Nat.Volatiles&Essent.Oils,2021;8(5):8805-8814

NVEO
Natural Volatiles &
Essential Oils

The Association between Lipocalin 2 and obesity for diabetic Female Type II

Ameer Athab Al-Ameri * and Lamia AM AL-Mashhedy

Department of Chemistry, College of Science, University of Babylon, Hilla-Iraq.

Abstract

Diabetes mellitus (DM) is a metabolic condition due to insulin deficiency, insulin resistance, or both, characterized by hyperglycemia. Lipocalin-2 elevated in type 2 diabetes mellitus-related conditions such as heart disease have been identified in previous research. The Lipocalin-2 are correlated with obesity and unregulated diabetes, in spite of may be useful to consider the consequences of glycemic control and obesity on lipocaline-2.

There participated 54 patients with diabetes type 2 and 36 non-diabetic, Individuals of two groups to obese and non-obese compare with apparently healthy control. Before the body mass index (kg/m^2) has been calculated, weight (kg) and height (m) were measured as a normal weight (18.49-24.99 kg/m^2), or obese (>30kg/m^2) according to the WHO classification. The ELISA sandwich was used to measure Lipocalin-2. Colorimetric methods were used for calculating FBS and lipid profiles. The LCN2 levels varied greatly for non-obese women in serum ($p<0,05$) compare with obese patients. Similarly, the disparity for non-obese female patients in LCN2 was substantial relative to female obese serum controls ($p<0.05$).

The study also has been found positive correlated with Diabetes duration (r=0.002,p= 0.99), age(r=0.084,p=0.67), VLDL(r=0.354, p=0.07), T.G(r=0.35,p=0.070),total cholesterol (r= -0.037, p=0.85), LDL(r= -0.129, p=0.523), BMI(r=-0.026, p=0.89),and FBG(r =0.261,p= 0.18) while negative corrlated with HDL(r=-0.046, p=0.820), in the prediabetic obese patients . Iraqi women patients with diabetes have high serum lipocalin-2 levels. The results may be suggested their susceptibility to LCN2-related complications, such as metabolic syndrome, insulin resistance, ischemic heart disease, and diabetic renal disease. However, no significant relation between LCN2 and glycemic control and obesity was found.

Keywords: Fasting blood glucose (FBG) , Type 2 diabetes mellitus, Obesity, Lipocalin-2(LCN2).

Introduction

Obesity has become one of the major health problems that the world suffers from. It is defined as the abnormal or excess fat accumulation in adipose tissues. The amount of fat in the bodies of people varies in distribution. This high body fat distribution contributes to weight gain and risk of obesity and disease forms caused by obesity. It is present in all genders and in socio-economic and ethnic groups of all ages. Worldwide, the number of obese adults has hit more than 3.3 billion (Klöting et al., 2008)(Yumuk et al., 2015).

Diabetes mellitus (DM) is a metabolic condition due to insulin deficiency, insulin resistance, or both, characterized by hyperglycemia. DM is a major public health problem to raise cardiovascular morbidity and mortality in addition to the development of retinopathy, nephropathy and neuropathy. There were 422 million people who have type2 diabetes mellitus (T2DM) in worldwide, about 85-90% of them have T2DM. The number of diabetes mellitus at the last years, particularly is elevated because of the changes in life style(Kyu et al., 2016).

While reduced insulin resistance is the direct cause of diabetes mellitus, about 80% of Type 2 diabetic patients are the main predisposing factor of obesity .The sixth largest risk factor related to

the global disease risk is seen as the increased body weight(Haslam & James, 2005).In the past 30 years, global obesity in males has grown from 28.8% to 36.9% and in females from 29.8% to 38.0%. (Ng et al., 2014). DM2 and obesity, the greatest cause of death in the Arab world, are both metabolic and vascular complications predisposing factors(Mokdad et al., 2014)(1377 ,کوچکی).

