Saudi Journal of Medicine (SJM)
Scholars Middle East Publishers
Dubai, United Arab Emirates
Website: www.saudijournals.com

ISSN 2518-3389 (Print)
ISSN 2518-3397 (Online)

Upcoming Role of Ceramides as Potential Biomarker in Cardiovascular Disease Prediction

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Article History
Received: 02.05.2018
Accepted: 15.05.2018
Published: 30.05.2018

DOI:
10.36348/sjm.2018.v03i05.003

Abstract: Coronary artery disease (CAD) is currently one major cause of death in the world [1, 2]. CAD is mainly caused by atherosclerosis, which is considered as a chronic inflammation in response to cholesterol accumulation in the arterial wall [3]. Therefore, biomarkers that can predict the presence for early atherosclerotic process and CAD are desirable. Lipidomics is playing vital role in development of atherosclerosis and in cardiovascular disease. Various inflammatory markers and lipid biomarkers are playing role in diagnosis of CAD. Inflammatory biomarkers such as CRP, cytokines [interleukin (IL)-1, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1)], soluble CD40 ligand, serum amyloid A (SAA), selectins (E-selectin, P-selectin), myeloperoxidase (MPO), matrix metalloproteinases (MMPs), cellular adhesion molecules [intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1)], placental growth factor (PlGF) and A_2 phospholipases may have a potential role for the prediction of risk for developing CAD and may correlate with severity of CAD..Dyslipidemia is major cause of Cardiovascular disease. Ceramide a lipid biomarker is playing a emerging role in diagnosis of CAD, In this review we tried to focus role of Ceramide in diagnosis of CAD and to further risk stratify patients who may otherwise not receive treatment but would remain at high risk for a serious cardiac event. These are patients who could benefit from more intensive treatment, for example: a higher-dose statin, a nutritionist consult or formal exercise therapy. Cardiologists at Mayo Clinic are already routinely checking Ceramides using the new test.

Keywords: Ceramide, CAD, Atherosclerosis.

INTRODUCTION

Ceramides are a family of waxy lipid molecules. Ceramides are known to associate with many central processes of atherosclerosis development including lipoprotein uptake, inflammation, and apoptosis. Despite major public health efforts, coronary heart disease continues to be the leading cause of death. Oxidized lipids contribute to heart disease both by increasing deposition of calcium on the arterial wall, a major hallmark of atherosclerosis, and by interrupting blood flow, a major contributor to heart attack and sudden death. Oxidized cholesterol (oxysterols) enhances the production of sphingomyelin, a phospholipid found in the cellular membranes of the coronary artery. This increases the sphingomyelin content in the cell membrane, which in turn enhances the interaction between the membrane and ionic calcium (Ca(2+)), thereby increasing the risk of arterial calcification [4]. Ceramide level in human plasma is a risk factor at the early stages of atherosclerosis. Ikuyo Ichi *et al*., examined the relationship between ceramide concentration and risk factors of atherosclerosis in normal human plasma using electrospray tandem mass spectrometry (LC-MS/MS). The ceramide concentration showed a significant positive correlation with total cholesterol (TC) and triglycerides (TG). In addition, plasma ceramide level increased drastically at a high level of LDL cholesterol (more than 170 mg/dL).Hence Ceramide play a vital role in diagnosis of coronary artery disease Fig-1 [5].

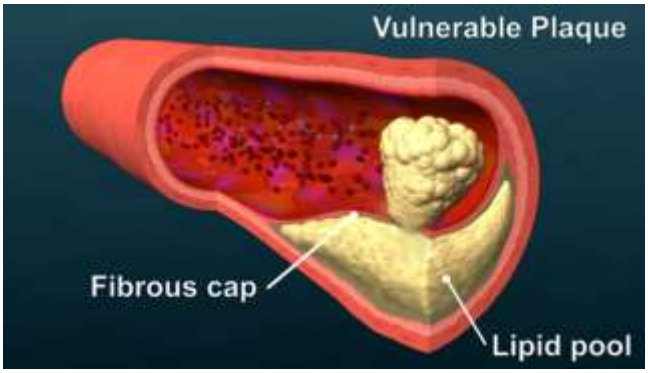

Fig-1:

Roles for ceramide and its downstream metabolites have also been suggested in a number of pathological states including cancer, neurodegeneration, diabetes, microbial pathogenesis, obesity, and inflammation. Recently their role in cardiovascular disease is emerging.

Ceramide Synthesis

De novo synthesis of ceramide begins with the condensation of palmitate and serine. Ceramide generation can also occur through breakdown of complex sphingolipids that are ultimately broken down into sphingosine, which is then reused by reacylation to form ceramide. This latter pathway is termed the Salvage pathway Fig-2.

