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Role of Ceramide in Insulin Resistance in Type 2 Diabetes; A Comparative Analysis

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ABSTRACT

Background: Diabetes is a metabolic condition with a variety of consequences that can lead to serious sickness in the long run. The disease has severe economic consequences and, sadly, is rapidly approaching a pandemic threshold over the globe. To overcome this disorder and associated pathogeneses, much research is now being conducted.
Aim of the study: to study the levels of ceramide in the blood of diabetic and prediabetic patients (Type 2 DM).
Patients and Methods: Outpatient volunteers with type II diabetes and their first or second-degree relatives served as controls. Between January and August 2021, they were recruited from Al-Hussein University Hospital's outpatient clinics. All individuals had a history and physical examination, which included a body mass index (BMI), blood samples for 8-hour fasting blood sugar, 2-hour postprandial blood sugar, and liver and kidney function tests (ALT, AST, Urea, Fasting Serum Insulin.).
Insulin Resistance Assessment Using a Homeostasis Model (HOMA-IR).
Results: Ceramides with shorter (C16:0, C18:0) and longer chains (C24:1, C30:10) had a moderate to substantial connection with HBA1C and HOMA-IR, respectively. Only long-chain polyunsaturated ceramides (C26:9) had a significant favorable influence on HBA1C and HOMA-IR in the regression model (Estimate: 1.7, P 0.001) and (Estimate: 8.9, P 0.001). Ceramides with shorter and longer chains are moderately linked to insulin resistance. Long-chain polyunsaturated ceramides were also found to have the strongest link to HBA1C and HOMA-IR.
Conclusion: Hyperlipidemia and ceramide metabolism were found to be closely linked to insulin resistance in our investigation. Ceramides with shorter and longer chains are moderately linked to insulin resistance. Long-chain polyunsaturated ceramides were also found to have the strongest link to HBA1C and HOMA-IR. Future prospective multi-centric studies should concentrate on the role of long-chain ceramides as predictors of morbidity and disease progression, according to the authors.

Keywords: *Diabetes; Shingolipids; Ceramide; Insulin Resistance*

INTRODUCTION

the most common metabolic and endocrine illness. The International Diabetes Federation (IDF) estimated that 366 million people worldwide have diabetes (representing over 8 percent of the global total population). Insulin resistance has a pathogenic mechanism that involves a decreased response in peripheral tissues, resulting in hyperinsulinemia and elevated blood sugar due to increased glucose production by hepatocytes. All these things happen before type 2 diabetes symptoms appear. [1]

However, the entire molecular mechanism is unknown; however, Ceramides and inflammatory cytokines are involved in the main underlying lipotoxicty of insulin resistance [2]

Ceramide aggregation has been linked to DM, high blood pressure, heart disease, and atherosclerotic arteries in persons with morbid obesity who consume a western diet. After their storage capacity is exceeded, triglycerides begin to accumulate in non-adipose tissue, within hypertrophic cells, causing organ failure. [3]

Ceramides have been linked to the development of insulin resistance in clinical investigations. Ceramides may be accumulated in peripheral tissues because of Sphingolipid recycling or salvage

processes. High myocyte Ceramides, for example, have been identified in obese people with a broad or even a truncal distribution of fat. [4]

Ceramides are important bioactive lipids of the sphingolipid family, which are produced from a fatty acid and sphingosine or by hydrolysis of sphingomyelin. Ceramides in biological membranes are part of the membrane's microdomains, the so-called lipid rafts, which stabilize the structure of the cell membrane and modulate the distribution of receptors and signaling molecules. In addition, ceramides influence cell signaling pathways that mediate growth, proliferation, motility, adhesion, differentiation, senescence, growth arrest, and apoptosis. [5]

Ceramide is synthesized in Many ways; De novo pathway, Salvage pathway OR recycling route, The sphingomyelin pathway and Catabolic pathway. [6]

Ceramide can be phosphorylated (by ceramide kinase), rapidly deacylated (by ceramidase, CDase: acidic, neutral, and alkaline), or glycosylated (by glucosylceramide synthase).

