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Research Article

Serum ceramide and meteorin-like protein as potential biomarkers of type 2 diabetes mellitus

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Abstract

Objectives: The recent research aims to detect ceramide and meteorin-like proteins as potential markers for identifying type 2 diabetes and monitoring its progression.

Methods: A cross-sectional study included three groups: type 2 diabetes without hypertension, type 2 diabetes with hypertension, and healthy control groups. Serum ceramide, meteorin-like protein, insulin, fasting blood glucose, lipid profile, and hemoglobin A1c levels were measured.

Results: Higher concentrations of ceramide, fasting blood glucose, hemoglobin A1c, and the homeostatic model assessment of insulin resistance were observed in both type 2 diabetes groups compared to the healthy control group. In the type 2 diabetes group with hypertension, total cholesterol was elevated compared to the other study groups; however, the concentration of low/very-low-density lipoprotein was statistically higher than in the healthy control group. Serum meteorin-like protein was statistically lower in the type 2 diabetes group with hypertension than in the other study groups and positively correlated with fasting blood glucose in type 2 diabetes with hypertension. The ceramide level showed a significant positive correlation with meteorin-like protein across all study groups and with systolic blood pressure in the type 2 diabetes group with hypertension. In type 2 diabetes without hypertension, ceramide negatively correlated with the homeostatic model assessment of insulin resistance and fasting blood glucose.

Conclusion: Elevated ceramide levels could accelerate type 2 diabetes progression. Meteorin-like protein levels were lower in type 2 diabetes with hypertension and higher in type 2 diabetes without hypertension. It positively correlated with fasting blood glucose in type 2 diabetes with hypertension, suggesting that meteorin-like protein may play a potential role in glycemic and blood pressure control.

Keywords: Ceramide, hypertension, IR, meteorin-like protein, T2D

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Type 2 diabetes (T2D) is a chronic, multisystem disorder and a major global public health issue that steadily declines the quality of life. In the twenty-first century, T2D has become one of the most significant epidemics due to its continually rising incidence. T2D patients often remain untreated for years because of the lack of early severe symptoms and the gradual progression of the disease, which lacks typical hyperglycemia manifestations, such as weight loss and dehydration [1]. Hypertension and dyslipidemia are some of the most common cardiovascular disease (CVD) risk factors [2]. An important area in clinical and public health research is understanding the correlation between various phases of glucose intolerance and serum lipid patterns, which has the potential to inform future prevention strategies for diabetes mellitus (DM) and related outcomes.

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Fasting blood glucose (FBG) and hemoglobin A1c (HbA1c) tests are unable to accurately guide the best possible treatment for T2D, as they fail to account sufficiently for variations in diabetes causes, insulin resistance, and insulin production. These tests are only useful post-disease manifestations [3]. Therefore, it is crucial to find biomarkers for rapid and precise DM outcome detection and to develop more accurate non-invasive markers that may be used for T2D diagnosis across various disease stages.

Ceramides (Cer) are involved in numerous pathophysiological processes, including cancer, inflammation, neurodegenerative diseases, obesity, diabetes, and CVD. Ceramides exhibit pro-inflammatory and pro-apoptotic properties, and their levels in the bloodstream are positively associated with age. However, the impact of ceramide alterations on T2D remains uncertain. Previous research suggests that ceramides play a role in T2D development by decreasing insulin production, disrupting insulin signaling, and impairing glucose transporter activity [4]. A better understanding of ceramide levels and T2D risk could direct the development of new pharmacologic treatments for both primary and secondary prevention of T2D.

Meteorin-like protein (Metrnl) has a diverse impact on metabolism, immunology, and inflammation. Various physiological activities, such as exercise, temperature changes, bariatric surgery, and high-fat diets, may also affect Metrnl levels. Studies report differing findings about Metrnl levels in patients; one study observed reduced Metrnl in diabetics [5], while another found elevated levels in diabetic patients [6]. Meteorin-like protein attracts eosinophils into adipose tissue, which is a primary producer of interleukin (IL)-13 and IL-4, which activate thermogenic genes. This suggests that Metrnl may regulate temperature and energy consumption [7]. Thus, Metrnl is a compelling subject to examine in the context of metabolic conditions. The current research aims to detect lipid abnormalities in T2D subjects and to examine the relationship between various serum lipid patterns and T2D, as well as address knowledge gaps regarding the profiles of ceramide and Metrnl in T2D patients. The hope is that these observations will translate into new screening assays and therapies to alleviate and potentially prevent or cure T2D.