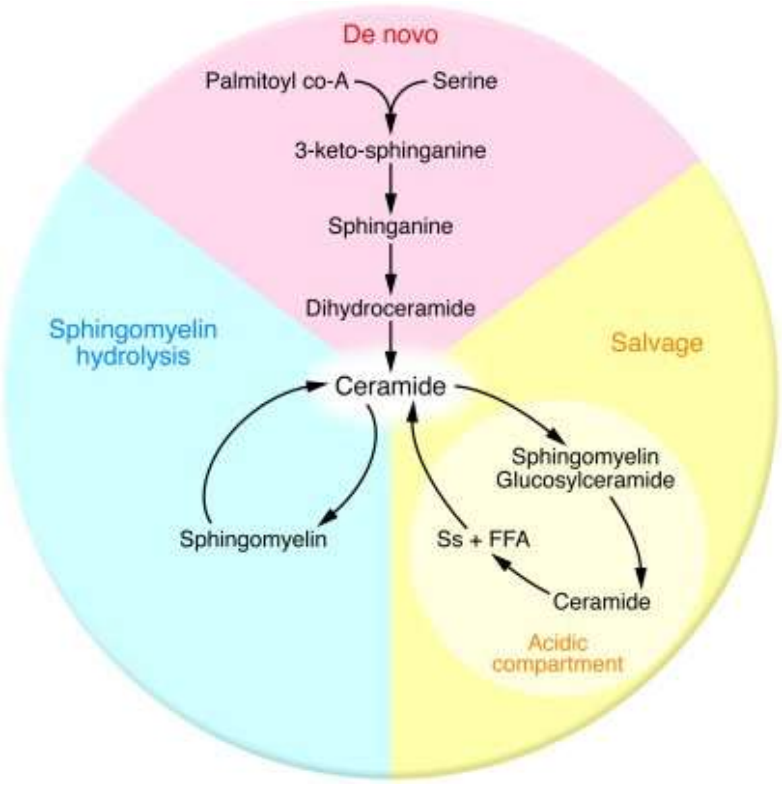

Fig-2

Ceramides in Cardiovascular disease

New research has revealed that elevated circulating ceramide levels correlate strongly with future major adverse cardiovascular events. Three specific ceramides—C16:0, C18:0, and C24:1—were shown to be independently predictive of atherosclerotic plaque instability and/or death In fact, quantifying increases of these particular ceramides in plasma may surpass the prognostic value of more conventional biomarkers: LDL and HDL_cholesterol, c-reactive protein, and lipoprotein-associated phospholipase A_2 [6-10].

Lipid species, particularly ceramide (d18:1/16:0), were also associated with necrotic core tissue type and lipid core burden in coronary angiography, and were predictive for 1-year clinical outcome in 581 ACS and stable CAD patients [7]. In

these studies, plasma CVD risk-related ceramide molecules (Cer(d18:1/16:0), Cer(d18:1/18:0), and Cer(d18:1/24:1)), and their ratios with Cer(d18:1/24:0), emerged as potential risk stratifiers for CAD patients.. Thus, monitoring ratios of ceramides species may provide insight into the metabolic regulation of atherosclerotic events [11].

Elevated ceramide levels can be detected within one to five years before a cardiac event in apparently healthy individuals. Aki S. Havulinna *et al*., showed that whether ceramides are associated with major adverse cardiovascular events (MACEs) among apparently healthy individuals. FINRISK 2002 is a population-based risk factor survey, which recruited men and women aged 25 to 74 years. The cohort was followed up until the end of 2014. They quantified 4 circulating ceramides, Cer(d18:1/16:0), Cer(d18:1/18:0), Cer(d18:1/24:0), and Cer(d18:1/24:1), in 8101 serum samples by a targeted liquid chromatography– tandem mass spectrometry assay.

Of the ceramide species, Cer(d18:1/18:0) had the strongest association with incident MACE. Results should encourage more detailed analyses of ceramides in cardiovascular pathobiology and suggest new biomarkers of MACE Distinct serum ceramides are associated with the risk of incident MACE in apparently healthy individuals [12].

Dr. Jeif Meeusen from Mayo Clinic studied 499 patients who were referred for coronary angiography to check possible stenosis and followed prospectively for 18 years with researchers recording occurences of myocardial infarctions, stroke revascularization and death. They measured four different types of ceramides in blood at baseline-three linked with cardiovascular disease and fourth that is abundant in all cells and not specially associated with disease but useful to normalize for intra-individual variability. By using ceramide risk score they found that elevated ceramides were associated with increased risk of myocardial infarction, acute coronary syndromes and mortality within 1-5 years.

They also found that ceramides could identify high risk patients among those with LDL-C <2.6 mmol/l. They also suggested that evaluating ceramide levels in patients who were not immediate risk of coronary artery disease events may help cardiologist decide who could benefit from proactive and preventive treatment such as statins or life style changes to prevent a serious cardiac events [13].

