as Mean±SE. Unpaired T-test (Man-Whitney U) test was used for comparing the parameter means between patient and control groups.

Results and discussion

Serum levels of glucose and HbA1c

The diabetic syndrome was confirmed by glucose and HbA1c levels determination in diabetic patients compared with healthy age-matched controls**.** The diabetic male patients showed significantly higher ($p<0.0001$) glucose level in both age groups (245.1±14.74 and233.9±16.81 mg/dL, respectively) in comparison with the age-matched control subjects r (98.80±4.39 and 107.1±3.28 mg/dL, respectively). Similarly, significantly higher glucose level in diabetic females with both age ranges (285.9±17.01 and 282.1±14.70 mg/dL, respectively) was observed as compared to the control (108.9±2.48 and 101.4±1.24 mg/dL, respectively). Also, there was highly significant increase ($p<0.0001$) of HbA1c level in diabetic males with both age ranges (9.06±0.38 mg/dL and 8.34±0.35 mg/dL, respectively) in comparison with their levels in the control (5.14±0.06 mg/dL and 5.16±0.11 mg/dL, respectively). Moreover, the mean value of serum HbA1c level was significantly higher in diabetic females with both age ranges (10.07±0.39 mg/dL and 10.13±0.37 mg/dL,respectively)in comparison with their levels in the control (5.08±0.08 mg/dL and 4.98±0.05 mg/dL, respectively)(Table 1).

Table 1- Levels of serum glucose and HbA1c in diabetic patients

Parameters		Group	Control	Patients	P-Value
Male	**Glucose (mg/dL)**	40-59	98.80±4.39	245.1±14.74	<0.0001
		60-80	107.1±3.28	233.9±16.81	<0.0001
	HbA1c (%)	40-59	5.14±0.06	9.06±0.38	<0.0001
		60-80	5.16±0.11	8.34±0.35	<0.0001
Female	**Glucose (mg/dL)**	40-59	108.9±2.48	285.9±17.01	<0.0001
		60-80	101.4±1.24	282.1±14.70	<0.0001
	HbA1c (%)	40-59	5.08±0.08	10.07±0.39	<0.0001
		60-80	4.98±0.05	10.13±0.37	<0.0001

Values are expressed as mean ± SE, Normal Values: (Glucose = 70-139 mg/dL), (HbA1c= 4.0-6.5 %).

The findings of the present study revealed that both male and female diabetic patients have remarkably increased serum glucose and HbA1c levels in comparison to controls. The present results agree with the study performed by Yassin *et al*. (2011) who indicated increased levels of serum

glucose and HbA1c in patients with type 2 DM [13]. Previous studies showed that increased glucose and HbA1c concentrations were correlated with a higher prevalence of type 2 DM. The best hypothesis explaining the increased level of glucose in diabetic patients is that insulin insensitivity precedes the progression of hyperglycemia in people who eventually progress to type 2 diabetic. In addition to diabetic patients, longstanding hyperglycemia contributes to the glycation of the non-enzymatic proteins, which causes the production of reversible Amadori compounds and Schiff bases. This glycation mechanism then produces irreversible advanced glycosylated end-products (AGEs) as a result of a series molecular rearrangements of complex compounds. Advanced glycosylated end-products can precipitate in the blood stream and in various tissues. It is confirmed that the concentrations of HbA1c in the blood represent the levels of glucose to which the red blood cell has been exposed during its period of life. Consequently, HbA1c test is valuable as it evaluates high blood sugar, instead of instantly blood glucose concentrations. HbA1c has been used as goal indicator of glycaemic index control, has a key role in the controlling of patients with diabetes, and is dependent upon in clinically significant resolutions, such as starting the treatment with insulin [14].

Serum levels of obestatin in diabetic patients

Mean ± SE values of obestatin concentration in sera samples are presented in Table-2. The mean value of serum obestatin level was observed to be remarkably lower ($p<0.0001$ and $p=0.0009$) in diabetic males with both age ranges (3.78±0.18 pg/mL and 5.86±0.23 pg/mL, respectively) in comparison with their levels in controls (7.90±0.72 pg/mL and 7.08±0.20 pg/mL, respectively). Similarly, the mean value of serum obestatin levels was observed to be remarkably lower ($p=0.0372$) in diabetic females with the age range of 40-59 years (1.94±0.13 pg/mL) in comparison with its level in age and sex-matched controls (7.40±3.67 pg/mL). Whereas serum obestatin levels was observed to be non-significantly lower ($p=0.6346$) in diabetic females with the age range of 60-80 years (7.62±0.40 pg/mL) in comparison with in the matched control (7.99±0.68 pg/mL).

Table 2-Level of serum obestatin in diabetic patients

Parameters		Group	Control	Patients	P-Value
Obestatin (pg/mL)	Male	40-59	7.90±0.72	3.78±0.18	<0.0001
		60-80	7.08±0.20	5.86±0.23	0.0009
	Female	40-59	7.40±3.67	1.94±0.13	0.0372
		60-80	7.99±0.68	7.62±0.40	0.6346

The values are expressed as mean ± SE

The results of the current study are in a decent agreement with those obtained by other investigators [15,16,17], in which they as well noticed low concentrations of obestatin in diabetic patients. The cause of this decrease in type II diabetic patients might be related to obestatin role in appetite regulation [16] or the increase of body mass index and insulin insensitivity [18]. The reason might be also related to the decrease of GPR-39 level in obese type 2 diabetic patients [17].