Materials and Methods

1. **Subjects/study design:** Between August 2023 and December 2023, cross-sectional research was conducted at the Al-Nasiriyah Teaching Hospital in Thi-Qar governorate, Iraq. Three study groups were enrolled in this research (15 males and 15 females for each group): the T2D without hypertension group (first group), which consisted of 30 T2D patients without hypertension aged 34 to 56 years; the T2D with hypertension group (second group), which included 30 T2D patients with hypertension aged 34–56 years; and the healthy control (HC) group (third group), comprising 30 subjects aged 35 to 55 years.

   Patients in the first and second groups had a confirmed T2D diagnosis from the Al-Nasiriyah Teaching Hospital. Exclusion criteria for this study included patients with recent blood transfusion, cancer, chronic or autoimmune diseases, pregnant or lactating mothers, patients under corticosteroid or biological therapy, recent surgery, or any diabetes complications. Eligible patients had to fast for at least 12 hours and meet none of the above criteria. Additionally, all T2D patients were diagnosed within the past six months, ensuring that case group participants were relatively newly diagnosed to allow for a more consistent comparison of early disease biomarkers and progression.

   Eligibility criteria for the HC group included no family history of DM, non-smoking status, non-pregnancy or lactation (for females), absence of inflammation or infection, no chronic or autoimmune diseases, no recent blood transfusions or surgeries, and no use of biological agents.

   The present study received full approval from the Ethics Committee of the Training and Human Development Unit, Thi-Qar Health Department, Ministry of Health, Iraq. This approval was granted under committee number 153/2023 on July 27, 2023, in line with the Helsinki Declaration, and informed consent was obtained from each participant to meet international research ethical standards.

2. **Blood pressure determination:** Blood pressure (BP) was measured using a digital patient monitor (UTAS Technologies, Slovakia). Normal systolic blood pressure (SBP) was defined as <140 mmHg, and normal diastolic blood pressure (DBP) was <90 mmHg [8].

3. **Sample collection:** Three to five milliliters (mL) of peripheral blood (PB) were collected from each subject after 12 hours of fasting. Two mL of collected PB was used for the HbA1c test, while the remaining PB was used for serum separation. Collected sera were stored at -80 degrees Celsius if not used immediately for subsequent analysis.

4. **Biochemical assays:** Quantitative determination of HbA1c in PB and FBG in serum was performed *in vitro* using the Roche/Cobas c111 system (Roche, Germany) with HbA1c and glucose kits (Roche, Germany). Findings were expressed as percentages (%) for HbA1c and in milligrams (mg) per deciliter (dL) for FBG.

   Human insulin was detected and quantified in micro-international units (µIU)/mL using an insulin enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, USA) based on sandwich-ELISA technology. The homeostatic model assessment (HOMA)-IR was determined using the following formula:

HOMA-IR=Fasting insulin (µIU)×FBG (mg/dl)/405 [9]

Serum TG, total cholesterol (TC), and high-density lipoprotein (HDL) were detected and titrated in mg/dl by Roche/Cobas c111 devices (Roche, Germany) using TG, cholesterol, and HDL kits, respectively (Roche, Germany). Serum very low-density lipoprotein (vLDL) and low-density lipoprotein (LDL) were determined by the formulas number (1) and number (2), respectively.