Martina Klevstig *et al*., showed association between Ceramide accumulation and heart function, myocardial left ventricle biopsies from subjects with chronic ischemia and found that ceramide levels were higher in biopsies from subjects with reduced heart function. Ceramides are produced by either *de novo* synthesis or hydrolysis of sphingomyelin catalyzed by acid and/or neutral sphingomyelinase. They used cultured HL-1 cardiomyocytes to investigate these pathways and showed that acid sphingomyelinase activity rather than neutral sphingomyelinase activity or *de novo* sphingolipid synthesis was important for hypoxia-induced ceramide accumulation. They used mice with a partial deficiency in acid sphingomyelinase (*Smpd1*$^{+/-}$ mice) to investigate if limiting ceramide accumulation under ischemic conditions would have a beneficial effect on heart function and survival. However, they found that targeting ceramide accumulation in the ischemic heart may not be a beneficial treatment strategy [14].

Wang *et al*., showed a strong positive association between plasma Ceramide concentrations and incident Cardiovascular disease (CVD) risk by using a prospective design nested in a well-known randomized trial and observed effect of the detrimental effect of higher ceramide concentrations on CVD risk was modified by the MedDiet intervention [15]. The potential mechanisms for the MedDiet's modulatory effects on the Ceramide pathway are two-fold. First, consumption of key components of the MedDiet may directly influence ceramide biosynthesis. Studies using cultured myotubes and animal models found that exposure to saturated free fatty acids (FFAs), especially long-chain saturated FFAs, promoted ceramide formation [16, 17], while unsaturated FFAs prevented the excess ceramide accumulation stimulated by saturated FFAs [18].

Bariatric surgery induces significant reductions in plasma ceramides, proinflammatory markers, and other indicators of CVD [19, 20]. Helen M *et al*., showed relationship between altered ceramide levels after bariatric surgery in morbidly obese patients, and decreased risk factors for CVD, such as lipoprotein and lipid levels, brachial artery reactivity, and the Framingham risk score for heart disease. The improvements observed in cardiovascular risk parameters as soon as 3– 6 months after gastric bypass further highlight the major benefits of bariatric surgery and support the development and application of surgical approaches for the treatment of obesity and for CVD risk reduction [21].

Pan W *et al*., suggested higher ceramide levels and SMase activity in patients with coronary heart disease may be an important factor in the development of atherosclerosis and changes in the plasma concentration of sphingolipids may indicate their involvement in the molecular mechanism of plaque destabilization. They demonstrated that patients with CAD has shown that plasma ceramide levels and SMase activity were elevated in the stable angina pectoris (SAP), unstable angina pectoris (UAP) and acute myocardial infarction (AMI) groups. In the group of patients with UAP, there was a significant increase in

SMase activity and ceramide concentration in comparison to the control and SAP groups, although the increased activity of SMase was transient. In the AMI group, the significantly elevated level of ceramide was noted up to 7 days following the cardiac incident in comparison to the control and SAP groups, whereas enhancement in activity of SMase occured only 3 days after infarction [22].

The plasma concentration of Ceramide in patients with acute myocardial infarction after admission to the intensive heart care unit was not significantly changed when compared to the control group, whereas the concentration of S1P was decreased by a significant 50 % [23]. Another investigation revealed that the plasma level of S1P was significantly decreased in patients admitted to hospital with STEMI (ST-elevation myocardial infarction) and Ceramide level was reduced 5 days post-infarct compared to the control group, although the reduction in ceramide level reached statistical significance 30 days following infarction. Two years after the infarction the plasma S1P level almost completely recovered, whereas the decreased Ceramide level was maintained. Erythrocytes from STEMI patients showed accumulation of S1P and ceramide during the thirty days following infarction, although only the elevated level of S1P reached statistical significance during the whole time of observation. After two years of observation the concentration of Ceramide and S1P decreased to the control levels, although these findings were not statistically significant [24].

Ceramide in diabetes mellitus associated cardiovascular disease

plasma ceramide in the pathogenesis of insulin resistance and systemic inflammation Ceramide inhibits insulin signaling by several independent mechanisms: by increasing PP2A activity [25], which decreases Akt phosphorylation and activity [26]; by blocking the recruitment of Akt to the plasma membrane, which is required for activation by the upstream kinases PDK1 (at Akt Thr^{308}) and TORC2 (at Akt Ser^{473}); and by ceramide accumulating in caveolin-enriched domains, activating PKCζ, which sequesters Akt in a repressed state within these membrane domains to prevent insulin signaling [27]. The reduction in insulin-stimulated glucose uptake with LDL-ceramide administration in vivo was accompanied by ceramide accrual in the plasma membrane and reduced Akt phosphorylation. Aside from effects on signaling pathways, ceramide can inhibit Rac activation in the pathway toward GLUT4 translocation [28].