Lippl *et al*. (2008) reported that basal obestatin concentrations in patients with type 2 diabetes were not different compared to non-diabetic people. This contradiction may be elucidated on the basis of body mass index, age, sample size, gender, and pre- or postprandial periods. Therefore, well-controlled human studies collecting different assessments of obestatin before and after a meal are required. These findings could be explained through the assessment of the biological mechanism of action of obestatin, i.e., through recognition of its receptor [19]. Furthermore, obestatin is positively correlated to insulin concentrations and actions [20]. It was reported that obestatin is involved in the treatment of cerulein-induced acute pancreatitis [21]. Obstatin and insulin show an interrelationship; insulin can lower obestatin concentration in insulin sensitive patients [22], indicating that insulin and insulin sensitivity are key factors influencing the production of obestatin. Likwise , abnormalities in the metabolic pathway generating insulin may influence obestatin concentrations as well [23].

In previous researches, serum obestatin concentration was reported to be lower in T2DM patients, along with impaired glucose tolerance . The correlation analysis showed obestatin to be independently correlated with impaired glucose tolerance and T2DM. Meanwhile, the expression of GPR39, a gene which was recognized as the receptor for obestatin, exhibited remarkably lower status in obese T2DM patients than in inclined and obese subjects with normal glucose levels.

Fasting glucose concentrations were also negatively correlated to mRNA expression levels of GPR39, but they displayed a positive relationship to adiponectin mRNA expression levels. This indicated the participation of obestatin signal pathway in the homeostasis of glucose and the progression of T2DM [24]. While St-Pierre *et al*. (2010) recorded that diabetic patients and normal subject show similar concentrations of serum obestatin in the fasting status. Nevertheless, in the current study, serum levels of obestatin were reduced in patients with diabetes [25]. Thus, we assume that the low concentration of obestatin could be a predictor for T2DM patients, while the mechanisms are not quite understood. We assumed that the high concentration of obestatin could be a mechanism for protection against high serum glucose level .

Several findings clearly indicated that obestatin is indeed a multifunctional peptide, exerting a variety of effects such as the stimulation of cell proliferation, survival and differentiation, as well as the influence on glucose and lipid metabolism, as along with several anti-inflammatory and cardioprotective actions [3]. Obestatin acts as a possible curative tool in various pathological conditions such as insulin resistance and diabetes, because of its regulatory role in the metabolism of glucose and lipid. Previous studies in humans revealed that circulating obestatin concentrations are remarkably lower in obese people and correlate negatively with body mass index and levels of insulin , glucose and HOMA-IR [1,22].

Serum levels of cardiac function markers

The results the Table-3 indicate significant increases (P=0.0408 and (P=0.0113) of CK concentrations in diabetic males with both age ranges (139.3±23.54 IU/L) & (135.5±15.66 IU/L) in comparison with their levels in matched controls (83.63±8.92 IU/L and 93.64±6.54 IU/L, respectively). Similarly, significant increases (P=0.0002 and P=0.0452) of CK concentrations were observed in diabetic females of both age ranges (100.8±4.21 IU/L) & (105.2±16.04 IU/L) in comparison with their levels in matched controls (78.32±5.50 IU/L and70.67±4.02 IU/L, respectively). As indicated in Table-3, there were significant increases (P=0.0453 and P=0.0319) in the mean levels of serum LDH in male diabetic patients with both age ranges (3.44±0.19 µKat/L and 3.26±0.30 µKat/L, respectively)in comparison with their levels in matched controls (2.82±0.14 µKat/L and 2.40±0.23 µKat/L, respectively). Similarly, the findings indicated that there were significant increases (p=0.0482 and p=0.0267) in the mean serum LDH in diabetic males with both age ranges (3.36±0.18 µKat/L and 3.38±0.19 µKat/L, respectively) in comparison with their levels in matched controls (2.81±0.19 µKat/L and 2.68±0.15 µKat/L, respectively).

Table 3- Serum levels of cardiovascular biomarkers in diabetic patients

Parameters		Group	Control	Patients	P-Value
Male	**CK (IU/L)**	40-59	83.63±8.92	139.3±23.54	0.0408
		60-80	93.64±6.54	135.5±15.66	0.0113
	LDH (µKat/L)	40-59	2.82±0.14	3.44±0.19	0.0453
		60-80	2.40±0.23	3.26±0.30	0.0319
Female	**CK (IU/L)**	40-59	78.32±5.50	100.8±4.21	0.0002
		60-80	70.67±4.02	105.2±16.04	0.0452
	LDH (µKat/L)	40-59	2.81±0.19	3.36±0.18	0.0482
		60-80	2.68±0.15	3.38±0.19	0.0267

Values are expressed as mean ± SE, Normal Values: (CK: Male = 38 - 174 IU/L, Female = 26 - 140 IU/L); (LDH: Male & female = 1.67 – 5.83 µKat/L).