VLDL (mg/dl)=TG/5 (1) [10]

LDL (mg/dl)=TG–(HDL + VLDL (2) [11]

Serum cer and metrnl detection

Serum ceramide (Cer) and meteorin-like protein (Metrnl) levels were detected in nanograms (ng)/mL using Cer and Metrnl ELISA kits (Bioassay Technology Laboratory, China), based on sandwich-ELISA technology. The kits have sensitivities of 0.023 ng/mL for Metrnl and 0.62 ng/mL for Cer, allowing detection at very low levels. The detection range for Metrnl is 0.05–15 ng/mL and for Cer is 1–400 ng/mL, which defines the range within which the assay can accurately measure these biomolecules. Cer and Metrnl levels were categorized as follows: below-normal (<40 ng/mL and <1 ng/mL), normal (40–70 ng/mL and 1–3 ng/mL), and above-normal (>70 ng/mL and >3 ng/mL), respectively.

For all the above-mentioned assays, the test protocol was followed according to the manufacturer's instructions, and all scoring values were based on levels observed in the HC group.

Statistical analysis

For data presentation and analysis, the Statistical Package for Social Sciences version 22 was used. Frequencies, relative frequencies, means, and standard deviation (SD) were calculated as descriptive statistics. The Chi-Square test was employed to compare categorical parameters, while the significance of differences in continuous variables with a non-normal distribution was evaluated using the Kruskal-Wallis test. Multiple comparisons within groups were tested using the Dunn test as a post hoc test. Spearman's correlation (r) was utilized to assess correlations between variables. Statistical significance was determined when the p-value was below 0.05.

Results

The findings revealed a significant elevation in age, LDL, and vLDL mean values in T2D patients with hypertension (48.2±5.13 years, 168.6±67.17 mg/dL, and 50.8±32.65 mg/dL, respectively) compared to the HC group (42.7±6.13 years, 103.5±43.45 mg/dL, and 26.7±12.27 mg/dL, respectively). The levels of FBG, HbA1c, HOMA-IR, and TG were significantly higher in both T2D without hypertension (223.9±107.67 mg/dL, 8.5%±1.87, 8.17±6.23, and 189.2±97.32 mg/dL, respectively) and T2D with hypertension (236.4±129.82 mg/dL, 8.4%±1.62, 12.50±9.75, and 254.3±163.3 mg/dL, respectively) compared to the HC group (98.47±13.24 mg/dL, 5.4%±0.57, 2.85±2.63, and 133.9±61.36 mg/dL, respectively). For TC, there was a significantly higher level in T2D with hypertension (208.8±42.81 mg/dL) compared to T2D without hypertension (184.9±34.09 mg/dL) and the HC group (162.1±34.77 mg/dL). Mean SBP in T2D patients with hypertension was significantly higher (140.9±15.34 mmHg) compared to T2D patients without hypertension (123.06±7.13 mmHg) and controls (118.4±5.26 mmHg), with P-values of <0.001 for both. Similarly, mean DBP was elevated in T2D patients with hypertension (89.6±8.55 mmHg) compared to T2D patients without hypertension (83.1±6.30 mmHg) and the HC group (81.3±5.19 mmHg), with significant P-values of 0.001 for both. All other comparisons revealed non-significant differences (Table 1).

Table 2 demonstrated that T2D without hypertension and T2D with hypertension groups had significantly higher percentages of above-normal Cer levels (53.3% and 40%, respectively) compared to the HC group (20%). However, there was no statistical difference between T2D without hypertension and T2D with hypertension. The mean Cer level was highest in T2D without hypertension (66.99±14.4 ng/mL) and in T2D with hypertension (61.66±20.69 ng/mL) compared to the control group (45.41±13.9 ng/mL), with a significant difference observed. No significant difference was found in mean Cer levels between T2D without hypertension and T2D with hypertension.

The percentage of Metrnl below-normal levels was significantly lower in T2D without hypertension (20%) compared to T2D with hypertension (60%) and the HC group (56.7%). No significant difference was observed in Metrnl levels between T2D with hypertension and the HC group. The mean Metrnl titer was significantly higher in T2D without hypertension (1.71±0.86 ng/mL) than in T2D with hypertension (1.12±0.96 ng/mL) and the HC group (1.55±1.80 ng/mL), and it was statistically elevated in the HC group compared to T2D with hypertension (Table 3).