Ceramide is a common molecular intermediate for conveying inflammatory signals. TNF-α increases cellular ceramide via de novo synthesis or sphingomyelin hydrolysis, and IL-1 is a potent inducer of ceramide. Activation of the innate immune receptor TLR4 increases SPT transcription and activity, ceramide synthesis, and plasma lipoprotein ceramide levels [29, 30]. Many of the cytokines produced by TLR4 also promote ceramide synthesis (e.g., TNF-α), thereby invoking multiple ceramide-producing stimuli. Most inflammatory mediators of ceramide production converge on the IκKβ–NF-κB pathway. On the other hand, ceramides induce inflammatory signals such as the IκKβ–NF-κB pathway. Ceramide causes NF-κB activation [31, 32] and TNF-α, IL-6, IL-1β, and MCP-1 are controlled by NF-κB. Blocking ceramide synthesis or inhibiting NF-κB reduces TNF-α and IL-6 production Fig-3 [33, 34].

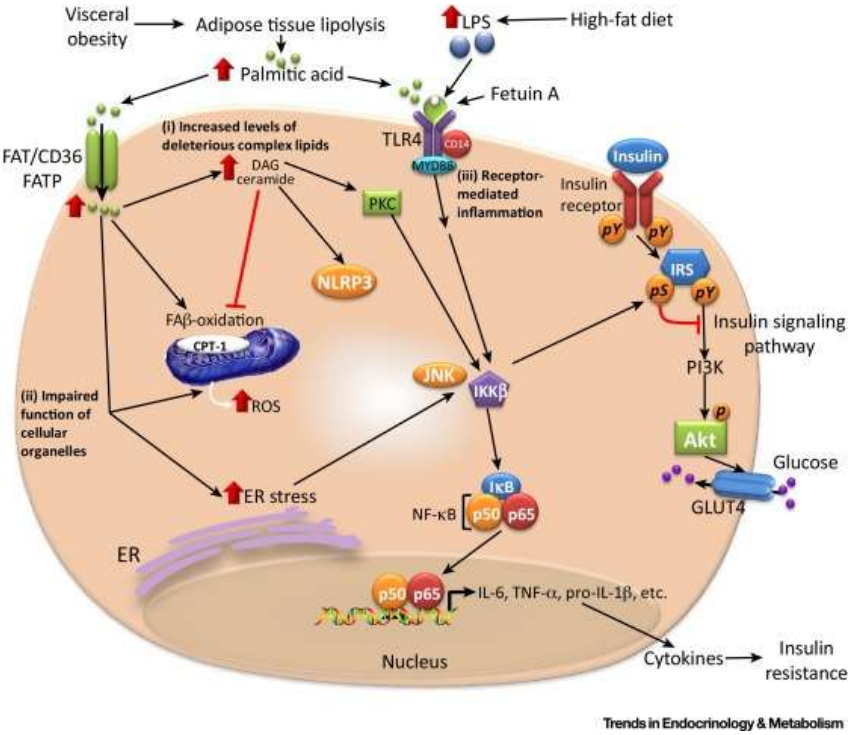

Fig-3:

Plasma ceramides are associated with proinflammatory cytokines in individuals with cardiovascular disease [35] type 2 diabetes [36].

Lemaitre *et al*., hypothesized that plasma ceramide and sphingomyelin species that contain the saturated fatty acid palmitic acid (16:0) are associated with higher risks of incident diabetes and diabetes-related cardiovascular disease. In contrast, they hypothesized that plasma ceramide and sphingomyelin species that contain a saturated fatty acid with 20 or more carbons (20:0, 22:0 or 24:0) are associated with lower risks of incident diabetes and cardiovascular disease [37].

Zora Biosciences has announced the expansion of its Coronary Event Risk Test (CERT) to include prediction of type II diabetes prevent Four specific ceramides have been identified by Zora as highly linked to cardiovascular disease and insulin resistance e.g. diabetes. These complex ceramide molecules can be quickly and accurately detected using Liquid Chromatography Mass Spectrometry (LCMS) and proprietary bioinformatics developed by Zora. The test allows for the first time reliable risk stratification for cardiovascular disease and diabetes in a single test. This exciting development enables a variety of interventional development of both diseases.

CONCLUSION

Plasma ceramides are significant predictors of Cardiovascular disease both in patients with stable CAD and ACS and Cardiac disease associated with Diabetes over and above currently used lipid markers. This may improve the identification of high-risk patients in need of more aggressive therapeutic interventions.

Conflicts of Interest

There is no conflict of interest.

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