The results presented in the current study demonstrates a significant elevation in the serum levels of creatine kinase (CK), which is in line with many previous studies [26,27,28,29]. In diabetes patients, disturbance in the metabolism of glucose, lipids and proteins occurs at the level of muscular cells. Because of the insulin resistance status in diabetes, the usage of glucose is declined, glucose phosphorylation is changed, biosynthesis of glycogen is diminished, and the glycolytic pathway is inhibited. Due to the low capacity of the glycolytic pathway, levels of oxaloacetate and pyruvate are decreased as well. The magnitudes of the citric acid cycle , the oxidative phosphorylation pathway, and electron transport chain are reduced. Through stimulated glycolysis and further fatty acids

oxidation, the body tries to challenge these pathways. Because of the reduction of adenosine triphosphate, biosynthesis of creatine phosphate is reduced and the potentiality of reproduction of adenosine triphosphate from adenosine diphosphate is decreased. The condition can be further complex through the phosphorylation of adenosine monophosphate-activated protein kinase [30]. This enzyme undergoes activation in cells when the percent value of creatine phosphate to creatine and the ratio of adenosine triphosphate / adenosine monophosphate are decreased. In healthy subject, this decline is observed during the contraction of muscle tissues due to the use of adenosine triphosphate and creatine phosphate [31]. It is possible to suppose that, due to the evident metabolic stress and all the above mentioned disturbances in diabetic patients, this condition is further complicated throughout the time required for muscle contraction.

After activation, CK undergoes phosphorylation in the muscle tissue and causes its blocking. During the activation processes, the cell is trying to protect itself from the reduction of adenosine triphosphate and by this means it ensures adequate quantities of this compound [32]. After the production of energy by metabolic pathways, they undergo reactivation. Since these metabolic pathways are deficient in patients with diabetes, this mechanism would not reverse the results in these subjects. Occasionally, defects in the production of adenosine triphosphate can cause a total blockage of the activity of creatine kinase. Hence , it can be proposed that one of the possible mechanisms describing the significant elevation in creatine kinase activity in diabetics could be linked to energy decline; i.e. energy generation through metabolic pathways is inhibited, creatine phosphate is missing and because of potential inhibition of creatine kinase activated by AMP (adenosine monophosphate) activated protein kinase), all of these factors are essential for normal muscle cells functioning. Because of these events, CK escapes from the cytoplasm into the bloodstream, resulting in the elevation of its activities in diabetic patients. The higher level of CK activity suggests that the activity of this enzyme is correlated with the status of the metabolic pathway in diabetes mellitus type II patients [29]. On the other hand, diabetes has long been known as a prediction factor for heart diseases and complications of the cardiovascular system that are well recognized in patients with chronic hyperglycemia. These complications include heart attack ,coronary heart disease, and stroke [10]. CK elevation is correlated with the presence of chronic cardiovascular disease in DM patients with multiple correlated heart complications [33].

Moreover, the findings of this study revealed that there is a remarkable elevation in the serum activities of LDH. These results are concordant with particular previous findings [34,35,36]. The increases in the activity of cardiac enzymes such as LDH), in general, can be explained by few theories. It is possible that cells release LDH into the blood stream after tissue damage or destruction of red blood cell . Elevated serum LDH activity is a clinical marker of tissue damage, especially those negatively influenced by the pathogenesis of diabetes mellitus and toxicity of chemical compounds , such as heart , liver and muscle tissues [37,38]. The elevated activity of serum LDH is a noticeable marker of tissue damage and necrosis. In clinical prognosis, LDH activity is relatively low in serum in the absence of tissue damage, whereas an elevated activity is an indication of tissue necrosis [39].

Another explanation of high LDH level may be the el which is mainly clinically manifested as dehydration, hyperglycemia and metabolic acidosis. Diabetic ketoacidosis patients may have various grades of myocardial damages, and the enzymes of cardiac muscles are important markers of cardiac damage , including creatine phosphokinase and LDH. DKA is a major complication of diabetes, caused by a serious lack of insulin, mainly characterized by loss of water and hyperglycemia metabolic acidosis [28]. DKA takes place when the degree of diabetes in patients becomes severe. At that time, the level of glucose is remarkably raised, insulin losses its activity, ketone bodies in the blood are increased, and the excretion of the ketones is elevated. When this happen, the body is in the state of depletion of oxygen and excessive loss of water with a relatively decreases ability for immune resistance. Hence, patients often fall into the state of an elevation in serum potassium or lowering in serum sodium [40].

When DKA occurs, the osmotic pressure of high sugar level can lead to hypoxic ischemic injury. The results of present study reported that the activities of LDH in the diabetic group are significantly higher than those in the healthy subjects. As the occurrence of DKA makes the body in the dehydrated state, it causes the loss of oxygen and hypoxic ischemic injury of myocardial tissues. At the same time, the increase in hypertonic state of the high glucose level will increase cell membrane permeability and the metabolism of the cell becomes disordered, causing necrosis in the mitochondria

and cell apoptosis. This leads to an elevation in the secretion of the intracellular cardiac enzyme markers , including LDH [28]. Moreover, persistent hyperinsulinemia might trigger endothelial dysfunction, atherosclerosis and development of cardiac dysfunction. These dysfunctions are accelerated in poor metabolic control. Elevated LDH activity seen in diabetic patients with poor glycemic control may originate from the heart and may therefore herald the onset of diabetes-associated cardiac dysfunction. The increased enzyme activity seen in diabetes can also be due to the effect of insulin on liver and muscle tissues. Muscular and hepatic dysfunctions are frequently correlated with diabetes and, thus, serum enzyme levels derived from liver and muscle may also be contributing to increased LDH activity in diabetes [34].