The enzyme sphingomyelin synthase (SMS) transfers a phosphocholine group from phosphatidylcholine to ceramide, so sphingomyelin (SM) is formed again. [(25)] Ceramides disrupt glucose absorption and impair the storage of nutrients such as glycogen or triglycerides, activate protein phosphatase 2A (PP2A) and activate pro-inflammatory cytokines. These sphingolipids also disrupt fat metabolism, especially in the liver, by inhibiting oxidation and stimulating the absorption of fatty acids. An abnormal accumulation of ceramides may contribute to lipid-induced skeletal muscles and hepatic insulin resistance, especially in obesity, and is a putative intermediate link between excessive obesity, inflammation, and Metabolic diseases such as type 2 diabetes mellitus. [7]

The goal of this study was to determine how serum ceramide affects diabetic and prediabetic patients (Type 2 DM). [8]

PATIENTS AND METHODS

Participants and settings: Outpatients with type II diabetes and their first or second-degree relatives served as controls. Between January and August 2021, they were recruited from Al-Hussein University Hospital's outpatient clinics. The protocol was approved by the faculty Institutional Review Board (IRB) after all subjects gave verbal informed permission.

Each participant was given a thorough medical and laboratory examination. Ceramides were extracted and quantified using the Brunkhorst method, which involved multiple reaction monitoring and liquid chromatography-tandem mass spectrometry. [(9)]

The efficiency of each extract has been adjusted to C12:0ceramide as an internal standard value to account for any variances in efficiency. Furthermore, if a plasma extract deviated more than 20% from the internal standard overall average, it was reanalyzed. Serum values were calculated by fitting the detected sub-types to standardized internal curves after removing their acylchains. All individuals had a history and physical examination, which included a body mass index (BMI), blood samples for 8-hour fasting blood sugar, 2-hour postprandial blood sugar, and liver and kidney function tests (ALT, AST, Urea, Fasting Serum Insulin). The fasting insulin plasma level (u IU/ml) and the fasting glucose plasma level (mg/dl)/405] have been used to calculate the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). A commercially available human enzyme-linked immunosorbent assay (ELISA) kit will be used to assess serum ceramide.

Any patient with clinically significant renal, hepatic, neurological, cardiovascular, autoimmune, or infectious disease, any acute inflammation, or other serious systemic diseases, including cancer, is excluded.

Patients who smoke and/or consume alcohol will be excluded from the research.

Statistical analysis

In addition to STATA v16.0, statistical analyses were conducted using R v4.0 open software (www.r-project.org) (Stata Corp LLC, College Station, TX 77845, USA). If the SD of an observation was >4.0 or 4.0, it was considered an outlier, and the effect of removing it was assessed (one per variable). The significance level was established at a p-value of less than 0.05. To analyze the differences between groups, we used the Chi-square test or the independent t-test, depending on the assumptions met. Pearson r and multivariable linear regression were used to examine the correlations between baseline ceramides and insulin resistance markers (HBA1C and HOMA-IR), which were adjusted for age, sex, and residency. To create the Gaussian distributions, the ceramide concentrations were subjected to an inverse-normal transformation, thus stabilizing the variance.

RESULTS

The total number of participants in the trial was 60, with 30 in each group. The distribution of age, sex, and BMI was consistent among groups.

Bivariate and multivariate analysis: The Independent Samples T-Test revealed that the lipid profile and levels of all ceramides differed significantly between the two groups. Higher total cholesterol and LDL levels were significantly linked with higher relative concentrations of all ceramides in multivariate analyses adjusted for age, sex, and residency (p0.05). HDL-cholesterol and triglycerides, on the other hand, displayed more erratic correlations with shorter and longer-chain ceramides (Table 1 and 2; Fig 1:5).

	Group	Mean	SD
Sample size	Cases	N=30	
	Control	N=30	
Sex (females)	Cases	N=17	
	Control	N=16	
Residency (Rural)	Cases	N=18	
	Control	N=15	
Age	Cases	42.03	5.898
	Control	41.83	5.344
BMI	Cases	32.07	1.015
	Control	32.30	1.119
HBA1C	Cases	7.87	0.860
	Control	5.00	0.000
Fasting Glucose, mg/dL	Cases	169.13	31.562
	Control	99.03	11.370
Fasting Insulin, µIU/mL	Cases	30.00	2.626
	Control	4.60	2.673
HOMA-IR	Cases	12.53	2.594
	Control	1.14	0.676
Triglycerides, mg/dL	Cases	62.87	39.670
	Control	67.40	35.577
Cholesterol, mg/dL	Cases	184.37	17.651
	Control	104.97	8.540
HDL-cholesterol, mg/dL	Cases	44.67	9.604
	Control	62.53	9.846
LDL-cholesterol, mg/dL	Cases	107.83	11.561
	Control	83.97	11.541
VLDL-cholesterol, mg/dL	Cases	18.50	0.509
	Control	8.63	4.679
Sex	Cases		
	Control		
Residency	Cases		
	Control		

