Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue 2, 78-86

The Role of Serum Ceramide Levels and Some Biochemical Markers Pathogenesis of Type 2 Diabetes Mellitus in Iraqi Patients

[1]Noor Q. Mudhaffer, [2]Ismail A. Abdul-Hassan

[1]Central teaching hospital of paediatric, Ministry of Health, Baghdad, Iraq.
[2]Institute of Genetic Engineering and Biotechnology for Post Graduate Studies, University of Baghdad, Iraq

Received: February 20, 2025 / Accepted: May 13, 2025 / Published: November 16, 2025

Abstract: Diabetes mellitus (DM) is a metabolic condition that, over time, leads to major illnesses due to its numerous consequences. Type 2 diabetes mellitus (T2DM) is the most prevalent kind of disease; it is a complicated disease that is caused by a combination of genetics and environmental factors. Ceramides are structural units of lipids in cell membranes and signaling molecules involved in the regulation of cell homeostasis. However, ceramides are most often recognized as the foundation for lipid bilayers. This study involved the collection of 120 blood samples, 60 patients with T2DM, and 60 apparently healthy subjects as a control group. The serum ceramide level was determined by ELISA. Body mass indexes (BMI) were calculated for all participants. The biochemical parameters studied (fasting blood glucose (FBG), lipid profile, and glycated hemoglobin (HbA1c) were also measured. The Results indicate that serum ceramide levels are significantly ($P<0.0001$) higher in T2DM patients versus controls. Also, FBG and HbA1c were levels significantly ($P<0.0001$) elevated in T2DM patients versus controls. Lipid parameters (Cholesterol, triglyceride, and very low-density lipoprotein (VLDL)) significantly increased in T2DM patients compared with a control group. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) showed no significant differences in individuals with type 2 diabetes compared to control subjects. The study concludes that the serum ceramide levels may represent a biomarker for the pathogenicity of T2DM in Iraqi patients.

Keywords: Type 2 diabètes mellites, T2DM, céramides, Insulin résistance, β-cell.

Corresponding authors: (E-mail: Noor.Qasem2300m@ige.uobaghdad.edu.iq).

Introduction

Diabetes mellitus (DM) is defined as the most chronic metabolic disorder resulting from a complex interaction between environmental and heredity factors. Diabetes mellitus type II, known as non-insulin-dependent DM, is the most common kind of diabetes described by hyperglycemia, insulin resistance, and a relative lack of insulin(1). The pathological mechanism of Type II DM mainly contains insulin resistance and impaired islet β-cell function (2). Insulin, mainly secreted through islet β-cells, is essential in regulating glucose metabolism in peripheral tissues. Defective islet β-cell function leads to decreased insulin secretion, a pathophysiological characteristic of T2DM (3). Ceramides are components of the structure of cell membrane lipids that act as signal molecules regulating cell homeostasis. Ceramides act as the main mediators

that connect lipid-induced inflammatory pathways to insulin resistance and the development of T2DM (4). Ceramide plays an essential role in beta-cell apoptosis, which is a programmed death of cells that performs an important role in maintaining tissue homeostasis by removing harmful or unwanted cells. The mechanism of apoptosis involves complex signaling pathways. The three currently accepted apoptotic signaling pathways are the extrinsic death receptor, the intrinsic mitochondrial, and the intrinsic ER pathways (5, 6).

In the pathogenesis of type II diabetes, one significant factor is the excessive apoptosis of pancreatic β-cells. Another route, ceramide, is phosphorylated at the plasma membrane by ceramide kinase. C1P phosphatase can hydrolyse its byproduct, ceramide-1-phosphate (C1P), to produce ceramide (7). An increasing amount of evidence highlights the role ceramides play as a pathogenetic role, especially in T2DM; however, whether ceramide disease modulation occurs as a Reaction to pathological mechanisms, such as inflammation or cellular stress, or if it is a direct factor in the pathogenesis of diabetes not yet clear. Ceramides have been suggested as a disease biomarker and can be found in blood samples (8). Ceramides may have a significant pathophysiological function in the devel opment of diabetes, according to recent data.

Therefore, this study aims to evaluate the potential role of serum ceramide levels as a biomarker for T2DM by investigating its association with fasting blood glucose (FBG), glycated hemoglobin (HbA1c), and lipid profile parameters in T2DM patients compared to healthy controls. Additionally, the study seeks to Examine the connection between insulin resistance and ceramide levels., providing insights into the pathophysiological mechanisms linking lipid metabolism to T2DM progression.