Serum levels of lipid profile markers in diabetic patients

The findings showed remarkable elevations (p=0.0335 and p=0.0039) in serum TC levels in male diabetic patients with both age ranges (201.6±6.36 mg/dL) & (180.1±9.34 mg/dL) in comparison with their levels in matched controls (167.6±12.37 mg/dL and 132.4±10.47 mg/dL,respectively). Similarly, there were statistically significant elevations (p=0.0001 and p=0.0286) in serum TC levels in diabetic females with both age ranges (201.2±10.02 mg/dL) & (190.7±9.30 mg/dL) in comparison with their levels in matched controls (144.0±8.86 mg/dL and 159.7±4.54 mg/dL,respectively)(Table-4). The results also demonstrated that serum LDL levels were remarkably increased (p=0.0481 and p=0.0337) in diabetic males with both age ranges (124.1±4.98 mg/dL) & (120.7±3.55 mg/dL) in comparison with their levels in matched controls (107.4±6.29 mg/dL and 106.5±5.55 mg/dL, respectively).

Similarly, the findings indicated that there were statistically significant elevations (p=0.0008 and p=0.0235) in serum LDL levels in diabetic females with both age ranges (123.6±4.80 mg/dL) & (120.6±5.83 mg/dL) in comparison with their levels in matched controls (98.13±3.63 mg/dL and 98.20±6.14 mg/dL, respectively) (Table-4). Table-4 also demonstrate that there were statistically remarkable increases (p=0.0241 and p=0.0009) in the mean serum levels of STG in diabetic males with both age ranges (228.0±28.96 mg/dL and 209.6±25.44 mg/dL, respectively) in comparison with their levels in matched controls (141.1±14.37 mg/dL and 114.0±10.07 mg/dL, respectively). The results also presented significant increases (p<0.0001); (p=0.0045) in the mean serum levels of STG in diabetic females with both age ranges (194.2±17.79 mg/dL and 180.5±17.05 mg/dL,respectively) in comparison with their levels in matched controls (100.9±7.58 mg/dL and 116.4±11.23 mg/dL, respectively) (Table-4).

Significant decreases (P=<0.0001 and P=0.0003) were recorded in serum HDL levels in diabetic males with both age ranges (35.12±1.43 mg/dL and 34.74±1.74 mg/dL, respectively) in comparison with their levels in matched controls (47.13±2.37 mg/dL and 47.90±2.74 mg/dL, respectively). Moreover, as indicated in Table-4, there was a remarkable decrease (P=<0.0001) in serum HDL level in diabetic females with both age ranges (38.07±0.88 mg/dL and 36.64±1.69 mg/dL, respectively) in comparison with its level in matched controls (48.53±1.68 mg/dL and 55.90±1.35 mg/dL, respectively).

Table 4-Mean values of lipid profile markers in diabetic patients and the control.

Parameters		Group	Control	Patients	P-Value
Male	STC (mg/dL)	40-59	167.6±12.37	201.6±6.36	0.0335
		60-80	132.4±10.47	180.1±9.34	0.0039
	LDL (mg/dL)	40-59	107.4±6.29	124.1±4.98	0.0481
		60-80	106.5±5.55	120.7±3.55	0.0337
	HDL (mg/dL)	40-59	47.13±2.37	35.12±1.43	<0.0001
		60-80	47.90±2.74	34.74±1.74	0.0003
	STG (mg/dL)	40-59	141.1±14.37	228.0±28.96	0.0241
		60-80	114.0±10.07	209.6±25.44	0.0009
Female	STC (mg/dL)	40-59	144.0±8.86	201.2±10.02	0.0001
		60-80	159.7±4.54	190.7±9.30	0.0286
	LDL (mg/dL)	40-59	98.13±3.63	123.6±4.80	0.0008
		60-80	98.20±6.14	120.6±5.83	0.0235
	HDL	40-59	48.53±1.68	38.07±0.88	<0.0001

	(mg/dL)	60-80	55.90±1.35	36.64±1.69	<0.0001
	STG (mg/dL)	40-59	100.9±7.58	194.2±17.79	<0.0001
		60-80	116.4±11.23	180.5±17.05	0.0045

Values are expressed as mean ± SE, Normal Values: (TC: Male & Female =<200 mg/dL); (LDL: Male & Female = <130 mg/dL); (HDL: Male & Female: Low level (Risk factor):<40 mg/dL, High level (Protective factor):≥60 mg/dL); (TG: Male & Female= 35 – 160 mg/dL).

The results showed that the serum levels of lipids profile parameters of, TCH, TG, LDL and lipoproteins are statistically remarkably increased in type 2 DM patients in comparison to controls, while the level of HDL-C was statistically diminished in T2DM. Patients with diabetes usually show abnormality at their lipids and lipoproteins due to the impairment of insulin activity on major metabolic enzymes. Insulin insensitivity , glucose tolerance, and serum insulin concentrations have been involved in abnormal serum lipoprotein concentrations, while high serum insulin levels have been correlated with the development of the complications of atherosclerosis in DM patients [41]. In diabetes mellitus, many factors have impacts on levels of blood lipids due to the strong relationship between carbohydrates and lipids metabolism. Therefore, any defect in sugar metabolism leads to a defect in lipid metabolism and vice versa . In combination with hyperinsulinemia, insulin resistance acts as a primary defect for future development of type 2 diabetes and, thus, has a strong predictive value in this condition [42]. Many studies reported that insulin has an impact on liver synthesis of apolipoprotein and is involved in the regulation of the enzymatic activities of the protein of cholesterol ester transport and lipoprotein lipase, which causes abnormalities in the metabolic pathways of lipid and lipoprotein in T2DM [43].

