Plasma Ceramides as Biomarkers for Microvascular Disease in Diabetes: Evaluating the Relationship Between Ceramide and Multiple Outcomes

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Abstract

Background

We aimed to evaluate ceramides as biomarkers for diabetic microvascular disease.

Methods

309 patients were prospectively enrolled from 2018 to 2020: healthy controls (group 1/N = 51), diabetes mellitus (DM) without Acute Myocardial Infarction (AMI) (group 2/N = 150), and DM with AMI (group 3/N = 108). Outcomes measured were coronary microvascular disease (CMD) using stress perfusion cardiac magnetic resonance imaging (outcome 1), retinal microvascular disease (RMD) using retinography (outcome 2), both (CMD & RMD) (outcome 3), or absence of microvascular disease (AMD) (outcome 4). Biomarker classification performance were evaluated using receiving operator curve analysis (AUC) and multiple logistic regression. Reference three ceramide ratios associated with diabetes were analyzed and compared with 11 ceramides (panel) previously identified by our study group.

Results

HbA1c mean values were 5.1% (group 1), 8.3% (group 2) and 7.6% (group 3). CMD was observed in 59.5% of patients, RMD in 25.8%, CMD&RMD in 18.8%, and AMD in 38.5%. The AUC using reference ceramide ratios for each outcome were: 0.66 (p = 0.012) (CMD), 0.61 (p = 0.248) (RMD), 0.64 (P = 0.282) (CMD&RMD) and 0.67 (P = 0.010) (AMD). However, AUC using 11 ceramides improved significantly: 0.81 (p = 0.001) (CMD), 0.73 (p = 0.010) (RMD), 0.73 (P = 0.04) (CMD&RMD) and 0.83 (P = 0.010) (AMD). Furthermore, specific ceramides features were identified for each outcome. Only increased C14.0 was positively associated with AMD (p < 0.001).

Conclusions

Plasma ceramides predict health status and microvascular disease sub-phenotypes in DM.

Background

Diabetes mellitus (DM) is a growing global health concern, with projections estimating a steady increase in its prevalence worldwide. In 2019, it was reported that 463 million people were living with diabetes, representing 9.3% of the global adult population(1). These numbers are expected to rise up to 578 million (10.2%) in 2030 and 700 million (10.9%) in 2045(1).

The complications of hyperglycemia can be broadly categorized as macrovascular and microvascular disease. The latter, which affects small blood vessels, includes long-term complications that can have
detrimental effects on vascular and endothelial function. Studies suggested that microvascular and macrovascular complications share some pathophysiological similarities(2), such as impaired endothelial function, inflammation, neovascularization, apoptosis, and a hypercoagulable state(3, 4).

Retinal microvascular disease (RMD) is one complication associated with diabetes, and it is generally classified as either proliferative or non-proliferative. RMD is present in 30% of patients with angina and is associated with increased morbidity and mortality(5). Coronary microvascular disease (CMD) is another complication that it is usually underdiagnosed. However, with a better understanding of the pathophysiology and development of new image methods, CMD diagnosis has become more accurate and efficient(6).

Mass spectrometry (MS) has shown great promise in identifying biomarkers, and previous studies have demonstrated the association between ceramide and major cardiovascular adverse outcomes(7). Ceramide is a class of sphingolipid with potential role in insulin resistance, macrovascular disease and microvascular disease(8, 9). Our study hypothesis is that ceramides measured by MS can be used to classify and label diabetes category of risk, providing a simple and non-invasive method for diabetic microvascular disease diagnosis.

Methods

Study Design

This was a prospective, multicenter, national, observational study that included 258 diabetic patients and 51 healthy individuals. The study was conducted in compliance with the Helsinki Declaration(10), with data collection and analysis approved by the domain-specific institutional review board of all hospitals. All participants provided signed informed consent centralized data collection and site monitoring was performed by HCOR Research Institute to assure compliance with study protocol.