The percentage of Metrnl below-normal levels was significantly higher in T2D without hypertension, T2D with hypertension, and HC subjects with below-normal Cer levels (50%, 100%, and 77.8%, respectively) compared to those with above-normal Cer levels (18.75%, 50%, and 0%, respectively). Metrnl concentration was statistically significant in T2D with hypertension and HC subjects with above-normal Cer levels (1.27 ng/mL and 3.70 ng/mL, respectively) compared to those with below-normal Cer levels (0.62 ng/mL and 0.76 ng/mL, respectively). However, no significant differences were observed in mean Metrnl levels for T2D without hypertension individuals with below-normal, normal, or above-normal Cer levels (Table 4).

A significant positive relationship was observed between Cer and SBP among T2D patients with hypertension. Additionally, a significant negative correlation was found between Cer, HOMA-IR, and FBG in T2D patients without hypertension. Among T2D patients with hypertension, Metrnl positively correlated with FBG, while in the HC group, it inversely correlated with FBG (Table 5). All other correlations were not significant, as shown in the Table.

Discussion

Individuals with T2D often exhibit insulin resistance (IR), impaired glucose tolerance, dyslipidemia, and hypertension [12]. These findings align with the current study, which showed elevated levels of TG, TC, HOMA-IR, LDL, and vLDL in both T2D groups compared to the HC group (Table 1). Hyperglycemia and elevated HbA1c levels negatively impact lipid profiles and increase the risk of CVD and dyslipidemia. Increased free fatty acids (FFA) stimulate triglyceride (TG) formation, leading to higher secretion of apolipoprotein B (ApoB) and LDL. Insulin typically promotes ApoB breakdown by activating phosphatidylinositol-3 kinase, but this

Table 1. Comparison of demographic and biochemical parameter between study groups

Variables	G1 (n=30) Mean±SD	G2 (n=30) Mean±SD	G3 (n=30) Mean±SD	p
Age (years)	45.7±6.88	48.2±5.13	42.7±6.13	G1/G2: 0.256 G2/G3: 0.002* G1/G3: 0.155
SBP (mmHg)	123.06±7.13	140.9±15.34	118.4±5.26	G1/G2: <0.001* G2/G3: 0.001* G1/G3: 0.06
DBP (mmHg)	83.1±6.30	89.6±8.55	81.3±5.19	G1/G2: 0.001* G2/G3: 0.001* G1/G3: 0.566
FBG (mg/dl)	223.9±107.67	236.4±129.82	98.47±13.24	G1/G2: 0.991 G2/G3: <0.001* G1/G3: <0.001*
HbA1c (%)	8.5±1.87	8.4±1.62	5.4±0.57	G1/G2: 0.969 G2/G3: <0.001* G1/G3: <0.001*
HOMA-IR	8.17±6.23	12.50±9.75	2.85±2.63	G1/G2: 0.292 G2/G3: <0.001* G1/G3: 0.001
TG (mg/dl)	189.2±97.32	254.3±163.3	133.9±61.36	G1/G2: 0.168 G2/G3: <0.001* G1/G3: 0.035*
TC (mg/dl)	184.9±34.09	208.8±42.81	162.1±34.77	G1×G2: 0.04* G2/G3: <0.001* G1/G3: 0.053
HDL (mg/dl)	86.5±21.91	91.04±16.28	85.3±19.03	G1/G2: 0.641 G2/G3: 0.486 G1/G3: 0.966
LDL (mg/dl)	136.1±48.04	168.6±67.17	103.5±43.45	G1/G2: 0.057 G2/G3: <0.001* G1/G3: 0.055
vLDL (mg/dl)	37.8±19.46	50.8±32.65	26.7±12.27	G1/G2: 0.132 G2/G3: <0.001* G1/G3: 0.073

The Dunn test is a post hoc test for multiple comparisons within groups, and the Kruskal-Wallis test is used to compare non-parametric continuous variables. *: Significant differences. G1: T2D without hypertension; G2: T2D with hypertension; G3: Control group; n: Number; SD: Standard deviation; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; HbA1c: Hemoglobin A1c; HOMA-IR: Homeostatic model assessment-insulin resistance; TG: Triglyceride; TC: Total cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; vLDL: Very low density lipoprotein; mmHg: Millimeters of mercury; mg: Milligram; dl: Deciliter.

process is impaired in IR conditions [13], possibly explaining the elevated TG levels under IR conditions.