Table 1: It showed Baseline Characteristics including the demographics, clinical features, and laboratory measurements of all patients at the start of the study.

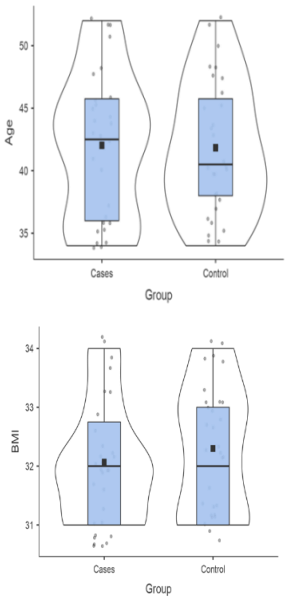


Fig 1: Violin plots showing the full distribution of the data, where the small black box represents the mean, the adjacent horizontal line represents the median, the large blue rectangle represents the IQR, and the outer shell displays the presence of different peaks with their relative amplitudes. The figure on the left illustrates a comparison of Age distribution between the cases and controls, while the figure on the right compares BMI distribution between the study groups.

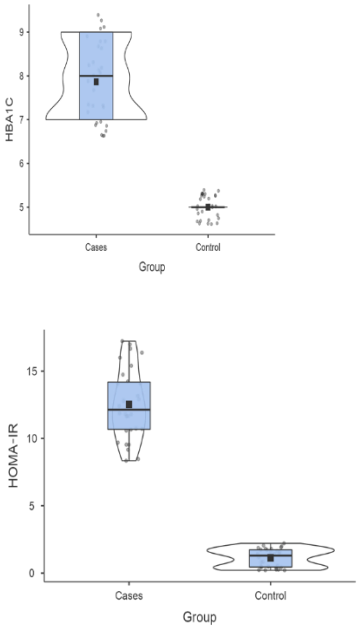


Fig 2: Violin plot displaying the overall distribution of the data. The small black boxes indicate the mean, the adjacent horizontal lines indicate the median, the large blue boxes indicate the IQR, and the outer shells indicate the presence of different peaks with relative amplitudes. The figure on the left compares the distribution of HBA1C between cases and the control group, and the figure on the right compares the distribution of HOMA-IR between the study groups.

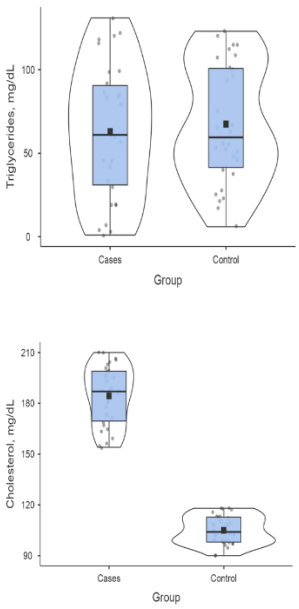
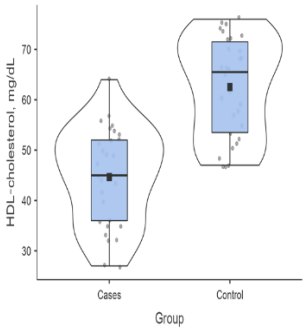


Fig 3: Violin plot depicting the complete distribution of data. The small black box represents the average, the adjacent horizontal lines represent the median, the large blue rectangle represents the IQR, and the outer envelope indicates the presence of various peaks. The figure on the

left shows a comparison of Triglyceride distributions between cases and controls, and the figure on the right compares Cholesterol distributions between study groups.