Study population

A total of 258 diabetic patients eligible for retinography, stress cardiovascular magnetic resonance (CMR) and blood collection were prospectively enrolled from 2018 to 2022. A total of 51 patients were enrolled in healthy control group (Group 1) and underwent echocardiogram instead of stress CMR to confirm normal heart function. The diabetic patients were analyzed into two groups, 150 without AMI (Group 2) and 108 with AMI (Group 3), with all patients in Group 3 undergoing coronary angiography as part of their usual treatment of acute coronary syndrome (ACS). All patients were fully revascularized and obstructive lesions excluded with angiography and/or FFR (Fractional Flow Reserve) and microvascular resistance evaluated by CFR. Moreover, in Group 3, 50 patients had triplicate blood collected at 24 hours, 1-month, and 6-months after AMI for longitudinal kinetic analysis, two patients without triplicate blood sample collection were excluded. Study work-flow was shown in Fig. 1.
The inclusion criteria for this study required participants to be over 21 years old, have a diagnosis of type 1 or 2 diabetes according to the ADA criteria (11) (Groups 2 and 3), and have experienced an AMI according to the Fourth AMI Universal definition (12) (Group 3). Group 1 individuals needed to have an echocardiogram, retinography, all laboratory exams within the normal range, and no disease diagnosed during the 6-month follow-up, therefore were not considered for CMR by reason of futility. The exclusion criteria included active cancer, glaucoma, previous stroke, severe renal impairment (eGFR < 30 ml/m), uncontrolled hypertension (systolic blood pressure ≥ 160mmHg and/or diastolic blood pressure ≥ 90mmHg), uncontrolled dyslipidemia (LDL greater than 150mg/dL) even while on-treatment, or inability to perform retinography or stress CMR.

Clinical and Laboratory Data

The clinical and laboratory data were grouped into clinical, socioeconomic, physical exam, comorbidities, medication in use and treatment adherence. The Morisky test was used as a tool to assess patient adherence to drug treatment. The test consists of a questionnaire with four questions related to the patient’s behavior in regularly taking prescribed medication.

The cohort was analyzed based on four outcomes to assess the spectrum of disease progression from non-diabetes to diabetes and to track microvascular disease progression to macrovascular disease. Only patients who could undergo CMR, retinography or both were included in the outcome analysis. Some patients were unable to undergo both tests due to claustrophobia or pandemic-related restrictions.

Study Procedures

Stress induced myocardial ischemia (SIMI) by cardiovascular magnetic resonance imaging (CMR). In a 1.5T scanner (GE 450) the CMR exam was performed in 114 patients (group 2) and in 45 pts (group 3) following a standard protocol that included LV short and long-axis cine images acquisition (steady-state free precession – SSFP sequence) and late gadolinium enhancement. First-pass myocardial perfusion was acquired in the LV short-axis plane and obtained 2 to 3 min after pharmacological stress using dipyridamole at 0.56mg.kg\(^{-1}\) injected over 4min. A single dose of 0.05 mM.kg\(^{-1}\) of nonionic, low-osmolar Gd-based contrast agent was injected into the antecubital vein by power injector at a rate of 5mL.s\(^{-1}\) followed by 20mL saline flush. Aminophylline was intravenously injected immediately after the stress perfusion image sequence. The heart was divided into 17 segments for myocardium perfusion assessment (20), which was determined not only in injected ischemic segment but also in non-injected myocardial segments; each segment was scored as presenting normal perfusion (0) mild (1), moderate (2) or severe (3) perfusion defect. Coronary flow reserve velocity is a reliable measure of coronary microvascular function in absence of any epicardial flow limitation, and cutoff values ≤ 2.5 are commonly used as indicative for impaired coronary microvascular function (13).