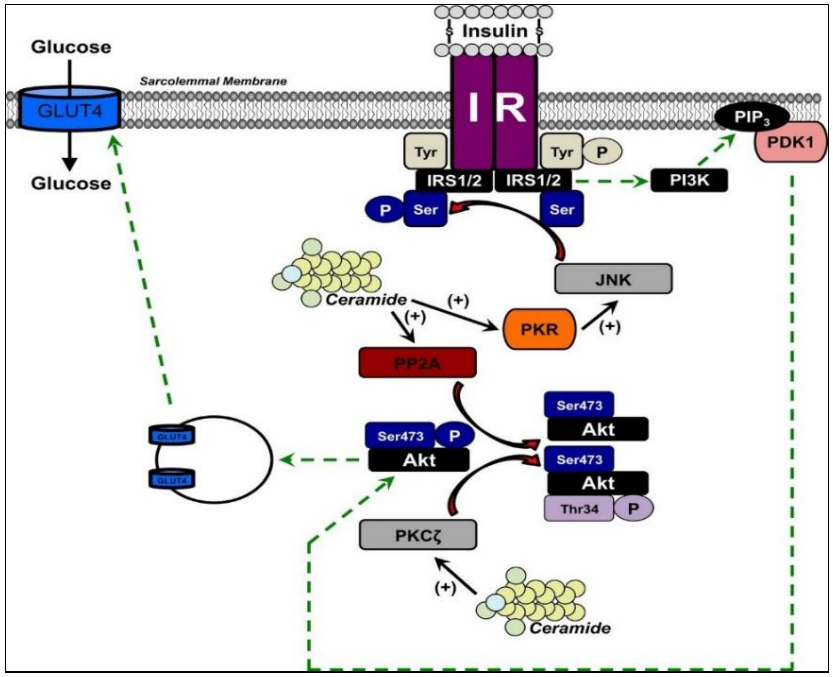

Figure (1): Mechanisms by which raised intracellular ceramide inhibits insulin signaling and subsequent glucose metabolism (9).

Material and Methods

Study Design and Participants

There were 60 T2DM patients and 60 apparently healthy controls in this case-control study. T2DM patients were recruited from AL-Furat General Hospital in Baghdad, Iraq. Physicians confirmed the diagnosis of T2DM based on the World Health Organization (WHO) diagnostic criteria, which define diabetes as an FBG level ≥126 mg/dL in addition to clinical symptoms.

Inclusion Criteria

Participants included in this study were Sixty Iraqi T2DM patients and 60 apparently healthy subjects, with ages ranging from (35 to 70) years. The current study classified the age into three groups (< 35 years, 40-50 years, and >50). The T2DM group comprised patients diagnosed according to WHO criteria, which define diabetes as an FBG level of ≥126 mg/dL, accompanied by clinical symptoms. The control group consisted of apparently healthy individuals with normal glucose metabolism and no history of diabetes. Individuals with type 1 diabetes, gestational diabetes, or any other endocrine disorder were excluded. Additionally, patients with severe cardiovascular, hepatic, renal, or inflammatory diseases, as well as those taking medications known to alter glucose metabolism or lipid levels, were not eligible for inclusion in the study.

Blood Sample Collection and Processing

5 mL of venous blood was collected from T2DM patients and healthy controls using EDTA-anticoagulated and gel tubes. 2 mL of whole blood For HbA1c measurement was stored in EDTA tubes and analyzed using the Cobas C111 analyzer. To obtain serum, 3 mL of blood was collected in gel tubes, centrifuged at 3000 rpm for 10 minutes, then kept in Eppendorf tubes at -20°C for additional biochemical examination. Biochemical assessments included FBG and lipid profile measurements, which encompassed total cholesterol, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL). These parameters were investigated using the Accent-200 Analyzer. Serum ceramide concentrations were quantified using a sandwich ELISA method (ELK Biotechnology), employing a double-specific monoclonal antibody to ensure measurement accuracy(10).

Result and Discussion

Sixty Iraqi T2DM patients and 60 apparently healthy subjects were selected for this study, with the ages ranging from (35 to 70) years. The current study classified the age into three groups (< 35 years, 40-50 years, and >50). Data is listed in table (1). The age variable results showed significant differences between the age and BMI in T2DM patients, as shown in Table (1).

All T2DM patients and healthy controls had the body mass indexes examined during the research study. The mean average BMI in T2DM patients and apparently healthy controls were (27.33 ±0.61) and (25.18 ±0.43) respectively. These results were inconsistent with (11), who found that BMI was effectively associated with type-II diabetes, and it was regarded as a risk factor for the incidence of the disease in people with different levels of obesity. There was a significant difference in Figure (2) between BMI in type 2 diabetes patients and healthy controls.