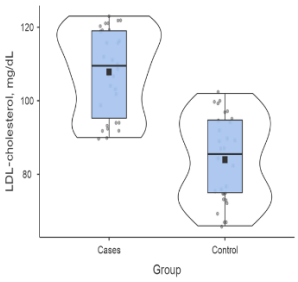


Fig 4: Violin plots showing the full distribution of the data, where the small black box represents the mean, the adjacent horizontal line represents the median, the large blue rectangle represents the IQR, and the outer shell displays the presence of different peaks with their relative amplitudes. The figure on the left illustrates a comparison of **HDL** distribution between the cases and controls, while the figure on the right compares **LDL** distribution between the study groups.

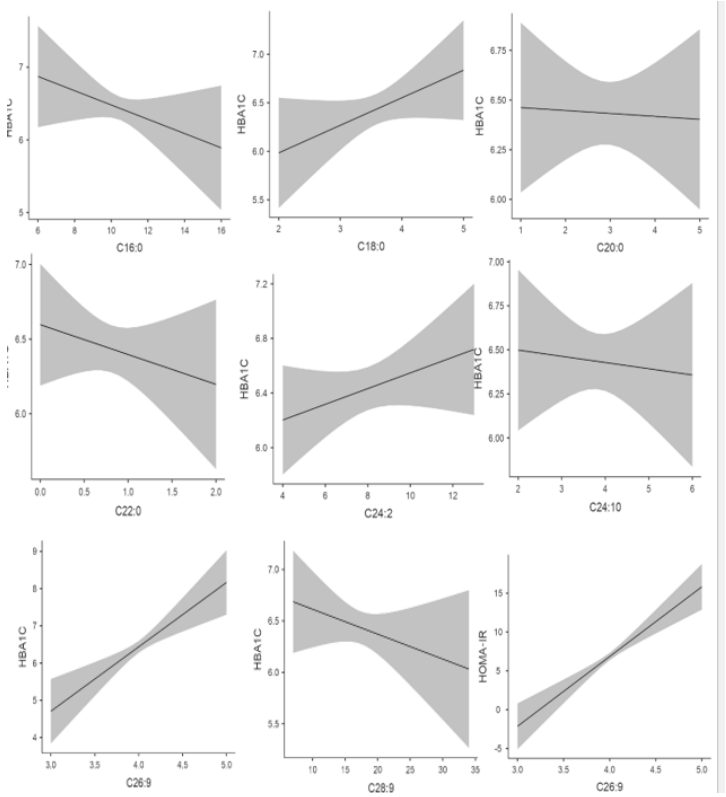


Fig 5: Association between Ceramides and markers of insulin resistance, it showed that Shorter (C16:0, C18:0) and longer chains (C24:1, C28:9) ceramides had a moderate to strong correlation with HBA1C and HOMA-IR. However, in the regression model, only long-chain polyunsaturated ceramides (C26:9) had a significant positive effect on HBA1C and HOMA-IR (Estimate: 1.7, P< 0.001) and (Estimate: 8.9, P< 0.001), respectively.

		Statistic	df	p	Mean difference	SE difference		Effect Size
C16:0	Student's t	23.266	58	< .001	6.733	0.2894	Cohen's d	6.007
C18:0	Student's t	14.751	58	< .001	1.900	0.1288	Cohen's d	3.809
C20:0	Student's t	10.752	58	< .001	2.267	0.2108	Cohen's d	2.776
C22:0	Student's t	4.473	58.	< .001	0.433	0.0969	Cohen's d	1.155
C24:2	Student'	5.260	58.	< .001	2.433	0.4626	Cohe	1.358

	s t						n's d	
C24:10	Student's t	14.099	58.	< .001	2.567	0.1820	Cohen's d	3.640
C26:9	Student's t	NaN					Cohen's d	
C28:9	Student's t	17.226	58.	< .001	16.433	0.9540	Cohen's d	4.448
C30:9	Student's t	14.784	58.	< .001	19.000	1.2852	Cohen's d	3.817
C30:10	Student's t	22.746	58.	< .001	30.700	1.3497	Cohen's d	5.873
HOMA-IR	Student's t	23.280	58.0	< .001	11.395	0.4895	Cohen's d	6.011
Triglycerides, mg/dL	Student's t	-0.466	58.	0.643	-4.533	9.7287	Cohen's d	-0.120
Cholesterol, mg/dL	Student's t	22.179	58.	< .001	79.400	3.5799	Cohen's d	5.727
HDL-cholesterol, mg/dL	Student's t	-7.115	58.	< .001	-17.867	2.5112	Cohen's d	-1.837
LDL-cholesterol, mg/dL	Student's t	8.002	58.	< .001	23.867	2.9825	Cohen's d	2.066
VLDL-cholesterol, mg/dL	Student's t	11.482	58.	< .001	9.867	0.8593	Cohen's d	2.965