Acquisition of phase contrast cine CMR data from the coronary sinus
The image of the acquisition of coronary sinus flow was adjusted in a perpendicular plane to the coronary sinus at cm from its ostium on axial cine CMR images. We acquired phase contrast (PC) of the coronary sinus at cine CMR during breath-hold (repetition time, 10.4 ms; echo time, 4.9 ms; flip angle, 20°; field of view, 380 × 228 mm; acquisition matrix, 256 × 128; reconstruction matrix, 256 × 128; reconstruction resolution, 1.48 × 1.78 mm; the number of phases per cardiac cycle, 30; velocity encoding, 40 cm/s; slice thickness, 8 mm)(14).

**Coronary sinus blood flow analysis by CMR**

All CMR exams were analyzed using a dedicated CMR-TT software (CVi42 5.13.5, Circle Cardiovascular Imaging Calgary, Canada), which allows flow 2D parameter analysis based in phase contrast cine images to quantify blood flow in the coronary sinus.

The contours of the coronary sinus were manually traced phase by phase for the entire cardiac cycle and the perform phase-offset correction for method static tissue mask in background correction available on software CVi42. Blood flow in the coronary sinus was calculated by integrating the product of the cross-sectional area and mean velocity in the coronary sinus, and then corrected using mean velocity in the adjacent tissue for all cardiac phases in the cardiac cycle. MBF was calculated as follows:

\[
\text{MBF (ml/min/g)} = \frac{\text{coronary sinus flow (ml/min)}}{\text{LV mass (g)}}
\]

Resting MBF correlates linearly with rate pressure product at rest, but hyperemic MBF does not correlate linearly with rate pressure product (15). Therefore, we corrected resting MBF by resting rate pressure product at rest from each subject using the following formulas (MBF during infusion dipyridamole was not corrected by rate pressure product during stress)(14).

Corrected MBF at rest (ml/min/g/) = MBF at rest (ml/mim/g) / rate pressure product at rest (mm Hg/mim) x 7,500

Rate pressure at rest (mm Hg/mim) = systolic blood pressure at rest (mm Hg) x heart rate at rest (beats/min)

The average rate pressure product at rest was 7,500 from healthy controls with mean age of 50.1 ± 9.7 years reported in a previous study (15, 16).

Δ MBF and CFR were calculated as:

\[
\Delta \text{MBF (ml/min/g)} = \text{MBF during infusion dipyridamole (ml/min/g)} - \text{corrected MBF at rest (ml/mim/g)}
\]

**Sample preparation and Mass Spectroscopy (MS) Analysis**

Analytes were extracted from plasma with addition of a solution composed of ethanol containing 0.1% ammonium hydroxide (v/v) and deuterated internal standards followed by sonication and agitation. For MS analysis, the Transcend TLX-4 system was used, which consisted of four Dionex UltiMate 3000 quaternary pumps, four Dionex UltiMate 3000 binary pumps, one VIM, and one CTC PAL autosampler.
The system was coupled to a TSQ Altis Triple Quadrupole Mass Spectrometer with a heated-electrospray ionization (HESI) source from Thermo Fisher Scientific, San Jose, CA, USA. Two TurboFlow Cyclone-C8 XL 0.5 x 50 mm columns and two Accucore C30 50 x 2.1 mm columns from Thermo Fisher Scientific were used in the TLX-4 system. For the first dimension (turboflow chromatography), mobile phase A consisted of water with 0.1% formic acid, mobile phase B was ethanol, and mobile phase C was acetonitrile/isopropanol/acetone (40:40:20, v/v). For the second dimension, mobile phase A was H2O/acetonitrile/methanol/formic acid (56:14:30:0.1, v/v) and mobile phase B was isopropanol/acetonitrile/formic acid (90:10:0.1, v/v). The extracts were injected into the system, and detection was achieved by monitoring protonated precursor ions and their respective fragments (264.27 for ceramides, 266.28 for dihydroceramide, 271.31 for ceramides-d7, and [M + H-H2O] + for qualifier transitions of ceramides and dihydroceramide).

