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Exploring the Role of Ceramides by Elisa Analysis: Orchestrating the Pathogenesis of Diabetes Mellitus and its Complications

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Abstract

The roles of adiponectin and ceramides, crucial molecules with differing impacts on the advancement of type 2 diabetes (T2D), are intricate and not entirely comprehended. It's important to highlight those ongoing investigations are essential to completely comprehend how they contribute to the development and progression of T2D. Therefore, the present study aimed to assess the role of ceramides and adiponectin in Type 2 diabetic conditions. The study included 20 patients with type 2 diabetes mellitus and 20 normal subjects. The study included healthy age-matched individuals as controls. Blood samples were collected from all the subjects and the concentration of Ceramides and adiponectin in plasma was determined by ELISA analysis. The results of the present study demonstrated considerably higher concentrations of Ceramides in type 2 diabetes patients in comparison to that of the healthy aged-matched control group. The adiponectin levels were found to be significantly ($p<0.05$) decreased in the T2D group than that of the normal healthy control group. The analysis showed that the markers, ceramides as well and adiponectin were significantly altered in type 2 diabetic conditions indicating an imbalance between these two molecules can significantly influence the development and progression of type 2 diabetes.

Keywords: *Adiponectin, Biomarkers, Ceramide, Diseases, Research, Type 2 Diabetes.*

Introduction

Diabetes mellitus is a chronic metabolic disorder that has been of concern since time immemorial. It is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. There are two main types of diabetes – Type 1 Diabetes and Type 2 Diabetes. The primary cause of T1D is insulin insufficiency, which is typically brought on by autoimmune mechanisms in people with certain genetic predispositions. A complicated interaction between genetic susceptibility, insulin resistance, and -cell dysfunction, among other contributing factors, is involved in the pathophysiology of T2D, in contrast [1]. Activation of pro-inflammatory and oxidative stress pathways is involved in the pathophysiology of both forms of diabetes as well as diabetic complications [2]. Diverse biomarkers have been studied for identifying patients with Type 2 diabetes mellitus at microvascular and macrovascular risk. Most of these markers are inflammatory, metabolic or procoagulant molecules, indicating poor

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metabolic and vascular health in patients with type 2 diabetes mellitus [3].

Ceramides and adiponectin are two biomarkers that have been implicated in the development and progression of diabetes mellitus. Ceramides are a class of lipids that are involved in a variety of cellular processes, including cell signalling, apoptosis, and inflammation. Several decades of research have established the involvement of various forms of ceramide in diabetes and its complications. Studies have shown that elevated levels of ceramides can impair insulin signalling, leading to glucose intolerance and hyperglycemia [4] [5]. Ceramides interfere with insulin signalling by preventing the phosphatidylinositol-3 kinase (PI3K) enzyme from transmitting signals and preventing the anabolic enzyme Akt/PKB from being activated. Ceramides further promote cell death by activating the enzymes cathepsin D, protein kinase C, caspase, and serine/threonine protein phosphatase (PP1) [6]. The emergence of ceramides as key mediators of the onset and progression of insulin resistance and diabetic complications provides unique opportunities to explore their potential use as therapeutic targets and disease biomarkers [7].

Similarly, Adiponectin is the most abundant peptide secreted by adipocytes, whose reduction plays a role in type 2 diabetes by reducing response to insulin action [8]. A study showed that increased concentration of circulating adiponectin in mice reduces ceramides in different tissues. It was found that adiponectin increases the activity of ceramidase and enhances ceramide catabolism to sphingosine [9]. Therefore, it is believed that adiponectin may become a way of treatment of insulin resistance and type 2 diabetes mellitus [8] [9]. Overall, the role of ceramides and adiponectin in diabetes mellitus is an area of active research, and further studies are needed to fully elucidate the complex mechanisms involved. This study aims to understand the alterations in levels of adiponectin and ceramides in type 2 diabetic patients.