The association between high blood pressure and dyslipidemia in T2D enhances cardiovascular and metabolic disease risk factors [12]. Consistent with these observations, the present research found a significantly higher level of TC in T2D with hypertension compared to T2D without hypertension (Table 1). A previous cross-sectional study [14] also reported significant differences in LDL levels among individuals diagnosed with T2D and hypertension. This investigation supports findings of increased TC levels in T2D patients with hypertension, indicating the presence of dyslipidemia.

Research has shown that as individuals with both hypertension and diabetes age, they face a higher risk of experiencing macrovascular and microvascular events [15]. The findings of this previous research support our current investigation, which shows an increasing trend in age among patients with both high BP and DM. The current study revealed significantly higher FBG levels and HbA1c percentages in both T2D groups compared to the HC group (Table 1). Consistent with recent findings, Khan et al. [16] observed a notable elevation in FBG and HbA1c levels in diabetic individuals. Previous studies have shown that individuals with T2D exhibit elevated levels of FBG, TC, LDL, and TG compared to control groups [17]. This study

Table 2. The result of ceramide in all study group

Biomarker/ groups	G1 (n=30)		G2 (n=30)		G3 (n=30)		Total (n=90)	
	n	%	n	%	n	%	n	%
Cer (ng/ml) FR (%)								
Below N (<40)	2	6.7	3	10	9	30	14	15.6
Normal (40–70)	12	40	15	50	15	50	42	46.7
Above N (>70)	16	53.3	12	40	6	20	34	37.8
Total	30	100	30	100	30	100	90	100
Mean±SD	66.99±14.40		61.66±20.69		45.41±13.90		58.02±16.33	
p-value	G1/G2: >0.05, G1/G3: <0.05*, G2/G3: <0.05*							

The Chi-square test is used to compare categorical variables, and the Kruskal-Wallis test is used to compare the mean of non-parametric continuous variables. *: Significant differences. G1: T2D without hypertension; G2: T2D with hypertension; G3: Control group; n: Number; Cer: Ceramide; ng: Nanogram; ml: Milliliter; FR: Frequency; N: Normal; SD: Standard deviation.

Table 3. The result of meteorin-like protein in all study group

Biomarker/ groups	G1 (n=30)		G2 (n=30)		G3 (n=30)		Total (n=90)	
	n	%	n	%	n	%	n	%
Metrnl (ng/ml) FR (%)								
Below N (<1)	6	20	18	60	17	56.7	41	45.6
Normal (1–3)	19	63.3	10	33.3	8	26.7	37	41.1
Above N (>3)	5	16.7	2	6.7	5	16.7	12	13.3
Total	30	100	30	100	30	100	90	100
Mean±SD	1.71±0.86		1.12±0.96		1.55±1.80		1.38±1.14	
p-value	G1/G2: <0.05*, G1/G3: <0.05*, G2/G3: <0.05*							

The Chi-square test is used to compare categorical variables, and the Kruskal-Wallis test is used to compare the mean of non-parametric continuous variables. *: Significant differences. G1: T2D without hypertension; G2: T2D with hypertension; G3: Control group; n: Number; Metrnl: Meteorin-like protein; ng: Nanogram; ml: Milliliter; FR: frequency; N: normal; SD: standard deviation.

confirms that diabetic patients, with or without hypertension, experience elevated FBG and HbA1c levels, highlighting the importance of these variables in assessing glycemic control.

The findings of this study showed no significant differences in HDL levels between the study groups (Table 1), consistent with other research [18] that reported no link between HDL concentrations and T2D occurrence.

The current study found that T2D patients with hypertension had significantly higher SBP and DBP compared to those without hypertension and the HC group (Table 1). This finding is consistent with recent research, such as a study by Rapsomaniki et al. [19], which demonstrated that hypertension is more severe and prevalent in T2D patients, contributing to a higher cardiovascular risk. Even normotensive T2D patients tend to have higher BP than healthy individuals, suggesting possible subclinical hypertension, as noted by Zaccardi et al. [20]. These findings highlight the importance of strict BP management in T2D patients to reduce the risk of complications, a point underscored in recent guidelines and reviews on the management of hypertension in diabetes by De Boer et al. [21].

