

Relationship Between Sialic Acid and Microvascular Complications in Type 2 Diabetes Mellitus

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ABSTRACT

Serum total sialic acid is a marker of acute phase response. Elevated levels have also been associated with several risk factors for diabetic vascular disease. **Objective:** To study relationship between sialic acid and metabolic variables in type 2 diabetic patients with and without microvascular complications. **Material and Methods:** This study included 200 subjects of which 50 were of diabetes mellitus with nephropathy, 50 patients of type 2 diabetes mellitus with retinopathy, 50 patients of type 2 diabetes without any complication and 50 healthy individuals without diabetes mellitus. (control subjects). The subjects aged 15-60 years were selected for study. Fasting venous blood samples were taken from all these 200 subjects. Simultaneously urine sample were also collected from each of them. All blood samples were analysed for fasting and postprandial glucose, total cholesterol, triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), creatinine, HbA1c on fully automated analyser. Serum and urine sialic acid along with microalbumin levels were also estimated. **Results:** Serum total sialic acid concentrations were significantly higher among all diabetic subjects with or without complications compared to control subjects. In diabetics patients there was a significantly increasing trend of serum & urinary sialic acid with severity of nephropathy ($P < 0.001$) and with degree of urinary albumin excretion ($p < 0.001$). Elevated serum sialic acid concentrations were also associated with several risk factors for diabetic vascular disease: diabetes duration, HbA1c, serum triglyceride, serum cholesterol, HDL and LDL concentrations. Significant correlations were found between sialic acid concentration and cardiovascular risk factors like LDL & TG in diabetic patients. **Conclusion:** The main finding of this study is that elevated serum and urinary sialic acid and microalbumin concentration were strongly related to the presence of microvascular complications like diabetic nephropathy and retinopathy and cardiovascular risk factors in type 2 diabetes patients.

Key words : Sialic acid, type 2 diabetes mellitus, cardio vascular risk factors, retinopathy, nephropathy.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by elevation of blood glucose concentration and is associated with increased prevalence of microvascular complications. These complications include diabetic retinopathy, nephropathy and peripheral neuropathy.

The development and severity of these complications are dependent on the duration of the disease and how well it is managed. It has been proposed that inflammatory process seems to play an important role in the development of diabetes and its late complications¹. Prospective studies have reported associations among various markers of inflammation and incidence of diabetes^{2,3}. Diabetes

is another risk factor for myocardial infarction and stroke^{4,5}. The relationship between diabetes and other traditional cardiovascular risk factors, *e.g.*, an adverse lipid profile, obesity, hypertension and physical inactivity explain the increased risk in diabetic individuals⁶. Even though it has been suggested that inflammation contributes to the increased incidence of cardiovascular diseases among diabetic subjects¹. Serum sialic acid is one of the markers for acute phase response⁷. Sialic acid is a terminal component of the non-reducing end of carbohydrate chains of glycoproteins and glycolipids⁸. Elevated total serum sialic acid (SA) concentration is a risk factor for cardiovascular mortality in humans^{9,10}. Increased total serum sialic acid leads to increased excretion of sialic acid in urine of the patient presented with high urinary microalbumin¹¹. It has been reported earlier that total serum sialic acid concentration increase in type 2 diabetes mellitus^{12,13}.

The aim of this study was to measure serum and urine sialic acid and their relation with urinary microalbumin, serum cholesterol, TG, LDL cholesterol in diabetic subjects with and without microvascular complications. Microalbumin is a risk factor for cardiovascular disease; it may be associated with chronic inflammation and investigated the relationship of urinary albumin excretion and urinary sialic acid.

MATERIALS AND METHODS

We investigated the relationship of sialic acid concentrations with serum lipids, and urinary albumin excretion in type 2 diabetic patients. The study includes 200 subjects (male and female) including 50 healthy individuals as control. The diabetic subjects were divided into three groups according to their level of different complications. Group A-50 patients with diabetes mellitus (DM) and nephropathy, Group B-50 patients with type 2 diabetes and retinopathy and Group- C-50 patients with type 2 diabetes without any complication. The subjects aged 15–60 years with type 2 diabetes were selected for the study as this type of diabetes mellitus is prevalent in this age group. The estimation of serum and urine sialic acid may prove to predictive and preventive of microvascular

diseases and their complications in people with type 2 diabetes. All the subjects were reported fasting in the morning after 10–12 hr overnight fast. Venous blood samples collected without the use of tourniquet from each of the patients were analyzed for total serum cholesterol, TG, LDL, HDL, fasting and postprandial glucose on fully automated analyzer (Hitachi 912 analyzer, Roche, Switzerland) with the reagents supplied by Roche. The HbA1c is estimated with the principle based on affinity chromatography technique. Serum and urinary sialic acid was measured by a colorimetric assay using standard chemicals and reagents. In this method a protein precipitate of serum containing sialic acid will react with diphenylamine producing a purple color, which is quantitatively measured on a spectrophotometer at 540 nm.

The fresh urine samples collected from the test and control group subjects were used for microalbumin estimation in an electrochemiluminescence analyzer (Roche, Switzerland).