Materials and Methods

Subjects

A total of forty (n = 20) patients with T2DM, fell under the criteria of the American Diabetes Association [10]. Attending the Outpatient Department of General Medicine, Saveetha Medical College & Hospitals, Chennai was taken for the study. All the T2DM patients >35 years of age were included in the study. All patients included were verbally informed and signed informed consent and all methods were performed according to the relevant guidelines and regulations.

Ethical Approval

This cross-sectional study received approval from the Institutional Review Board, and ethical clearance was obtained from the Ethics Committee of Saveetha Medical College & Hospitals, Chennai.

Inclusion Criteria

Age: 35-65, both sexes. All type 2 DM patients with a history of diabetes for the past 1 to 3 years with BMI≥25, Type 2 diabetes with fasting blood sugar of more than 126mg/dl and glycosylated haemoglobin (HbA1c) between 7.0 to 10.0%

Exclusion Criteria

Uncontrolled hypertension and diabetes, serum creatinine more than or equal to 2mg/dL, hepatic impaired patients, serum triglycerides ≥ 500mg/dl, pregnant and lactating females. Patients with inflammatory diseases like inflammatory bowel syndrome, pancreatitis and autoimmune disorders which could affect the serum levels of inflammatory markers were excluded from the study. In addition, patients with advanced diabetes, any genetic disorder, cancer and gestational diabetes were also excluded from the study.

Sample Size

The total sample size was 40. The sample size was calculated to detect an effect size of

0.40 at a type 1 error of 50% and power of 80% using “G Power v. 3.1.9.2”.

Sample Collection

5 ml of blood from both type 2 diabetes (T2DM) patients and healthy individuals was collected after an overnight fast of at least 10-12 hours. The collected blood was promptly transferred into heparinized tubes and centrifuged at 1,500 rpm for 10 minutes to separate the plasma, which was then divided into 2 ml microfuge tubes. The plasma samples were stored at a temperature of -20°C until they were ready for further analysis.

ELISA Analysis of Adiponectin and Ceramides

Quantitative measurement of adiponectin and total ceramides in plasma was done by sandwich enzyme-linked immunosorbent assay (ELISA) using Raybiotech Human adiponectin and total ceramides ELISA kits following manufacturer's protocol (Raybiotech, USA).

Statistical Analysis

The data collected were entered into Microsoft Excel and subjected to data cleaning procedures before analysis. Clinical and laboratory data were described using non-parametric methods. Continuous variables were presented as either means and standard deviations or medians. Paired t-tests were conducted to identify significant differences in paired variables. $P<0.05$ was considered significant. All statistical analyses were carried out using SPSS version 22.0.

Results

Estimation of Ceramide Levels

Ceramide is the centre labyrinth of the Sphingolipid metabolic pathway. Elevated plasma ceramide concentrations may serve to identify individuals who develop type 2 diabetes. In the present study, we have found considerably increased concentrations of plasma ceramides in type 2 diabetes whereas the healthy controls did not exhibit elevation.

Normally distributed continuous variables were described by Mean± SEM and student’s t-tests were performed. *p<0.05, **p<0.01, ***p<0.001 vs Control.

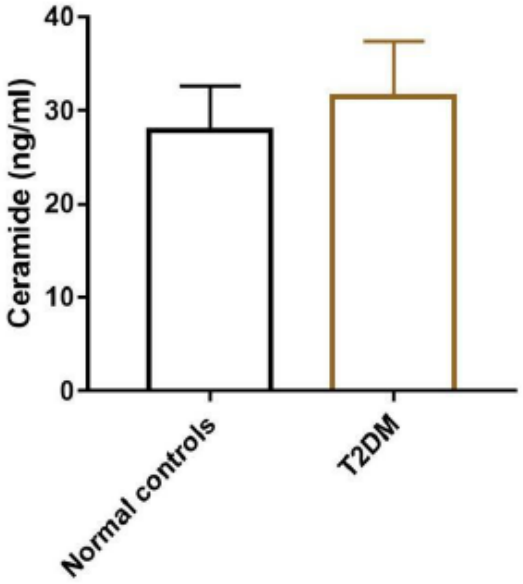

Figure 1. Representative Bar Graph showing Elevated Levels of Ceramide in Healthy Controls and Type 2 Diabetic Patients

Estimation of Adiponectin Levels

The study results showed a significant ($p<0.05$ by student t-test) decrease in the levels of adiponectin exhibited in the plasma of type 2 diabetic patients. The healthy controls demonstrated increased levels of adiponectin.