Statistical Analysis

The data were expressed as mean ± SD for continuous variables that were normally distributed or median (range) for skewed data. Percentages were presented for categorical variables. Comparisons among group variables were performed using the Fisher Exact Test for categorical variables, ANOVA for parametric continuous variables or the Kruskal-Wallis test for non-parametric continuous variables. Three ceramide ratios associated with pre-diabetes in a large populational study(17) were selected for benchmark analysis and mean ± SD were compared between patients with and without diabetes in our cohort to assure this association: ceramide ratios C18.0/C16.0; C18.0/C24.0 and C18.0/C24.1. Then, all four study outcomes were analyzed using ROC curve with the three ceramide ratios already mention and results compared with the 11 ceramides (panel) discovered by our group of study(9) associated with AMI. All outcomes were analyzed with each of the 11 ceramides by multiple logistic regression and significant ceramides were selected to build the study conclusion showing differences in plasma ceramides during the progression from non-diabetes to diabetes with and without microcirculatory disease. SPSS, Prism Plus 9 and Wizard 2 software were used for data and for graphical analysis.

Results

Baseline characteristics and clinical outcomes

This study included healthy individuals and a wide spectrum of diabetic patients with different ages, socioeconomic status, comorbidities and medication in use (Table 1). Group 1 consisted of younger and healthy individuals at mean age of 35-y old with high socioeconomic status, no comorbidities and no medication in use. Group 2 included patients at mean of age 54-y old, multiple comorbidities such as hypertension (66%), dyslipidemia (38%), and all patients had diabetes, treated with oral hypoglycemic drugs or insulin. Group 3 consisted of older patients at mean age of 65-y old with diabetes and an acute AMI and with higher amount of cardiovascular disease drugs in use. Group 1 had an HbA1c value of 5.1%, as expected for healthy volunteers (Table 2). In contrast, groups 2 and 3 had HbA1c values of 8.3% and 7.6%, respectively, consistent with diabetes. All laboratory results were within normal range in group
1, while group 3 had on-treatment a lower LDL-cholesterol level 76.3 ± 28.6 compared to other groups and all had normal renal function. Regarding cardiac cavity diameters, group 3 had increased heart dimensions, although left ventricle ejection fraction was normal. Group 1 had no abnormalities observed in the retinography exam, while group 2 and 3 had about 30% of patients with non-proliferative microvascular lesions. Group 3 had twice as many patients with proliferative lesions as group 2 (7% vs 3.5%) (p-value < 0.001). In addition, in the 6-month follow-up, only one major cardiovascular adverse event (stroke) was observed in group 2, while no adverse events were reported in other groups.

**Analysis of Ceramide Kinetics**

The longitudinal kinetics of ceramides (Group 3/N = 48pcs) showed a non-significant increase in total plasma concentrations after AMI at 24 hours, 1-month and 6-months follow-up (P = 0.664) (Fig. 2). The analysis of all individual 11 biomarker kinetics demonstrated its plasma stability, as shown in Supplementary Table 1.

**Comparison of Ceramide Plasma Concentrations among patients with and without DM**

The mean full cohort concentration was 8.0 ± 4.5 µmol/L (Fig. 3a). Ceramide plasma median concentrations of diabetic patients (Groups 2 and 3, N = 258) was 7.0 µmol/L and differed significantly compared to non-diabetic patients (Group 1, N = 51) 6.3 µmol/L (p = 0.01) (Fig. 3b). Among the three groups the mean difference in ceramide plasma concentrations were also significant (Group 1: 6.44±3.71 µmol/L; Group 2: 9.28±5.12 µmol/L and Group3 7.470±3.595 µmol/L (p = < 0.001), as well as between Groups 1 and 2 (p = 0.001) and Groups 2 and 3 (p = 0.002), but not between Group 1 and 3 (p = 0.309) (Fig. 3c). We also tested three ceramide ratios that had already been validated in large studies (17, 18) and had a proven association with diabetes. The mean average ceramide ratio C18.0/C16.0 was 0.409 ± 0.007 in diabetic patients versus 0.341 ± 0.01 in non-diabetic patients (p < 0.001) (Fig. 3d). The mean average ceramide ratio of C18.0/C24.0 was 0.038 ± 0.001 in diabetics versus 0.031 ± 0.001 in non-diabetic patients (p = 0.007) (Fig. 3e), and the mean ceramide ratio C18.0/C24.1 was 0.141 ± 0.002 in diabetics versus 0.131 ± 0.004 in non-diabetics (p = 0.046) (Fig. 3f). These results confirm the association of these biomarkers with diabetes status.