LCN2 is a gene-encoded glycoprotein that is found in many other tissues, including the lungs, kidneys, adipocytes, and macrophages(Balducci, Stefano, Sacchetti, Massimo, Haxhi, Jonida, Orlando, Giorgio, D'Errico, Valeria, Fallucca, Sara, Menini, Stefano, Pugliese, 2014). This protein has been found to be a component of the innate immune system .

Lipocalin-2 was first isolated from mouse kidney cells in 1989(Sompayrac, Jane, & Danna, 1996). It was subsequently isolated as a human neutrophil gelatinase protein in humans in 1993(Kjeldsen, Johnsen, Sengelov, & Borregaard, 1993).

Recent studies identify adipokine, mostly secreted from adipose tissue, as lipocalin-2. LCN2 expression and secretion increased after preadipocyte conversion into mature adipocytes(Yan et al., 2007)(Law et al., 2010). LCN2 as adipokine has bacteriostatic consequences and antioxidant roles in chronic inflammation of the airway. A biomarker for kidney damage(Oberoi et al., 2015). LCN2 plays an important role in glucose homeostasis and insulin-sensitivity(Yan et al., 2007).

Many studies reported a close correlation between LCN2, atherosclerosis in diabetes mellitus, obesity, and metabolic syndrome(Balducci, Stefano, Sacchetti, Massimo, Haxhi, Jonida, Orlando, Giorgio, D'Errico, Valeria, Fallucca, Sara, Menini, Stefano, Pugliese, 2014)(Ni et al., 2013).

The research aimed at assessing the levels of lipocalin 2 in women with diabetes mellitus type 2 and the relation between lipid profile, fasting blood sugar and BMI with LCN2 levels as an indicator for diagnosis the relationship between obesity and type 2 diabetes.

Methods

Two groups of individuals were included in this study. The first group contain from 54 females diabetics patients and group two has 36 females as a healthy control .The first group was divided into two groups, according to BMI to obese and non-obese. Similarly, group 2 also divided into two groups, including females obese and another group to females non obese.

The research was case-control study at the Center for Diabetes and Endocrinology of Hilla District, Babylon, Iraq, Marjan Teaching Hospital. Included in the following criteria is both patients and healthy people: The age (35 to 60) years of type 2 DM who visited diabetes center during the study period from September to December 2020 on Sundays, Saturdays and Tuesdays.

There were criteria questioners with both patients and healthy individuals. Research was carried out using parameters for data collection; age, sex, disease existence and period, form of medicine, family background and climate. The body mass index (BMI, kg/m ^{2}) has been measured as an average weight (18.49-24.99 kg/m^2), or obese (> 30kg/m^2) according to the WHO classification. 2012.

Blood Sampling

Patient and control samples of blood (5 ml) have been collected. Samples of blood were permitted to coagulate and then centrifuged 10 minutes at 4500 rpm. Sera was separated and stored deep freeze until analysis .

Study of the laboratory

FBS was tested with enzymatic glucose oxidation. Classified diabetic patients were controlled (FBS <75-115), and non-regulated (FBS >75-115). The profile of the lipid was determined using enzyme methods. Lipocalin-2 was assessed in serum through ELISA sandwich.

Analysis of statistics

For statistical analysis, statistics from the SPSS version (V.26.0) were used. The findings were shown to be medium ± SD, one way ANOVA to test the significance of difference in means between more than two groups. Two separate average classes were contrasted with students t assessments. For evaluating relationships between variables, Pearson correlation coefficients were used. The P values recorded were two-tailed, and the P values were deemed significant at 0.05.

Results

The research included ninety women classified into two groups, 54 diabetic women (27 obesity, 27 non-obesity) patients and 36 female monitors (18 obese and 18 non obese).In Tables 1 and 2, The studied participants were faced with clinical and biochemical variables. In the patient group, LCN2 Significant in the patients group compared with controls , p(<0.05). FBS was significant in patients relative to controls, p(<0.001). Total cholesterol(T.C), very low-density lipoprotein (vLDL), low-density lipoprotein (LDL), triglycerides (T.G) were also important in patients with diabetes relative to controls, p(<0.02). High density lipoprotein (HDL) and BMI were non-significant in the patients group compared with controls (p> 0.05).