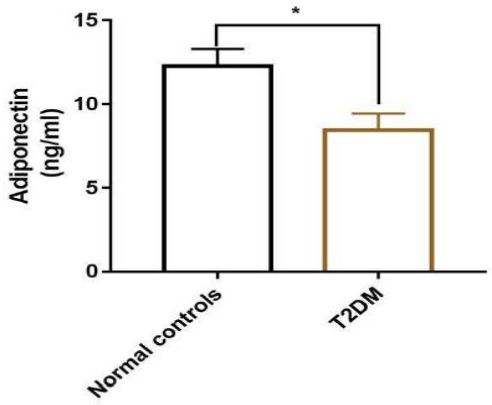

Figure 2. Representative Bar Graph Showing Decreased Levels of Adiponectin in Healthy Controls and Type 2 Diabetic Patients

Normally distributed continuous variables were described by Mean± SEM and student's t-tests were performed. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs Control

Discussion

In this study, we have evaluated the levels of adiponectin and ceramides in Type 2 diabetes conditions. We have observed higher ceramide levels and lower adiponectin levels in our results that were reported to be associated with the incidence of type 2 diabetes mellitus as per the review by Mandal et al. [1]. Our study focused on obese diabetic patients (BMI≥25) as obesity is a major risk factor for diabetes. A multicohort study of diabetic patients showed that dysfunctional visceral adiposity and insulin resistance are associated with increased plasma ceramide levels suggesting that alterations in ceramide levels could act as a DM biomarker [11].

The advances in sphingolipid and diabetes research over the past few years have given further support to establish ceramides to play a key role in the pathophysiology of diabetes and various mechanisms leading to diabetic complications in target tissues (heart, blood vessels, eyes, kidney, and peripheral nerves). For example, diabetic retinopathy, the leading cause of blindness, has been found to result from increased levels of sphingolipids, particularly ceramide [12]. Cardiovascular dysfunction is also a deleterious consequence of diabetes and cardiac death has also been correlated with increased ceramide levels [13] [14].

Adiponectin, the most abundant product secreted from adipocytes, lowers hepatic and muscle triglycerides [15]. And is associated with improved insulin sensitivity, decreased cardiometabolic risk, and decreased risk of progression from prediabetes to type 2 diabetes [16]. It is believed to modulate ceramide levels via the AdipoRI and AdipoR2 receptors, which help regulate ceramidase activity in tissues and help in lowering ceramide levels. A longitudinal cohort study showed that adiponectin levels could act as a biomarker for diabetes risk [17]. Furthermore, higher levels of adiponectin were found to be associated with a decreased risk of progression from normoglycemia to prediabetes in African American and European American offspring of parents with T2DM [18]. Besides improvements in insulin sensitivity, other mechanisms of adiponectin's pro-cardiometabolic profile include anti-inflammatory effects, decreased apoptosis, preservation of β-cell function, and reversal of

lipotoxicity. These numerous mechanisms appear to be unified by interactions among adiponectin, fibroblast growth factor 21 and ceramides [18,19]. Based on our data by comparing healthy controls and type 2 diabetic individuals it is quite evident that there are alterations in the levels of ceramides and adiponectin in diabetic individuals which may be a significant contributing factor to the progression of T2DM.

Conclusion

With the given knowledge from our study and previous reports about the potential of ceramides and adiponectin, the changes in sphingolipid metabolism and their role in regulation in diabetes we conclude that they may indeed contribute to the pathology of diabetes. Therefore, further studies may ultimately prove ceramides and adiponectin as a useful target for therapeutics and its study may provide insights into the complexity behind insulin resistance. Such an understanding undoubtedly will have a direct impact on future therapies for diabetes.

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Conflict of Interest

The authors declare no conflict of interest.

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