Table (1): Distribution of sample study according to BMI and Age in control and patient groups.

Factor		Patients (No=60)	Control (No= 60)	P-value
BMI (kg/m^2)	Severe Thinness: >16	0 (0.00%)	0 (0.00%)	0.0001 **
	Moderate Thinness: 16-17	0 (0.00%)	0 (0.00%)	
	Mild Thinness: 17-18.5	0 (0.00%)	0 (0.00%)	
	Normal: 18.5 – 25	32 (53.33%)	20 (33.33%)	
	Overweight: 25 – 30	24 (40.00%)	28 (46.67%)	
	Obese Class I: 30 – 35	4 (6.67%)	11 (18.33%)	
	Obese Class II: 35 – 40	0 (0.00%)	1 (1.67%)	
	Obese Class III: > 40	0 (0.00%)	0 (0.00%)	
Age groups (year)	<40 yr.	40 (66.67%)	8 (%)	0.0001 **
	40-50 yr.	12 (%)	17 (%)	
	>50 yr.	8 (%)	35 (%)	
** (P≤0.01).				

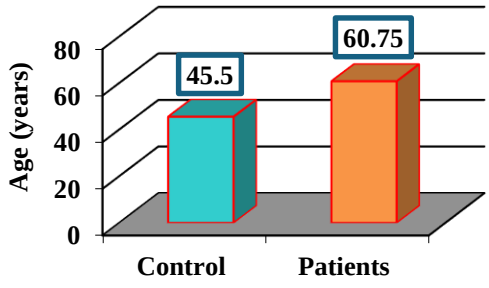

Figure (1): Comparison between control and patient groups in Age.

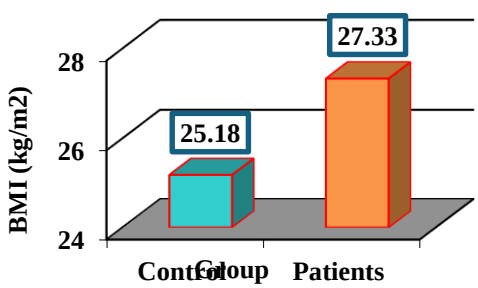

Figure (2): Comparison between control and patient groups in BMI

Fasting blood glucose was measured in the serum of all T2DM patients and controls. The FBG in the T2DM patients' group is (158.75 ±6.12) compared to (87.33 ±1.72) in the apparently healthy control group, as shown in Table (2). the results show that fasting blood glucose ($p<0.001$) significantly increased in patients with T2DM in comparison with its levels in normal healthy controls, as shown in Figure (3). The levels of HbA1c measured for diabetic patients and apparently healthy controls. The results showed in Figure (4) that the average glycated hemoglobin HbA1C level significantly ($P>0.0001$) increased from (7.52 ±0.17) in serum sample of apparently healthy controls to (5.43 ±0.06) in serum samples of the T2DM patients' group. as shown in table (2) These results correspond with those that (12) Proteins react spontaneously in blood with glucose to form glycated derivatives. The extent of glycation of proteins is controlled by the concentration of glucose in the blood and by the number of reactive amino groups present in the protein that are accessible to glucose for reaction. All proteins with reactive sites can be glycated, and the concentration of the glycated proteins that can be measured in blood is a marker for the fluctuation of blood glucose concentrations during a specific period.

Table (2): Comparison between control and patient groups in FBG and HbA1c.

Group	Mean ±SE	
	FBG (mg/ml)	HbA1c (%)
Control	87.33 ±1.72	5.43 ±0.06
Patients	158.75 ±6.12	7.52 ±0.17
T-test	12.603 **	0.365 **
P-value	0.0001	0.0001
** (P≤0.01).		

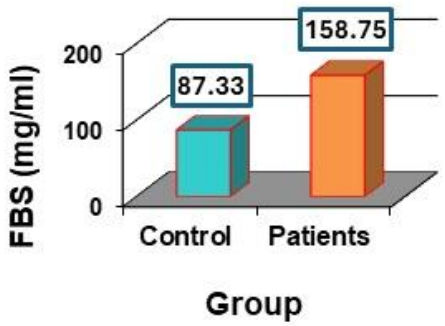

Figure 3: Comparison between control and patient groups in FBS

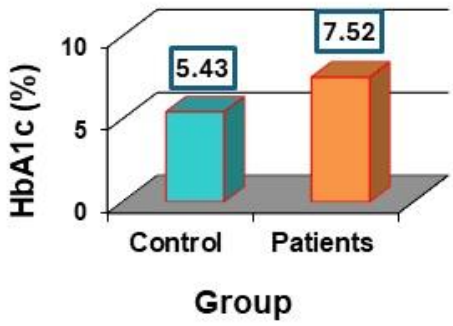

Figure 4: Comparison between control and patients groups in HbA1c

Table (3): Comparison between control and patient groups in Lipid profile.

