

## 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. The objective of the study was to find out a relationship between sialic acid and metabolic variables in type 2 diabetic patients with and without microvascular complications.*

*Materials and Methods: This study included 200 subjects of whom 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 the 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 diabetic patients there was a significantly increasing trend of serum and urinary sialic acid with severity of nephropathy ( $p < 0.001$ ) and with the degree of urinary albumin excretion ( $p < 0.001$ ). Elevated serum sialic acid concentrations were also associated with several risk factors.*

*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 diabetic patients.*

*Key words: Sialic acid, type 2 diabetes mellitus, cardiovascular risk factors, retinopathy, nephropathy.*

### INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterised 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 complications.<sup>1</sup> Prospective studies have reported associations among various markers of inflammation and incidence of diabetes.<sup>2,3</sup> Diabetes is a risk factor for myocardial infarction and stroke.<sup>4,5</sup> 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 individuals.<sup>6</sup> Even though it has been suggested that inflammation contributes to the increased incidence of cardiovascular diseases among diabetic subjects.<sup>1</sup> Serum sialic acid is one of the markers for acute phase response.<sup>7</sup> Sialic acid is a terminal component of the non-reducing end of carbohydrate chains of glycoproteins and glycolipids.<sup>8</sup> Elevated total serum sialic acid concentration is a risk factor for cardiovascular mortality in humans.<sup>9,10</sup> Increased total serum sialic acid leads to increased excretion of sialic acid in urine of the patient presented with high urinary microalbumin.<sup>11</sup> It has been reported earlier that total serum sialic acid concentration increase in type 2 diabetes mellitus.<sup>12,13</sup>

The aim of this study was to measure serum and urine sialic acid and their relation with urinary microalbumin.

**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	120.2 ± 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

Mean ± SD \*\*\* p < 0.001, NS = not significant, n = 50

## 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 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 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 analysed for total serum cholesterol, TG, LDL, HDL, fasting and postprandial glucose on fully automated analyser (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 pro-

tein precipitate of serum containing sialic acid will react with diphenylamine producing a purple colour, which is quantitatively measured on a spectrophotometer at 540 nm.

The fresh urine samples collected from the test and control groups 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 analysed using the statistical package for social science, SPSS and p-value < 0.05 was taken as the cut off level for significance. As the distribution of most variables was not symmetric. We used non-parametric statistical methods. Chi-square tests.

## RESULTS

The table 1 shows the relationship between serum, urine sialic acid, and microalbumin concentrations with metabolic variables in diabetic subjects with and without microvascular complications. The table depicts or 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). The table 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 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 paid 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 diabetic complications among subjects with high fibrinogen<sup>13</sup> and other markers of inflammation.<sup>14,15</sup> 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 diabetes<sup>16</sup> and atherosclerosis.<sup>17</sup> Inflammation could be a common antecedent for both diabetes and cardiovascular disease. Hyperglycaemia 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 stress.<sup>18</sup> 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 subjects.<sup>19</sup> The results of our study showed serum and urine sialic acid 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). In addition, 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 type 1 DM complications has been observed before for microalbuminuria and clinical proteinuria in type 1<sup>22,24</sup> and type 2 diabetes.<sup>23</sup>

It is *Concluded* 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 diabetic patients.

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