**The Spectrum of Cardiac and Retinal Microvascular Disease**

A total of 159 CMR exams were conducted in diabetic patients (group 2 and 3) and as a control group 51 healthy individuals (group 1) all with normal echocardiographic and retinography were included in the analysis (total = 210 pts), 59.5% (125 pts) were diagnosed with coronary microvascular disease (outcome 1), as shown in Fig. 4a. In Retinography exams (N = 248 pts), 25.8% (64) were diagnosed with RMD (outcome 2), as shown in Fig. 4b. Both coronary microvascular disease and RMD (outcome 3) were observed in 18.8% (N = 36 pts) of the 192 patients who underwent both CMR and Retinography exams, as
shown in Fig. 4c. Absence of both conditions was observed in 38.5% (N= 74 pts) out of the 192 patients, as shown in Fig. 4d.

**Ceramides Predict Microvascular Phenotypes**

The three ratios identified in a large populational study associated with diabetes in the literature were analyzed and compared with a 11 ceramides panel. The AUC using reference ceramide ratios for each outcome were: 0.66 (p = 0.012) (CMD), 0.61 (p = 0.248) (RMD), 0.64 (P = 0.282) (CMD&RMD) and 0.67 (P = 0.010) (AMD). However, AUC using 11 ceramides (panel) improved significantly: 0.81 (p = 0.001) (CMD), 0.73 (p = 0.010) (RMD), 0.73 (P = 0.04) (CMD&RMD) and 0.83 (P = 0.010) (AMD).

**Differential Ceramide Profiles of Retinal and Cardiac Microvascular Phenotypes**

All four outcomes in our study were analyzed using each 11 ceramides (panel). The different patterns of ceramides were shown by their coefficients and p-values (Fig. 5) derived from the multiple logistic regression model. Patients with CMD had reduced C14.0 (p < 0.001) and increased plasma levels of C18.0 (P = 0.009), C22.1 (P = 0.01) and C26.0 (P = 0.05). Patients with RMD had reduced C14.0 (p = 0.01) and C16.0 (P = 0.01) and increased plasma levels of DhC16.0 (P = 0.009) and C24.1 (P = 0.02). Patients with both CMD & RMD had lower levels of C14 (P = 0.03) and C16 (P = 0.05) and in contrast higher levels of C26 (P = 0.04). Finally, patients and health individuals with absent of microvascular disease (AMD) had higher levels of C14 (p < 0.001) and lower levels of C18 (P = 0.01), C22.1 (P = 0.007) and C26.0 (P = 0.02). Interestingly, C14.0 was only positively associated with AMD. The study visual conclusion supports different trends of ceramides as a signature for each disease spectrum (Fig. 6).

**Discussion**

The microcirculation is emerging as a major determinant of cardiovascular outcomes among patients with and without DM (19). This study aimed to fill this gap and evaluate the effectiveness of using ceramide as a biomarker for microvascular disease. The four outcomes analyzed cover the disease progression and identified distinct ceramide profiles, that reflect core pathological disease stages, from healthy individuals through early diabetic disease to advanced stage. The main study findings are: ceramides levels are stable in plasma for at least at 6-months period, ceramide levels are higher in diabetic patients, ceramides identified in our previous study in macrovascular disease were also associated with microvascular disease progression, as well as absence of microvascular disease among participants with and without DM and coronary artery disease.