Statistical method

Results were expressed as mean±S.D. except where otherwise stated. Data were analyzed using the statistical package for social science, SPSS and $P < 0.05$ was taken as the cut off level for significance. Because the distribution of most variables was not symmetric. We used non parametric statistical methods. Chi square tests was be used to examine, type 2 diabetes mellitus, the various clinical and biochemical markers.

RESULTS

Table 1 shows the relationship between serum sialic acid, urine sialic acid and microalbumin concentrations with metabolic variables in diabetic subjects with and without microvascular complications. The table depicts significant increase of serum sialic acid (<0.001) among the diabetic subjects compared to the control subjects. Furthermore, in the diabetic subjects urine sialic acid and microalbumin were significantly higher (<0.001). Table 1 also shows the association of sialic acid and several risk factors for diabetic vascular disease; diabetes duration, serum TG and cholesterol concentration. It is observed that the

Table 1: Serum and urinary sialic acid and microalbumin levels in Type 2 diabetes with nephropathy and retinopathy.

Parameters	Diabetes without any complications	Diabetic nephropathy	Diabetic retinopathy	Non-diabetic subjects	P value
Serum sialic acid (mg%)	55.05 ± 2.9*	85.05±2.7	75.05±2.5	46±2.08	<0.001
Urine sialic acid (mg%)	6.02 ± 2.58**	13.06±1.58	11.03±1.78	3.2±0.65	<0.001
Microalbumin(mg%)	8.2 ± 3.24	132.20±35.24	102.2±29.24	7.67±3.28	<0.001
FBS(mg%)	140.02 ±70.08	155.60±50.7	150.6±49.9	90.02±80.08	<0.01
PPBS(mg%)	150.02± 102.10	207.30±57.6	200.1±57.6	120.02±102.10	<0.01
HbA1c(%)	9.10±5.20	11.10±2.3	10.1±2.5	6.10±5.20	<0.05
Triglyceride(mg%)	122.04±75.01	178.02±78.01	180.04±75.01	120.04±76.01	<0.05
Cholesterol(mg%)	148.04±12.01	256.03±134.01	246.03±130.01	140.04±119.01	NS
HDL(mg%)	35.01±20.04	38.01±26.02	35.01±20.04	36.01±19.04	NS
LDL(mg%)	90.00±76.06	165.00±97.01	160.00±95.01	87.00±76.05	<0.05
Creatinine(mg%)	2.00±1.6.06	10.05±2.03	10.03±2.01	1.40±1.20	<0.001
Urine creatinine(mg%)	145.00±102.6	155.03±65.02	150.03±66.01	146.00±113.06	<0.05

*** p < 0.001, NS = not significant, n = 50

sialic acid values were statistically significantly higher with increasing urinary albumin excretion ($p < 0.001$). Similarly HbA1c, FBS, PPBS, TG and cholesterol showed marked increase in patients with elevated level of microalbumin and urine sialic acid when compared to normal subjects without any complications.

DISCUSSION

In recent years, much attention has been given to the relationships among adiposity, inflammation, and diabetes. High inflammation sensitive plasma protein levels increased the cardiovascular risk slightly more in diabetic. Studies of diabetic subjects have reported increased incidences of cardiovascular diseases or increased diabetes complications among subjects with high fibrinogen¹³ and other markers of inflammation^{14,15}. Measurement of inflammation sensitive markers may be useful for assessment of the cardiovascular risk in diabetic patients. Results from prospective studies suggest that inflammation involved in the pathogenesis of diabetes¹⁶ and atherosclerosis¹⁷. Inflammation could be a common antecedent for both diabetes and cardiovascular disease. Hyperglycemia and insulin resistance could also promote inflammation, and may be factor linking diabetes to the development of atherosclerosis. Elevated glucose levels could promote inflammation

by increased oxidative stress¹⁸. Yet another possibility is that the inflammatory response is a result of vascular complications following diabetes. In type-2 diabetes, the circulating sialic acid concentration is elevated in comparison with non-diabetic subjects¹⁹. The results of our study showed serum and urine SA concentration increased in diabetic patients as compared to the general population, especially in type-2 diabetic patients with either microalbuminuria or albuminuria. Furthermore, the serum and urine sialic acid levels were independent of the duration of diabetes mellitus and degree of metabolic control (as estimated by HbA1c). Also, a good correlation was observed between sialic acid and important cardiovascular risk factors such as cholesterol, LDL and TG. It has been reported that serum sialic acid levels are increased in type1 DM patients with albuminuria²⁰. Several authors found the increased urinary concentration of sialic acid in type 2 diabetes with microangiopathy. The vascular permeability is regulated by sialic acid moieties, with increased vascular permeability resulting from the shedding of vascular endothelial sialic acid into the circulation. It is well established that vascular endothelium carries a high level of sialic acid²¹, and the vascular damage leads to its release into the circulation. A relationship between serum sialic acid levels and microvascular complications has been observed before for microalbuminuria and clinical proteinuria in type 1^{22,24} and type 2 diabetes²³.

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