The results of the current study observed a statistically significant elevation of total cholesterol (TC) levels in diabetic male and female patients with both age ranges. The elevation of TC in individuals with DM was documented by several studies [41, 44]. This elevation may be due to a rise in the serum level of very low-density lipoproteins and low-density lipoproteins, which may be attributed to an elevation in the hepatic synthesis of very low-density lipoproteins or a diminished removal of very low-density lipoproteins and low-density lipoproteins from the blood stream [45]. A statistically significant elevation in serum LDL level in both male and female diabetics with both age ranges was previously documented by numerous studies [41,46]. The significant elevation of LDL level in type 2 diabetes mellitus may be due to the ability of insulin to increase receptors of LDL. Longstanding insulin deficiency may be correlated with a decreased level of LDL receptor. This contributes to the elevation of LDL levels in diabetes mellitus [47]. Thus, patients with low LDL level will also possess lower serum levels of HDL and higher levels of triglyceride , which may further increase the risk of atherosclerosis [48].

The current study found a significant elevation in serum TG level in diabetic males and females with both age ranges. Also, it was previously reported in several studies that the level of TG is increased in diabetic subjects [41,49,50,51]. Significantly increased level of triglycerides in patients may be due to an increase in the biosynthesis of VLDL which contributes to raised serum concentrations of triglyceride which, through an exchanging process assisted by cholesterol ester transfer protein, results in a decline in the concentration of high density lipoprotein. It also may be attributed to defects in insulin which results from the abnormal glucose usage causing high blood glucose level and movement of fatty acids from adipocytes [52]. The fatty acids from adipocytes are provided for the generation of energy where excess fatty acids are precipitated in the liver and then transformed to triglyceride. The most frequent changes of lipid metabolic parameters are the condensation of increased triglyceride (VLDL - triglyceride), diminished clearance of triglyceride-rich lipoproteins, and decreased HDL [41,46]. In a recent research , it was clear that there was a direct relationship between HbA1c and high triglycerides and that HbA1c can be used as a potent indicator for lipid profile abnormalities and lowers the macro- and micro-vascular complications of disease. In other words, the risk of the elevation of serum triglyceride would be increased by 2.69 % in average in a poor glycemic control. This proposes an elevated risk of atheroclorosis due to lipid profile abnormalities linked with poor control of diabetes [51].

The findings of the current research detected a significantly lower level of HDL in diabetic male and female patients with both age ranges. This significant finding is consistent with results reported by previous studies [41,46,53,54]. Lower HDL level is due to high enrichment of triglyceride by

cholesterol ester transfer enzyme and elevated activity of hepatic triglyceride lipase. The main diabetic lipid profile abnormalities are the low HDL level due to its elevated degradation rate [52], linked with impaired HDL function as a result of advanced glycosylation end products [55]. The level of lipoprotein lipase, which is an enzyme mainly responsible for degradation of chylomicron lipoprotein and VLDL, is remarkably diminished in patients with T2DM. In addition, plasma free fatty acid levels are elevated as a result of diminished blocking of lipase in patients with T2DM. The net result is a high pool of lipoproteins with a high content of triacylglycerol, which elevate the activity of cholesteryl ester transfer enzyme and accelerate triacylglycerol enrichment of HDL particles caused by this enzyme. Consequently , hepatic lipase and HDL catabolism are enhanced by the raised triacylglycerol content of HDL [52]. A previous research found that T2DM patients with microalbuminuria had decreased serum HDL levels as compared to those without microalbuminuria. The results provided an evidence that HDL is an independent factor linked to diabetic nephropathy. The results also supported the assumption that T2DM patients with an elevate HDL levels might be protected against diabetic nephropathy [56].

Conclusions

The results of the current research study suggest that obestatin hormone participates in insulin resistance and has a major role in diabetes mellitus. Furthermore, obestatin may act as a useful treatment for patients with diabetes. Our findings demonstrated the pattern of alterations in lipid metabolic parameters and cardiac markers in diabetic patients with cardiovascular complications. From the present research, it is noticeable that Type 2 DM has a real effect on lipid metabolism. This was proved by the fact that all the lipid metabolism parameters as well as cardiac markers (creatine kinase and lactate dehydrogenase) activities were increased in diabetes patients when compared to healthy subjects.

Conflict of interest

The authors declare no conflict of interest during this study.