Elevated levels of Cer in the bloodstream can result in IR and metabolic syndrome; however, disruption of Cer biosynthesis through drugs or genetics can promote glucose metabolism and improve insulin sensitivity [22]. Consistent with these findings, the current study showed a significantly higher Cer level among both T2D groups compared to the HC group (Table 2). Evidence suggests that several metabolic conditions, such as DM and CVD, can be ameliorated by inhibiting Cer formation and promoting Cer breakdown in humans [23]. These findings confirm that Cer could potentially serve as a specific T2D biomarker.

Meteorin-like protein (Metrnl) is a newly identified adipomyokine that may assist in managing metabolic and inflammatory diseases, such as T2D. Since its discovery, many researchers have investigated the link between blood concentrations of Metrnl and T2D, although findings have yet to reach a consensus [24]. Multiple studies on the role of serum Metrnl in T2D have report-

Table 4. The comparison between ceramide and meteorin-like protein levels

Biomarkers	Metrnl (ng/ml)								p
	Below N (<1)		Normal (1–3)		Above N (>3)		Total		
	FR (%)	Mean	FR (%)	Mean	FR (%)	Mean	FR (%)	Mean	
Ceramide (ng/dml)									
Group 1									
Below N (n=2)	1 (50)	0.68	0 (0)	0	1 (50)	3.09	2 (100)	1.88	<0.05*
Normal (n=12)	2 (16.7)	0.71	9 (75)	1.62	1 (8.3)	3.19	12 (100)	1.60	
Above N (n=16)	3 (18.75)	0.85	10 (62.5)	1.55	3 (18.75)	3.45	16 (100)	1.77	
Total (n=30)	6 (20)	0.78	19 (63.3)	1.58	4 (16.7)	3.33	30 (100)	1.71	
Group 2									
Below N (n=3)	3 (100)	0.62	0 (0)	0	0 (0)	0(0)	3 (100)	0.62	<0.05*
Normal (n=15)	9 (60)	0.71	6 (40)	1.17	0 (0)	4.38	15 (100)	1.11	
Above N (n=12)	6 (50)	0.76	4 (33.3)	1.20	2 (16.7)	4.65	12 (100)	1.27	
Total (n=30)	18 (60)	0.71	10 (33.3)	1.2	2 (6.7)	4.51	30 (100)	1.12	
Group 3									
Below N (n=9)	7 (77.8)	0.48	2 (22.2)	1.73	0 (0)	0	9 (100)	0.76	<0.05*
Normal (n=15)	10 (66.7)	0.54	5 (33.3)	1.29	0 (0)	0	15 (100)	0.90	
Above N (n=6)	0 (0)	0	1 (16.7)	1.46	5 (83.3)	4.09	6 (100)	3.70	
Total (n=30)	17 (56.7)	0.51	8 (26.6)	1.42	5 (16.6)	4.09	30 (100)	1.55	

The Chi-square test is used to compare categorical variables, and the Kruskal-Wallis test is used to compare the mean of non-parametric continuous variables. *: Significant differences. Metrnl: Meteorin-like protein; N: Normal; FR: Frequency; ng: nanogram, ml: Milliliter; n: Number.

Table 5. Illustrates the Pearson correlation between ceramide, meteorin-like protein, and anthropometric parameters