Ceramides were previously identified by our group and others as tissue-based biomarkers linked to unstable atherosclerotic plaque(9, 20). Ceramides are produced in response to hyperglycemia(21), TNF (tumor necrosis factor)-α signaling(22), NO-signaling, inflammation(23), vascular FAT-redox state and are link to poor cardiovascular outcomes(24). Hence, ceramides are interrelated to cardiometabolic syndrome, diabetes, microvascular disease development and mortality(25), but microvascular disease is
often difficult to detect and therefore underdiagnosed(13). The study design is to identify differences in biomarkers levels in patients with different degrees of microvascular disease, hence our comparison groups are not similar. This study remarkable finding is that different ceramide profiles are associated with each pathological microvascular disease territory, independently or in association. Therefore participants were assignment to diverge health and disease conditions is documenting a naturally occurring relationship about diabetes microvascular disease progression by non-invasive information using plasma biomarkers that is of great interest for risk stratification of those patients(26).

Common atherosclerotic risk factors do not accurately classify patients at risk of MACCE or microvascular disease(27). Interestingly, LDL-cholesterol was found to have little informative value of MACCE risk when included in models that use ceramides(18). Ceramide ratios identified in previous studies were tested and associated with diabetes but not with microvascular disease(17). It is noteworthy to mention about this study that ceramide ratio C14 was correlated with all outcomes. Interestingly, CMD diagnosis was associated with increase of C18, C22.1 and C26 in contrast to negative relationship of ceramide 14:0 in agreement to the large number of studies in the literature that observed similar findings(28–30). In addition, an increase in ceramide levels has been shown to be detrimental for the retina, leading to inflammatory events, apoptosis, and retinal degeneration by disrupting the balance of cell death and survival(31). Previous studies have demonstrated that a high ceramide ratio C16:0 is strongly associated with MACCE(28, 32) and other metabolic defects that contribute to diabetes pathology, such as insulin resistance. Furthermore, cholesterol dysmetabolism and diabetes can act synergistically to develop diabetic retinopathy(33). C16:0 was found to be associated with RMD in this study and has been implicated in the development of obesity, type 2 diabetes and new drugs to reduce its production is under development (34).

Recent studies have identified poor cardiovascular health through lipidomic profiling and a low ceramide C14:0 level has been linked to poor blood pressure, total cholesterol, and fasting blood glucose(35, 36). This study yielded similar findings, indicating that a high concentration of plasma ceramide C14:0 is a signature of a healthy status, while a low concentration indicates a disease state. Epidemiological studies have shown that microvascular disease and ACS risks starts at pre-diabetic stage (37). Detection of pre-diabetes is possible using plasma ceramides(17) and might represent an opportunity for reducing the burden of microvascular and macrovascular disease through heightened attention to screening for vascular complication. This can have a critical implication in clinical practice since prevention studies have demonstrated that their risk can be reduced with lifestyle interventions(38).

However, this study has several limitations that deserve to be mention. First, some patients did not underwent to heart and retina microvascular disease exams due to pandemic risk of infection and/or claustrophobia during stress cardiac MRI (39). The reported incidence of anxiety-related reactions during MRI ranges from 4–30% in the general population. Secondly, in the present study subjects had a lower incidence rate of MACCE and intermediate outcomes could not be associated with hard outcomes likely owing to the high percentage of lipid-lowering therapy. However, the study protocol was not predefined to evaluate MACCE but this data could had increased the value of our findings.
Altogether, the biological effect of microvascular disease on organ dysfunction is a crucial element in diabetic disease progression and the development of non-invasive biomarkers that can diagnose at early stage of microvascular disease is critical to reduce disease progression. These findings provide support that ceramide is a biomarker that can be used to refine the classification of the diabetic microvascular disease.

**Conclusion**

Plasma ceramides demonstrate distinct signatures associated with the presence of retinal and/or cardiac microcirculatory dysfunction in the presence and absence of DM and coronary artery disease. Our findings may inform future studies investigating links between circulating ceramides and microvascular disease.