References

1. Nakahara, T., Harada, T., Yasuhara, D., Shimada, N., Amitani, H., Sakoguchi, T., Kamiji, M.M., Asakawa, A. and Inui, A. **2008**. Plasma obestatin concentrations are negatively correlated with body mass index, insulin resistance index, and plasma leptin concentrations in obesity and anorexia nervosa. *Biological psychiatry*, **64**(3): 252-255
2. Kellokoski, E., Kunnari, A., Jokela, M., Mäkelä, S., Kesäniemi, Y.A. and Hörkkö, S. **2009**. Ghrelin and obestatin modulate early atherogenic processes on cells: enhancement of monocyte adhesion and oxidized low-density lipoprotein binding. *Metabolism*, **58**(11): 1572-1580
3. Zorlu, M., Kiskac, M., Güler, E., Gültepe, I., Yavuz, E., Çelik, K. & Kocyigit, A. **2017**. 'Serum obestatin and omentin levels in patients with diabetic nephropathy', *Nigerian Journal of Clinical Practice*, **20**(2): 182–7.
4. Granata, R.; Settanni, F.; Gallo, D.; Trovato, L.; Biancone, L.; Cantaluppi, V.; Nano, R.; Annunziata, M.; Campiglia, P.; Arnoletti, E., Ghe, C.; Volante, M.; Papotti, M.; Muccioli, G.; Ghigo, E. **2008**. Obestatin promotes survival of pancreatic beta-cells and human islets and induces expression of genes involved in the regulation of beta-cell mass and function. *Diabetes*, **57**: 967-979
5. Ren, A.J.; Guo, Z.F.; Wang, Y. K.; Wang, L.G.; Wang, W.Z.; Line, Li; Zheng, X.; Yuan, W.J. **2008**. Inhibitory effect of obestatin on glucose- induced insulin secretion in rats. *Biochem. Biophys. Res. Commun.*, **369**: 969-972.
6. Granata, R.; Gallo, D.; Luque, R. M.; Baragli, A.; Scarlatti, F.; Grande, C.; Gesmundo, I.; Cordoba-Chacon, J. ; Bergandi, L.; Settanni, F.; Togliatto, G.; Volante, M.; Garetto, S.; Annunziata, M.; Chancclon, B. ; Gargantini, E.; Rocchietto,S.; Matera, L.; Datta, G.; Morino, M.; Brizzi , M.F.; Ong, H.; Camussi, G.; Castano, J.P.; Papotti, M.; Ghigo, E. **2012**. Obestatin regulates adipocyte function and protects against diet- induced insulin resistance and inflammation. *The FASEB J*., **26**: 150-73
7. Sarfraz, M., Sajid, S. and Ashraf, M.A., **2016**. Prevalence and pattern of dyslipidemia in hyperglycemic patients and its associated factors among Pakistani population. *Saudi journal of biological sciences*, **23**(6): 761-766.

8. Vergès, B., **2009**. Lipid modification in type 2 diabetes: the role of LDL and HDL. *Fundamental & clinical pharmacology*, **23**(6): 681-685.

9. Hirano, T. 2018, Pathophysiology of Diabetic Dyslipidemia, *Journal of atherosclerosis and thrombosis*, pp. 771–82.

10. Aguiar, C., Duarte, R. & Carvalho, D. **2019**. 'New approach to diabetes care: from blood glucose to cardiovascular disease', *Revista Portuguesa de Cardiologia*, **38**(1): 53–63.

11. DeFronzo, R.A., Ferrannini, E., Groop, L., Henry, R.R., Herman, W.H., Holst, J.J., Hu, F.B., Kahn, C.R., Raz, I., Shulman, G.I. and Simonson, D.C., 2015. Type 2 diabetes mellitus. *Nature reviews Disease primers*, **1**: 15019.

12. Al-Nozha, M.M., Ismail, H.M. & Al Nozha, O.M. **2016**. 'Coronary artery disease and diabetes mellitus', *Journal of Taibah University Medical Sciences*, **11**(4): 330–8.

13. Yassin, M.M., Altibi, H.I. and El Shanti, A.E., 2011. Clinical and biochemical features of type 2 diabetic patients in Gaza Governorate, Gaza Strip. *West African journal of medicine*, **30**(1): 51-56.

14. d'Emden, M.C., Shaw, J.E., Colman, P.G., Colagiuri, S., Twigg, S.M., Jones, G.R., Goodall, I., Schneider, H.G. and Cheung, N.W., **2012**. The role of HbA. *Med J Australia*, **197**(4): 220-221.

15. Allwsh, T.A. and Mohammad, J.A., 2013. Clinical study of obestatin hormone and its relation to diabetes mellitus. *Rafidain journal of science*, **24**(4 E): 74-87

16. Qi, X., Li, L., Yang, G., Liu, J., Li, K., Tang, Y., Liou, H. and Boden, G., **2007**. Circulating obestatin levels in normal subjects and in patients with impaired glucose regulation and type 2 diabetes mellitus. *Clinical endocrinology*, **66**(4): 593-597

17. Catalán, V., Gómez-Ambrosi, J., Rotellar, F., Silva, C., Gil, M.J., Rodriguez, A., Cienfuegos, J.A., Salvador, J. and Frühbeck, G., **2007**. The obestatin receptor (GPR39) is expressed in human adipose tissue and is down-regulated in obesity-associated type 2 diabetes mellitus. *Clinical Endocrinology*, **66**(4): 598-601

18. Cuping, Z. and Dongmei, K., **2009**. Changes of plasma obestatin and ghrelin in diabetes mellitus. *Chinese J. Clinical Health Care*, **22**(6): 589-591

19. Lippl, F., Erdmann, J., Lichter, N., Tholl, S., Wagenpfeil, S., Adam, O. and Schusdziarra, V., 2008. Relation of plasma obestatin levels to BMI, gender, age and insulin. *Hormone and metabolic research*, **40**(11): 806-812