Table 1- Clinical properties for DM2 patients and controls

parameter	Patients n=54	Controls n=36	P value
Duration (years)	8.15±7.32	_____	_____
Age(years)	49.35±6.68	47.05±7.36	0.141
BMI(kg/m^2)	30±5.4	29.71±5.32	0.56
Duration of medication (years)	6.02±6.06	_____	_____

Table 2- Laboratory properties for DM2 patients and controls

parameter	Patients 54	Controls 36	P value
Lipocalin-2 (ng/mL)	316.02±153.83	242.01±108.41	<0.01

FBG(mg/dL)	191.52±71.95	87.70±19.64	<0.001
T.C(mg/dL)	240±63.48	131.86±39.54	<0.001
T.G (mg/dL)	239.22±111.25	138.86±54.73	<0.001
HDL (mg/dL)	48.85±15.73	52.45±19.03	0.35
LDL (mg/dL)	144.15±73.07	51.62±45.88	<0.001
vLDL (mg/dL)	47.84±22.35	27.77±10.81	<0.001

The mean difference is significant at P≤ 0.05.

Table 3- The Comparison of Patient and Control Groups for LCN2 (ng/mL)

Parameter	Groups	Mean± SD	CI 95%		Compared groups		P value
			Lower	Upper			
LCN2(ng/mL)	GP1	340.63±161.22	276.856	404.414	GP1	GP2	0.36
						GC1	0.011
						GC2	0.035
	GP2	307.22±135.42	253.651	360.799	GP2	GC1	0.076
						GC2	0.183
	GC1	251.05±113.07	192.920	309.197	GC1	GC2	0.70
	GC2	233.47±106.36	180.579	286.369			

GP1 patients obese , GP2 patients non obese, GC1 controls obese, GC2 controls non obese

The mean difference is significant at P≤ 0.05

LCN2 level comparison between different groups in Table 3. LCN2 was significantly increased in the patients obese compared to controls obese, (p=0.011). Similarly, in the patients obese compared controls non obese (p=0.035). while LCN2 levels were non-significant elevated in the GP1 compared to GC1, (p=0.076). and for GP2 compared with GC2 (p=0.183).

Table 4 -The FBS Levels(mg/dL) and BMI(kg/m^2) for Patient Groups Compared to Control Groups

Parameter	Groups	N	Mean± SD	CI 95%		Compared groups		P value
				Lower	Upper			
FBG(mg/dL)	GP1	27	159.49±53.13	138.477	180.517	GP1	GP2	0.001
							GC1	<0.001
							GC2	<0.001
	GP2	27	222.34±76.31	193.944	253.150	GP2	GC1	<0.001
							GC2	<0.001
	GC1	18	86.37±22.12	75.374	97.382	GC1	GC2	0.87

	GC2	18	89.11±17.20	80.268	97.956			
BMI(kg/m^2)	GP1	27	34.62±4.16	32.945	36.239	GP1	GP2	<0.001
							GC1	0.508
							GC2	<0.001
	GP2	27	26.18±2.18	25.321	27.049	GP2	GC1	<0.001
							GC2	0.342
	GC1	18	33.94±3	32.452	35.436	GC1	GC2	<0.001
	GC2	18	25.23±3.03	23.676	26.794			

GP1 patients obese , GP2 patients non obese, GC1 controls obese, GC2 controls non obese

The mean difference is significant at P≤ 0.05

FBS levels and BMI were comparison between different groups for patients and controls as illustrated in Table 4. Obese patients have low FBS (159.49±53.13) with BMI(34.62±4.16) compared to non obese patients FBS (222.34±76.31) with BMI(26.18±2.18), according to criteria compared to obese control FBS (86.37±22.12) with BMI (33.94±3) compared another non obese control FBS(89.11±17.20) with BMI (25.23±3.03).