**Abbreviations**

CMD: Coronary Microvascular Disease  
RMD: Retinal Microvascular Disease  
AMD: Absence of Microvascular Disease  
HbaA1: Glycated Hemoglobin  
AUROC: Area under the receiver operating characteristic curve  
MS: Mass Spectrometry  
AMI: Acute Myocardial Infarction  
MACCE: Major Adverse cardiac and cerebrovascular events  
DhC: Dihydroceramide  
C: Ceramide

**Declarations**

**Ethics Approval and consent to participate**

HCOR CAAE: 95533918.2.3002.5505

**Consent for publication**

We have signed consent forms from all participants in this study.
Availability of data and materials

The datasets generated during and/or analysed during the current study are not publicly available due but are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors Contributions

D.L.M.J: Substantial contribution to conception and design, acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the version.

A.B.C: Substantial contribution to conception and design, acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the version.

J.M.S: drafting the article or revising it critically for important intellectual content and final approval of the version.

E.Y: drafting the article or revising it critically for important intellectual content and final approval of the version.

I.A.J: drafting the article or revising it critically for important intellectual content and final approval of the version.

A.S: drafting the article or revising it critically for important intellectual content and final approval of the version.

K.N: drafting the article or revising it critically for important intellectual content and final approval of the version.

L.O.S: drafting the article or revising it critically for important intellectual content and final approval of the version.

M.A.L: drafting the article or revising it critically for important intellectual content and final approval of the version.
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M.C: drafting the article or revising it critically for important intellectual content and final approval of the version.

J.C: drafting the article or revising it critically for important intellectual content and final approval of the version.

V.M.C: drafting the article or revising it critically for important intellectual content and final approval of the version.

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C.E.R: drafting the article or revising it critically for important intellectual content and final approval of the version.

J.E.K: drafting the article or revising it critically for important intellectual content and final approval of the version.

L.P.C: Substantial contribution to conception and design, acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content and final approval of the version.

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Not applicable
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Tables

Tables 1-3 is available in the Supplementary Files section.

Figures
Figure 1

Study Work-Flow
### Longitudinal Kinetics after AMI

**Triplicate Ceramides Mean Values (µmol/L)**

<table>
<thead>
<tr>
<th>Timeline</th>
<th>24-Hour</th>
<th>1-Month</th>
<th>6-Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceramide (d18:1/14:0) (0.2%)</td>
<td>0.02</td>
<td>0.05</td>
<td>0.35</td>
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<tr>
<td>Ceramide (d18:0/16:0) (0.7%)</td>
<td>0.17</td>
<td>0.19</td>
<td>0.19</td>
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<tr>
<td>Ceramide (d18:1/16:0) (4.7%)</td>
<td>0.81</td>
<td>0.07</td>
<td>1.00</td>
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<td>Ceramide (d18:1/18:0) (2.3%)</td>
<td>3.67</td>
<td>1.11</td>
<td>0.06</td>
</tr>
<tr>
<td>Ceramide (d18:1/20:0) (2.5%)</td>
<td>7.50</td>
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<tr>
<td>Ceramide (d18:1/24:1) (15%)</td>
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<tr>
<td>Ceramide (d18:1/26:0) (6.6%)</td>
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<tr>
<td>Ceramides ∑ (100%)</td>
<td>6.97</td>
<td>0.95</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*P-value=0.664

**Figure 2**

Heat Map with longitudinal Ceramide kinetic analysis (Group 3/N=48 pts)

**Figure 3**

Differences between plasma ceramide concentrations between diabetic and non-diabetic patients
Figure 4

Full Cohort Main Outcomes

1. Outcomes: Contribution by Groups

![Graph showing the contribution by groups with percentage values for each group.]

2. Outcomes: Full Cohort (309)

![Graphs showing the outcomes of Full Cohort (309) with details for each category.]

Figure 5

Roc curve analyzes of three reference ceramide ratios (C 18.0/16.0; C 18.0/24.0 & C 18.0/24.1) versus 11 ceramides (Panel)
Figure 6

Visual conclusion of the study with the progression from non-diabetes to diabetes with microcirculatory disease and the patterns of biomarkers signature with significant statistical association

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf
- Table2.pdf
- Tabela3.png