Biomarkers/ groups	Ceramide (ng/ml)						Metrnl (ng/ml)					
	Group 1		Group 2		Group 3		Group 1		Group 2		Group 3	
	r	p	r	p	r	p	r	p	r	p	r	p
Age (year)	0.09	0.62	-0.02	0.90	0.15	0.42	-0.09	0.67	-0.14	0.46	0.08	0.69
SBP (mmHg)	0.21	0.26	0.49	0.01	-0.10	0.60	-0.14	0.50	0.14	0.45	0.09	0.64
DBP (mmHg)	0.22	0.24	0.34	0.07	-0.22	0.25	0.14	0.50	-0.07	0.70	-0.33	0.07
HbA1c (%)	-0.30	0.10	0.20	0.28	-0.02	0.91	0.35	0.08	-0.33	0.07	0.00	0.98
HOMA-IR	-0.35	0.04	-0.07	0.68	0.201	0.28	-0.004	0.98	0.21	0.25	-0.28	0.13
FBG (mg/dl)	-0.35	0.04	-0.08	0.66	-0.17	0.36	-0.26	0.15	0.36	0.04	-0.52	0.00
TG (mg/dl)	-0.09	0.63	-0.11	0.58	-0.14	0.48	0.08	0.71	0.03	0.87	-0.10	0.61
TC (mg/dl)	0.26	0.16	0.06	0.76	0.04	0.84	-0.08	0.69	-0.14	0.45	-0.26	0.17
HDL-c (mg/dl)	0.29	0.12	0.09	0.65	0.09	0.64	0.09	0.66	-0.08	0.69	0.01	0.97
LDL (mg/dl)	0.03	0.88	0.01	0.96	-0.06	0.77	-4.10	0.98	-0.09	0.63	-0.12	0.52
vLDL (mg/dl)	-0.09	0.63	-0.11	0.58	-0.14	0.48	0.08	0.71	0.03	0.87	-0.10	0.61

Spearman correlation is used for correlation between non-parametric continuous variables. Metrnl: Meteorin-like protein; ng: Nanogram; ml: Milliliter; r: Correlation coefficient; mg: Milligram; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HbA1c: Hemoglobin A1c; HOMA-IR: Homeostatic model assessment-insulin resistance; FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol; HDL-c: High density lipoprotein cholesterol; LDL: Low density lipoprotein; vLDL: Very low density lipoprotein; dl: Deciliter; mmHg: Millimeter of mercury.

ed controversial results. In the current research, Metrnl levels were markedly increased in T2D subjects without hypertension compared to T2D patients with hypertension and the HC group. Additionally, Metrnl levels were statistically lower in T2D subjects with hypertension than in the HC group (Table 3). Similar to these findings, another study found that Metrnl levels in the bloodstream were inversely correlated with BP [6]. According to our data, we speculate that Metrnl is elevated in T2D without hypertension and decreased in T2D with hypertension; however, further clinical studies are required to verify this speculation.

Pancreatic beta (β)-cells produce Cer in response to lipotoxicity and hyperglycemia, and β-cell death can be prevented by inhibiting Cer synthesis. Individuals with high lipid accumulation in the liver have higher Cer levels than those with healthy livers [25]. This literature indicates that Cer has a crucial role in lipid-associated IR. Consistent with these observations, the current research found a significant positive correlation between Cer and Metrnl across all study groups (Table 4), indicating that a higher Cer level is associated with elevated lipid levels and higher Metrnl levels.

Metabolomics studies have shown varying relationships between Cer levels and HOMA-IR, ranging from positive to negative or no associations [26]. The current research reported a significant inverse relationship between Cer and HOMA-IR in T2D patients without hypertension (Table 5). Plasma Cer species exhibit a correlation with insulin sensitivity in small cross-sectional investigations involving fewer than 50 individuals [27]. Together, these data suggest that certain Cer species may have either deleterious or protective roles in DM and could offer future treatment targets. Several studies have evaluated the role of sphingolipids on phosphatidylinositol-3 kinase, phosphoinositide-dependent protein kinase-1, phosphoinositide, and glucose transporter-4 (GLUT4) [28, 29]. However, most researchers have not identified a direct role. The current research reported a significant inverse relationship between Cer and FBG in T2D patients without hypertension (Table 5). While cell culture experiments have shown that Cer limits GLUT4 translocation and suppresses insulin-induced glucose uptake [30], human research is limited, and the precise impact of sphingolipids on glucose homeostasis remains unclear. Population-based research has found correlations between various Cer species and insulin levels, but it is still uncertain if sphingolipids are related to subsequent biomarkers of metabolic disorders, such as glucose intolerance or the occurrence of diabetes itself [31].