The results of T.C, T.G, LDL-C in Table 5 demonstrated significantly (P<0.05) higher levels obese diabetic patients group compared to nonobese diabetic patients . Nonsignificant variations in level of HDL-C in obese diabetic patients group when compared with those of obese controls group. There was a decrease in the mean value of serum HDL-C of obese diabetic patients group compared with mean value of nonobese diabetic patients group.

Table 5- The Comparison of Patient and Control Groups for Lipid Profile

Parameter	**Groups**	**Mean± SD**	**CI 95%**		**Compared groups**		**P value**
			Lower	**Upper**			
T.C (mg/dL)	GP1	261.74±54.45	240.205	283.289	GP1	GP2	0.005
						GC1	0.001
						GC2	0.001
	GP2	219.95±65.90	193.885	246.031	GP2	GC1	0.001
						GC2	0.005
	GC1	134.72±32.93	117.789	151.661			
	GC2	129.14±45.71	106.408	151.878			
T.G(mg/dL)	GP1	244.71±96.37	206.587	282.835	GP1	GP2	0.25

						GC1	0.001
						GC2	0.002
	GP2	220.47±80.60	188.594	252.364	GP2	GC1	0.001
						GC2	0.001
	GC1	126.43±33.92	67.37	181.05			
	GC2	122.55±37.33	212.63	47.02			

HDL-C (mg/dL)	GP1	46.62±15.65	40.429	52.81	GP1	GP2	0.51
						GC1	0.46
						GC2	0.88
	GP2	49.63±13.85	44.136	55.09	GP2	GC1	0.12
						GC2	0.32
	GC1	50.34±20	40.399	60.29			
	GC2	54.68±18.28	45.284	64.08			
LDL-C (mg/dL)	GP1	168.64±65.08	142.893	194.38	GP1	GP2	0.004
						GC1	<0.001
						GC2	<0.001
	GP2	119.66±73.50	90.592	148.74	GP2	GC1	<0.001
						GC2	0.001
	GC1	59.20±24.55	25	89.57			
	GC2	53.38±37.86	34.245	72.51			
VLDL-C (mg/dL)	GP1	52.44±23.78	43.030	61.84	GP2	GP2	0.072
						GC1	0.012
						GC2	0.005
	GP2	43.24±20.	35.247	51.25	GP2	GC1	<0.001
						GC2	<0.001
	GC1	28.82±9.73	23.985	33.66			

	GC2	26.66±12.05	20.463	32.855		

Lipocalin2 and FBS in Table 6 show, strongly associated with FBG and positive correlation with diabetes duration, T.G, BMI, T.C Age, vLDL, while negative correlation with HDL.

Table 6- The Correlation between LCN2 and Variables for Obese Diabetic Patients.

parameters	r	p
Age(years)	0.084	0.67
Duration(years)	0.002	0.99
BMI(kg/m^2)	0.026	0.89
FBS(mg/dL)	0.261	0.18
T.C (mg/dL)	0.037	0.85
T.G (mg/dL)	0.35	0.07
HDL(mg/dL)	-0.046	0.820
LDL(mg/dL)	0.129	0.523
vLDL(mg/dL)	0.354	0.07

The mean difference is significant at P≤ 0.05

Lipocalin2 and FBS in Table 7 show, weak associated with FBG and positive correlation with diabetes duration, BMI, Age, T.C, LDL, while negative correlation with T.G , HDL, vLDL .