Table 2: Table 2: Independent Samples T-Test, it showed that lipid profile and all ceramides' levels significantly differed between the two groups. In multivariate analysis adjusted for age, sex, and residency, higher total cholesterol, and LDL levels were significantly associated with higher relative concentrations of all ceramides ($p<0.05$). In contrast, HDL-cholesterol and triglycerides had more variable relationships with shorter and longer-chain ceramides**.** [a] Levene's test is significant ($p < .05$), suggesting a violation of the assumption of equal variances. [b] All observations are tied

DISCUSSION

The goal of this study was to see if there was a link between elevated ceramide levels and insulin resistance in people with prediabetes and TIIDM. We first completed a full history and clinical assessment of each patient included in the study to assess the potential association between the two factors. BMI, fasting and postprandial blood sugar and insulin levels, lipid profile, liver, and renal function tests, HBA1C, HOMA-IR, and serum ceramides levels were all assessed.

High levels of cholesterol and LDL were shown to be strongly linked to high levels of all ceramides in our investigation. In contrast, the correlations between HDL and triglycerides and shorter and longer-chain ceramides were more varied. Ceramides and insulin resistance indicators had a substantial relationship. Ceramides with shorter and longer chains had a moderate to strong relationship with HBA1C and HOMA-IR. Only long-chain polyunsaturated ceramides exhibited a significant favorable effect on HBA1C and HOMA-IR in the regression model. [(10)]

Sphingolipids, one of the most important lipid groups in mammalian lipidomic storage, have a sphingoid base as a common component in their production. These lipids are abundant in eukaryotic cell membranes and are required for signaling pathway regulation. Sphingolipid dysregulation has been linked to many cases of serious illnesses, including cancer, heart disease, and neurological diseases. Sphingolipids Ceramides play a crucial role in the development of diabetes, according to recent research. [(11)] Several studies have found that FFA buildup, glucocorticoids, obesity, and a sedentary lifestyle are all key contributors to insulin resistance. Ceramides are the most important intermediate linkages in the development and evolution of insulin resistance, according to numerous clinical investigations. [11]

The most serious and potentially life-threatening complications associated with diabetes are the greatest dangers. Sphingolipids, particularly ceramide, are increasingly being implicated in the pathophysiology of both micro- and macrovascular diabetic problems, according to a growing body of research. The major causes of death in diabetics are cardiovascular illnesses such as atherosclerosis, myocardial infarction, and stroke. The apoptosis of cardiomyocytes is a hallmark of diabetic cardiomyopathy. [12] These findings highlight the importance of Ceramides in the development of diabetic long-term consequences such nephropathy [13] and diabetic retinopathy. [14]

This is the first clinical investigation in the Middle East and Africa to examine the potential role of ceramides in insulin resistance, to our knowledge. We investigated the robustness of our findings by measuring several outcomes of interest, using various statistical methods, and evaluating the robustness of

our findings. However, because our study was limited to a single institution, the findings cannot be extrapolated without more multi-center research. In addition, the cross-sectional methodology hampered the ability to assess the incidence and draw a causal conclusion, necessitating further prospective research. [15.]

CONCLUSION

the global incidence of TIIDM has increased in recent decades because of economic growth and lifestyle changes, particularly in developed countries. Hyperlipidemia and ceramide metabolism were found to be closely linked to insulin resistance in our investigation. Ceramide is a major sphingolipid metabolic product that contributes to insulin resistance and obesity. Ceramides with shorter and longer chains are moderately linked to insulin resistance. Long-chain polyunsaturated ceramides were also found to have the strongest link to HBA1C and HOMA-IR. Future prospective multi-centric studies should concentrate on the role of long-chain ceramides as predictors of morbidity and disease progression, according to the authors. The development of innovative treatment targets and biomarkers for diabetes and insulin resistance will be aided by advances in our knowledge of these relationships.

Conflict of interest : none

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