Moreover, a statistically significant positive correlation was found between Cer concentrations and SBP in T2D subjects with hypertension (Table 5). By increasing oxidative stress in blood vessels, Cer may exacerbate hypertension, leading to impaired vasodilation and endothelial dysfunction [32]. Ceramides, suggested as potential biomarkers for hypertension, have been linked to inflammation and vascular dysfunction. An investigation by Vozella et al. [33] revealed a positive correlation between age and plasma Cer in 164 individuals (84 women). However, the current investigation did not find a clear correlation between age and serum Cer levels across study groups (Table 5). This discrepancy may stem from the previous study's focus on women only, whereas our study included both sexes. Additional factors, such as study quality, sample collection conditions, and measurement materials, may have contributed to this variation.

Al Khairi et al. [7] reported no significant link between Metrnl, FBG, HbA1c, and HOMA-IR in T2D subjects. Similarly, the current research found that Metrnl was positively correlated with FBG in T2D with hypertension and negatively correlated with FBG in the HC group (Table 5). An observational study also reported a significant elevation in serum Metrnl in newly diagnosed T2D, with a notable correlation with glucose profile and IR [34]. The elevated Metrnl concentrations in T2D patients may indicate a protective compensatory response to metabolic stress, including IR. Thus, a strong correlation may exist between circulating Metrnl and glucose regulation in the body. To our knowledge, this research is the first to establish the valuable roles of Cer and Metrnl as possible biomarkers for Iraqi patients with T2D.

Additionally, no significant relationship was reported between Cer and DBP, HbA1c, or lipid profiles in any study group (Table 5). This aligns with an observational cross-sectional study of 84 T2D participants and 75 controls, which reported no significant relationship between Cer and DBP, lipid profiles, or HbA1c in T2D patients [35]. Furthermore, there was no statistical relationship between Metrnl and other variables listed in Table 5 across all study groups. This is consistent with a cross-sectional study of 150 subjects, which found no significant correlation between Metrnl and biochemical parameters such as lipid profile, SBP, and DBP [36]. Another study on 80 T2D patients revealed no significant correlation between Metrnl and biochemical tests involving HbA1c, TG, TC, HDL, LDL, SBP, and DBP in T2D groups [37].

Table 5 also presents correlations between Metrnl concentrations and age, HbA1c, and HOMA-IR across three groups, with no significant correlations found in any group. The negative correlation between Metrnl and age in T2D groups aligns with findings by Wu et al. [38], while the lack of a significant correlation with HbA1c is in line with mixed results reported by Phuong et al. [39]. The negligible correlation between Metrnl and HOMA-IR in T2D without hypertension echoes findings from Paczkowska et al. [40]. These results suggest complex interactions between Metrnl, metabolic factors, and hypertension.

In the control group, the table indicates a positive correlation between Metrnl levels and age, though this correlation is not statistically significant. These findings align with previous research, such as a study by Raschke et al. [41], which reported no significant correlation between Metrnl and age in a healthy population. The control subjects also show no significant relationship between Metrnl and HbA1c. The correlation between Metrnl and HOMA-IR is a non-significant negative. These results are consistent with studies such as Ding et al. [42], which found no significant correlation between Metrnl and HOMA-IR in obese individuals.

This study has several limitations. First, the sample size was relatively small, and only Iraqi individuals were included; thus, results may not be generalizable to other ethnic groups. Additional confounding factors, particularly exercise, cannot be ruled out, as Cer and Metrnl levels may fluctuate with activity. Future studies with larger, more diverse populations should be conducted to address these aspects.

Conclusion

The current study found a significant increase in Cer levels, which could potentially accelerate T2D progression. Cer levels exhibited an inverse correlation with both FBG and HOMA-IR, while SBP and Metrnl showed a positive relationship with Cer. Relationships were observed between Cer, BP, and IR. Metrnl levels were lower in T2D patients with hypertension and higher in T2D without hypertension. The positive correlation between Metrnl and FBG in T2D patients with hypertension suggests that Metrnl may play a potential role in glycemic and BP control. Enhancing knowledge of Metrnl's role in DM development may aid in identifying potential therapeutic targets for managing DM and its complications.

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