Group	Mean ±SE				
	Cholesterol (mg/ml)	Triglyceride (mg/ml)	HDL (mg/ml)	LDL (mg/ml)	VLDL (mg/ml)
Control	166.28 ±6.23	129.48 ±8.89	41.03 ±1.55	123.42 ±6.42	25.89 ±1.78
Patients	203.02 ±7.54	171.61 ±9.83	42.84 ±2.03	116.08 ±8.45	34.21 ±1.90
T-test	19.391 **	26.254**	5.68 NS	21.018 NS	5.153 **
P-value	0.0003	0.0019	0.4808	0.491	0.0018
** (P≤0.01), NS: Non-Significant.					

As shown in Table (3), the Serum Cholesterol results show that the mean level value in diabetic patients (203.02 ±7.54) was significantly (P<0.0001) higher than the mean serum of the control group (166.28 ±6.23), as shown in Figure (5) This increase may be due to an increase in the plasma concentration of VLDL and LDL, which may be due to the increase in hepatic production of VLDL or decrease in the removal of VLDL and LDL from the circulation, Cholesterol level in blood is based on other risk factors such as age, gender, family history, smoking, high blood pressure, physical inactivity, obesity and diabetes, total cholesterol normal range should be lower than 200 mg/dl (13).

The level of serum TGs in a sample of type-II diabetes patients and apparently healthy controls was determined. Results showed that serum triglyceride level in diabetic patients was significantly (P<0.0001) increased in comparison with its level in healthy controls, where the average mean of TGs in the serum sample of T2DM patients' group was (171.61 ±9.83) compared with (129.48 ±8.89) in

healthy controls group as shown in figure (6) triglyceride levels should be under 135 mg/dl in the normal range. The measurement of triglyceride (TG) levels is thought to be helpful in the diagnosis of diabetes mellitus in addition to pancreatitis, heart disease, and other medical conditions. Triglyceride level was the most important risk factor for diabetes mellitus, followed by family history, BMI, and age (14). An elevation of any one of the lipid parameters to a level above these limits was considered dyslipidemia.

Results of high-density lipoprotein (HDL) showed in Figure (7) that there is no significant difference in serum mean level of HDL between T2DM patients (42.84 ±2.03) and healthy controls (41.03 ±1.55). These results agree with the study achieved by (15) that high triglyceride levels and low HDL cholesterol levels are associated with type 2 diabetes and that the link between high triglycerides and low HDL cholesterol levels reflects a physiological process and explains the low levels of HDL cholesterol in patients with type 2 diabetes.

On the other hand, the Serum LDL-Cholesterol mean showed no significance in diabetic patients (116.08 ±8.45) than the mean value of the control group (123.42 ±6.42), as shown in Figure (8). The serum level of very low-density lipoprotein (VLDL) was measured in serum samples of T2DM patients and controls. The results showed that the mean level of VLDL significantly increased ($P<0.0001$) from (34.21 ±1.90) in the T2DM specimen in comparison with the mean level in healthy controls (25.89 ±1.78), Insulin resistance, in which the skeletal-muscle system drives the conversion of energy from eaten glucose to enhance liver triglyceride production, resulted in higher VLDL levels. As a result, atherogenic TG-rich lipoprotein units, such as VLDL, will be produced (16). As shown in Figure (9), All lipid profiles agree with (17). Lipid abnormalities are prevalent in DM patients because of IR, which affects key enzymes and pathways in lipid metabolism: Apo protein production, regulation of lipoprotein lipase, action of cholesterol ester transfer proteins, and hepatic and peripheral actions of insulin (18).