20. Chanoine, J.P., Wong, A.C. and Barrios, V., **2006**. Obestatin, acylated and total ghrelin concentrations in the perinatal rat pancreas. *Hormone Research in Paediatrics*, **66**(2): 81-88

21. Bukowczan, J., Cieszkowski, J., Warzecha, Z., Ceranowicz, P., Kusnierz-Cabala, B., Tomaszewska, R. and Dembinski, A., **2016**. Therapeutic effect of obestatin in the course of cerulein-induced acute pancreatitis. *Pancreas*, **45**(5): 700-706

22. Anderwald-Stadler, M., Krebs, M., Promintzer, M., Mandl, M., Bischof, M.G., Nowotny, P., Kästenbauer, T., Luger, A., Prager, R. and Anderwald, C., **2007**. Plasma obestatin is lower at fasting and not suppressed by insulin in insulin-resistant humans. *American Journal of Physiology-Endocrinology and Metabolism*, **293**(5): E1393-E1398

23. Huang, J., Zhang, Y., Yu, S., Gan, X., Su, Y., Yuan, J. and An, R., **2012**. Circulating obestatin concentration is lowered by insulin in rats. *Experimental and clinical endocrinology & diabetes*, **120**(01): 56-58

24. Gu, P.Y., Kang, D.M., Wang, W.D., Chen, Y., Zhao, Z.H., Zheng, H. and Ye, S.D., **2013**. Relevance of plasma obestatin and early arteriosclerosis in patients with type 2 diabetes mellitus. *Journal of diabetes research*, *2013*.

25. St-Pierre, D.H., Settanni, F., Olivetti, I., Gramaglia, E., Tomelini, M., Granata, R., Prodam, F., Benso, A., Ghigo, E. and Broglio, F., **2010**. Circulating obestatin levels in normal and type 2 diabetic subjects. *Journal of endocrinological investigation*, **33**(4): 211-214

26. Ugur, K., Aydogan, Y., Akgun, A. and Aydin, S., **2019**. A High Creatine Kinase Concentration Might Be a Sign of McArdle Disease in Patient With Type 1 Diabetes. *Biochemistry insights*, **12**: 1178626419861407.

27. Odum, E.P. and Young, E.E., **2018**. Elevated cardiac troponin I, creatine kinase and myoglobin and their relationship with cardiovascular risk factors in patients with type 2 diabetes. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, **12**(2): 141-145.

28. Zhao, N., Ma, L., Bai, S. and Wang, C., **2018**. Correlation of blood glucose level and blood pH value with cardiac enzyme, amylase and other markers in patients with diabetic ketoacidosis. *Int. J. Clin. Exp. Med*, **11**(9): 10208-10214.
29. Jevrić-Čaušević, A., Malenica, M. and Dujić, T., **2006**. Creatine kinase activity in patients with diabetes mellitus type I and type II. *Bosnian journal of basic medical sciences*, **6**(3): 5-9.
30. Ponticos, M., Lu, Q.L., Morgan, J.E., Hardie, D.G., Partridge, T.A. and Carling, D., **1998**. Dual regulation of the AMP-activated protein kinase provides a novel mechanism for the control of creatine kinase in skeletal muscle. *The EMBO journal*, **17**(6): 1688-1699.
31. Umpierrez, G.E., Stiles, R.G., Kleinbart, J., Krendel, D.A. and Watts, N.B., 1996. Diabetic muscle infarction. *The American journal of medicine*, **101**(3): 245-250.
32. Winder, W.A. and Hardie, D.G., **1999**. AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. *American Journal of Physiology-Endocrinology And Metabolism*, **277**(1): E1-E10.
33. Segre, C.A.W., Hueb, W., Garcia, R.M.R., Rezende, P.C., Favarato, D., Strunz, C.M.C., Sprandel, M.D.C.O., Roggério, A., de Oliveira Carvalho, A.L., Maranhão, R.C. and Ramires, J.A.F., **2015**. Troponin in diabetic patients with and without chronic coronary artery disease. *BMC cardiovascular disorders*, **15**(1): 72.
34. Nsonwu-Anyanwu, A.C., Egbe, E.R., Offor, J.S. and Usoro, C.A., **2015**. Glycemic control, micronutrients and some metabolic enzyme activity in type 2 diabetes. *Int J Res Med Sci*, **3**: 2757-64.
35. Yousaf, M.N. and Powell, M.D., **2012**. The effects of heart and skeletal muscle inflammation and cardiomyopathy syndrome on creatine kinase and lactate dehydrogenase levels in Atlantic salmon (Salmo salar L.). *The Scientific World Journal*, *2012*.
36. Sreenivasan, R.S., Krishna Moorthy, P., Deecaraman, M., Prakash, N. and Renganathan, N.G., **2010**. Variations in the enzyme activity of carbohydrate metabolic disorder on cardiac function. *Eur. J. Appl. Sci*, **2**(2): 62-69.
37. Chikezie, P.C., Ojiako, O.A. and. Ogbuji, A.C., 2015. "Oxidative stress in diabetes mellitus." *Int J Biol Chem* **9**(3): 92-109.
38. Claudino, M., Ceolin, D.S., Alberti, S., Cestari, T.M., Spadella, C.T., Rubira-Bullen, I.R.F., Garlet, G.P. and de Assis, G.F., **2007**. Alloxan-induced diabetes triggers the development of periodontal disease in rats. *PloS one*, **2**(12).
39. Chikezie, C.M., Ojiako, O.A., Emejulu, A.A. and Chikezie, P.C., **2018**. Serum Lactate Dehydrogenase Activity and Visceral Organs and Body Weights of Diabetic Rats Administered Single and Combinatorial Herbal Formulations. *Pharmacognosy Communications*, **8**(1).
40. Jefferies, C., Cutfield, S.W., Derraik, J.G., Bhagvandas, J., Albert, B.B., Hofman, P.L., Gunn, A.J. and Cutfield, W.S., **2015**. 15-year incidence of diabetic ketoacidosis at onset of type 1 diabetes in children from a regional setting (Auckland, New Zealand). *Scientific reports*, **5**(1): 1-7.
41. Ozder, A., **2014**. Lipid profile abnormalities seen in T2DM patients in primary healthcare in Turkey: a cross-sectional study. *Lipids in health and disease*, **13**(1): 183.
42. Haffner, S.M., Mykkänen, L., Festa, A., Burke, J.P. and Stern, M.P., **2000**. Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-sensitive prediabetic subjects: implications for preventing coronary heart disease during the prediabetic state. *Circulation*, **101**(9): 975-980.
43. Mooradian, A.D., **2009**. Dyslipidemia in type 2 diabetes mellitus. *Nature Reviews Endocrinology*, **5**(3): 150-159.
44. Jain, H.R., Shetty, V., Singh, G.S. and Shetty, S., **2016**. A study of lipid profile in diabetes mellitus. *International Journal of Scientific Study*, **4**(9): 56-61.
45. Ganong, W.F., **2003**. Review of Medical Physiology. Endocrine Function of pancreas & Regulation of Carbohydrate Metabolism.324-33.
46. Bhowmik, B., Siddiquee, T., Mujumder, A., Afsana, F., Ahmed, T., Mdala, I.A., Moreira, D.V., Cristina, N., Khan, A.K.A., Hussain, A. and Holmboe-Ottesen, G., **2018**. Serum lipid profile and its association with diabetes and prediabetes in a rural Bangladeshi population. *International journal of environmental research and public health*, **15**(9): 1944.