Table 7- The Correlation between LCN2 and Variables for Non-obese Diabetic Patients

parameters	r	p
Age(years)	0.118	0.347
Duration(years)	0.058	0.776
BMI(kg/m^2)	0.143	0.477
FBS(mg/dL)	0.045	0.824
T.C (mg/dL)	0.125	0.536
T.G (mg/dL)	-0.058	0.773
HDL(mg/dL)	-0.042	0.836
LDL(mg/dL)	0.138	0.491
vLDL(mg/dL)	-0.058	0.773

The mean difference is significant at P≤ 0.05

In this study we found nonsignificant correlated with BMI and LCN2 in both groups obese and nonobese patients diabetes. Areej E. Elkhidir, *et al*, found nonsignificant correlated with BMI and LCN2 in both group obese and nonobese patients diabetes. This may be indicated their susceptibility to develop complications associated with LCN2 such as metabolic syndrome, insulin resistance, ischemic heart diseases and diabetic renal diseases (Elkhidir, Eltaher, & Mohamed, 2017)(Mishra et al., 2003)(de Carvalho et al., 2016).

While Wang, *et al* found a strong positive correlation between BMI and LCN2, and they provide the clinical and experimental evidence to demonstrate the LCN2 is a marker for obesity and its associated pathologies. Also they reported that adipose tissue and liver are probably the LCN2 principal sources that contribute to elevate circulating levels of this protein in obesity (Wang et al., 2007). Although the opposite results of the current study, it gives an indication of the importance of this parameter with obesity state.

The previous studies, proposed that LCN2 consider as a beneficial role in the regulation of various aspects of energy metastasis. Those include protection from diet-induced obesity, fatty liver disease, atherogenic dyslipidemia and insulin resistance, suppression of hepatic gluconeogenesis, and promotion of adaptive thermogenesis, activation of brown adipose tissue, and fatty acid oxidation(Zhang et al., 2014)(Paton et al., 2013)(Mosialou et al., 2020).

We found also the levels of LCN2 were increased significantly in T2DM patients with higher levels in obese diabetics compared nonobese diabetics. But this difference does not reach significance(p=0.36). El-mesal-lamy, *et al*, found LCN2 level was increased significantly in T2DM patients with higher levels in obese diabetics compared nonobese diabetics, but this difference does not reach significance(El-Mesallamy, Hamdy, & Sallam, 2013). LCN2, it is recently known to be an independent risk factor for IR and diabetes and to be correlated with metabolic and inflammatory parameters(Law et al., 2010).

In our study, the levels of LDL were significantly elevated for obese patients compared with nonobese patients (p=0.004), this result it may be to uncontrolled blood sugar which mean that prolonged hyperglycemia significantly increased the concentration of LDL, that increased LDL level is associated with silent myocardial ischemia(Mohammed, Mohamed, Fadlallah, & Mohamed, 2014). As well as the lower HDL levels in diabetics patients compared to controls shown in Table 2 . Mohammed, *et al* found low HDL level in diabetics patients , that in an indicator for peripheral arterial disease , Coronary Artery Disease (CAD) and macro and micro vascular complications(Mohammed et al., 2014).

The present study is agreement with the results of the Jacob, et al. (2013) study which demonstrated that the total cholesterol, triglycerides and LDL-C concentrations were higher in 82 obese patients, compared to 30 non-obese subjects, while HDL-C levels were low in obese patients, compared with non-obese subjects. (Algayed, Alharbi, & Almutairi, 2017).

In this study ,we found strong positive correlation between LCN2 and TG (r=0.35, p=0.07) in obese diabetics patients compared to nonobese patient (r= -0.058 , p=0.7). Wang, *et al*, found strong positive correlation between LCN2, T.G and found a strong negative correlation between LCN2 and lipid profile (increased serum triglyceride and decreased HDL cholesterol), suggesting that LCN2 may be independent risk factor for hyperglycemia and insulin resistance in human and development different metabolic syndrome (Wang et al., 2007).

The conclusion of our study suggested the LCN2 levels, which consider as a pro-inflammatory marker for obesity enhance complications such as metabolic syndrome, insulin resistance, ischemic heart disease, and diabetic renal disease for women obese diabetes patients compared with nonobese diabetes patients .

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