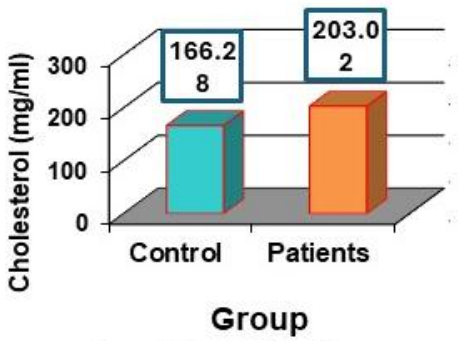

Figure 5: Comparison between control and patient groups in Cholesterol

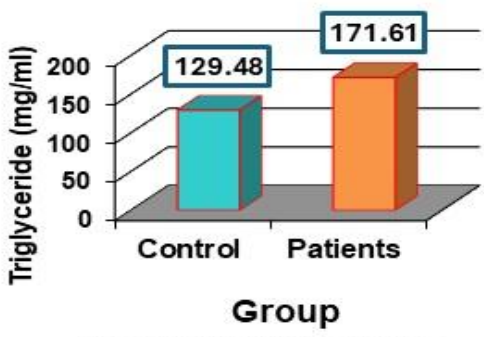

Figure 6: Comparison between control and patient groups in Triglyceride

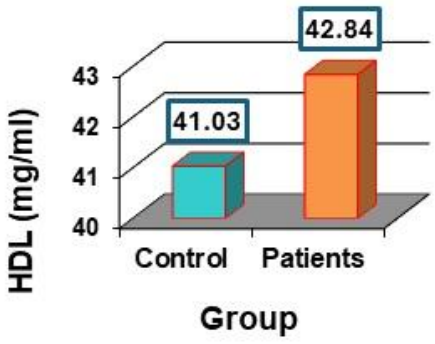

Figure 7: Comparison between control and patient groups in HDL

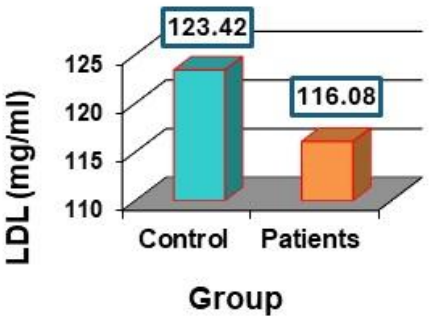

Figure 8: Comparison between control and patient groups in LDL

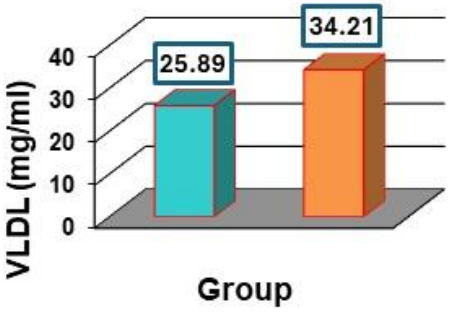

Figure 9: Comparison between control and patient groups in VLDL

While The Serum ceramide enzyme level results showed that the mean level of ceramide significantly (P<0.0001) increased from (75.31 ±1.70) in the T2DM patients compared with the mean level in apparently healthy controls (37.05 ±0.60), which agrees with (10), as shown in Figure (10), Recent studies have demonstrated that ceramides and their metabolites play key roles in metabolic diseases such as AD, T2DM and neurodegenerative diseases, such as insulin. Serum ceramides are a promising new clinical diagnostic tool for the identification of patients at risk of adverse type 2 diabetes mellitus. As shown in Table (4).

Table (4): Comparison between control and patient groups in Ceramide kinase enzyme.

Group	Mean ±SE of Ceramide kinase enzyme
Control	37.05 ±0.60
Patients	75.31 ±1.70
T-test	3.574 **
P-value	0.0001
**** (P≤0.01).**	

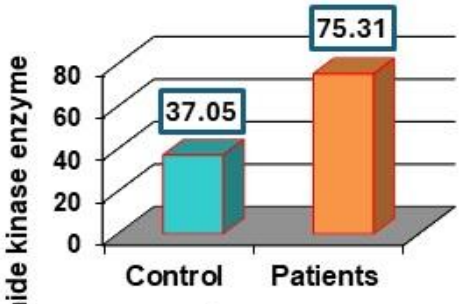

Figure 10: Comparison between control and patient groups in Ceramide kinase enzyme

Conclusion

In conclusion, ceramides are lipid molecules mostly found in cell membranes. They are essential for numerous intracellular signaling pathways and the lipid bilayer. Recent data indicates that they also exert pathogenic impacts so that they could be causally related and implicated in the beginning of diabetes mellitus .

Our analysis of the available data indicates that ceramides can interfere with glucose homeostasis, cause insulin resistance, and increase the opportunity to develop diabetes. The development of new medicinal substances that allow pharmacological lowering of ceramide synthesis by using ceramide synthase inhibitors, which specifically suppress ceramide production and, therefore, concentration levels, could be helpful in the treatment and/or prevention of diabetes and its complications.

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