47. Suryawanshi, N.P., Bhutey, A.K., Nagdeote, A.N., Jadhav, A.A. and Manoorkar, G.S., **2006**. Study of lipid peroxide and lipid profile in diabetes mellitus. *Indian Journal of Clinical Biochemistry*, **21**(1): 126.

48. Bays, H., **2003**. Atherogenic dyslipidaemia in type 2 diabetes and metabolic syndrome: current and future treatment options. *The British Journal of Diabetes & Vascular Disease*, **3**(5): 356-360.

49. Pantoja-Torres, B., Toro-Huamanchumo, C.J., Urrunaga-Pastor, D., Guarnizo-Poma, M., Lazaro-Alcantara, H., Paico-Palacios, S., del Carmen Ranilla-Seguin, V., Benites-Zapata, V.A. and Metabolic Syndrome Research Group, **2019**. High triglycerides to HDL-cholesterol ratio is associated with insulin resistance in normal-weight healthy adults. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, **13**(1): 382-388.

50. Young, K.A., Maturu, A., Lorenzo, C., Langefeld, C.D., Wagenknecht, L.E., Chen, Y.D.I., Taylor, K.D., Rotter, J.I., Norris, J.M. and Rasouli, N., **2019**. The triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio as a predictor of insulin resistance, β-cell function, and diabetes in Hispanics and African Americans. *Journal of Diabetes and its Complications*, **33**(2): 118-122.

51. Naqvi, S., Naveed, S., Ali, Z., Ahmad, S.M. & Asadullah, R. **2017**. *Correlation between Glycated Hemoglobin and Triglyceride Level in Type 2 Diabetes Mellitus*, **9**(6).

52. Vergès, B., **2015**. Pathophysiology of diabetic dyslipidaemia: where are we?. *Diabetologia*, **58**(5): 886-899.

53. Shen, Y., Shi, L., Nauman, E., Katzmarzyk, P.T., Price-Haywood, E.G., Bazzano, A.N., Nigam, S. and Hu, G., **2019**. Inverse association between HDL (high-density lipoprotein) cholesterol and stroke risk among patients with Type 2 Diabetes Mellitus. *Stroke*, **50**(2): 291-297.

54. Haase, C.L., Tybjærg-Hansen, A., Nordestgaard, B.G. and Frikke-Schmidt, R., **2015**. HDL cholesterol and risk of type 2 diabetes: a Mendelian randomization study. *Diabetes*, **64**(9): 3328-3333.

55. Zhou, H., Tan, K.C., Shiu, S.W. and Wong, Y., 2008. Increased serum advanced glycation end products are associated with impairment in HDL antioxidative capacity in diabetic nephropathy. *Nephrology dialysis transplantation*, **23**(3): 927-933.

56. Sun, X., Xiao, Y., Li, P.M., Ma, X.Y., Sun, X.J., Lv, W.S., Wu, Y.L., Liu, P. and Wang, Y.G., **2018**. Association of serum high-density lipoprotein cholesterol with microalbuminuria in type 2 diabetes patients. *Lipids in health and disease*, **17**(1): 229.