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## Correlation Between Frequent Risk Factors of Diabetic Nephropathy and Serum Sialic Acid

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**Abstract:** It is recently established that serum sialic acid is a potent cardiovascular and renal risk factor in general population and also found elevated in diabetic type 2 patients. This study was designed to assess the coexistence of frequently documented risk factors of diabetic nephropathy with serum sialic acid. A total of 100 diabetic patients (50 with and 50 without nephropathy) aged  $47.56 \pm 10.68$  (mean  $\pm$  SD) year attending several diabetic clinics of private sector in Karachi were included after informed consent was obtained. Systolic and diastolic blood pressures were recorded by standard mercury sphygmomanometer. Fasting blood samples were collected for the estimations of blood glucose, HbA1c, serum urea, creatinine and sialic acid levels. Serum sialic acid, glucose, HbA1c, urea and creatinine levels were increased significantly ( $p < 0.01$ ) in diabetic nephropathy patients as compared to diabetic patients without nephropathy. Regression and correlation analysis showed a significant positive correlation between serum sialic acid and fasting blood glucose, HbA1c, serum urea and creatinine levels. BMI and blood pressures were also significantly high in diabetic patients with nephropathy as compared to those without nephropathy. Fifty four percent diabetic nephropathy patients were found to be smokers where as 41% were smokers in diabetic patients without nephropathy. It is concluded that the elevated serum sialic acid level is strongly associated with the presence of microvascular complications of diabetes, nephropathy for example. There is a significant unvaried link between serum sialic acid and diabetic nephropathy. The consideration of sialic acid as potent disease marker for diabetic nephropathy is therefore justified.

**Key words:** Serum sialic acid, diabetic nephropathy, risk factors

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### Introduction

The serum or plasma Sialic acid (N-acetyl neuraminic acid), an inflammatory marker, has recently been shown to be a strong predictor of cardiovascular mortality (Crook *et al.*, 2001; Linderberg *et al.*, 1991). Several general population studies and those carried out in diabetic patients with complications have pointed to serum sialic acid as a marker of inflammation in cardiovascular disease (Gavella *et al.*, 2003; Crook *et al.*, 2002). Sialic acid is basically released from terminal oligosaccharide chain of some glycoproteins and glycolipids of the acute phase (Spunda *et al.*, 1996).

Diabetes mellitus is a chronic metabolic disorder that can lead to serious cardiovascular, renal, neurologic and retinal complications (Khawaja *et al.*, 2004; Shera *et al.*, 2004). Diabetic nephropathy affects more than 30% of type 1 diabetic patients and mainly due to renal failure among type 2 diabetic patients, is the leading cause of end stage renal disease (Jeremy, 2003). A number of disease markers have previously been associated with the metabolic syndrome including hypertension, poor glycemic control, central obesity, smoking, dyslipidemia and glycation end products (UKPDS, 2000). Serum

sialic acid is newly established as a potent risk factor for the development of macro and microvascular complications of diabetes. The current study was designed to investigate the significance of serum sialic acid as a major risk factor in development of diabetic nephropathy and to observe the clinical relationship of serum sialic acid with the other strong disease markers in diabetic nephropathy.

## Materials and Methods

A total of 100 previously diagnosed type 2 diabetic patients (50 with and 50 without nephropathy) according to WHO's criteria (WHO, 1985) attending several diabetic clinics of private sector in Karachi were included in the presented study after informed consent was obtained. Their mean age was 47.56±10.68. However, patients suffering from type 1 diabetes mellitus, gestational diabetes and any known mental illness, macro vascular disease prior to diagnosis of type 2 diabetes or the patients who refused to participate in the study were excluded. Fifty healthy subjects with no known history of hyperglycemia and renal insufficiency were included as control. The study was conducted during 2004-05.

A previously structured questionnaire was used to record the demographic features of all subjects. Height and weight were noted for BMI (BMI = weight in kg/height in m<sup>2</sup>). BMI from 20.0 to 29.9 was considered non-obese over weight and >30.0 was considered as obese. Blood pressure was measured with standard mercury sphygmomanometer while the patient was sitting after resting for 10 min. Hypertension was defined as the blood pressure ≥ 140/90 mmHg (Bakris *et al.*, 2000). Patients were classified as smokers if they smoked more than one cigarette a day.

Fasting blood samples were collected in Lithium-Heparin coated tubes after no medication were taken for last 12 h or long. Serum sialic acid was estimated by Ehrlich's method (Crook, 1993). HbA1c was estimated by Fast Ion-Exchange Resin Separation Method (Human Gessellschaft fur Biochemica und Diagnostica mbH, Germany). Fasting blood glucose was measured by O-Toluidine method (Dubowski *et al.*, 1962). Serum urea was estimated by Thiosemicarbazide-Diacetyl monoxime method (Mather and Roland, 1969). Serum creatinine was measured by modified Jaffe's method (Spierto *et al.*, 1979).

Results are presented as mean±SD. Statistical significance and difference from control and test values were evaluated by Student's t-test. Correlation coefficient and regression analysis were used to describe the effects of elevated serum sialic acid levels on glucose, HbA1c, urea, creatinine, SBP, DBP and BMI. Scattergram was plotted by taking dependent variables on y-axis and independent variable (Sialic acid) on x-axis.

## Results

Serum sialic acid, blood glucose and HbA1c were increased significantly (p<0.01) in both diabetic and diabetic nephropathy patients as compared to control subjects. The elevation was found to be high in diabetic nephropathy patients as compared to those without nephropathy (Table 1).

Serum urea and creatinine levels were increased significantly (p<0.01) in patients with diabetic nephropathy as compared to control subjects. No significant elevation in serum urea and creatinine levels was observed in diabetic patients as compared to control subjects (Table 1).

Table 1: Biochemical parameters in diabetic patients with and with out nephropathy as compared to control

Parameters	Control	Diabetic patients	Diabetic nephropathy patients
Serum sialic acid (mmol L <sup>-1</sup> )	1.72±0.36	1.95±1.25*	2.21±0.37*
Blood glucose (mmol L <sup>-1</sup> )	5.4±0.45	9.0±3.9*	11.86±3.36*
HbA1c (%)	4.59±1.38	7.29±1.99*	9.31±1.87*
Serum urea (mmol L <sup>-1</sup> )	10.58±2.63	10.69±1.93	19.41±4.9*
Serum creatinine (µmol L <sup>-1</sup> )	107.67±48.81	112.83±38.43	198.25±29.85*

Values are Mean±SD, \* p<0.01 as compared to control subjects

Table 2: Physical parameters in diabetic patients with and with out nephropathy as compared to control

Parameters	Control	Diabetic patients	Diabetic nephropathy patients
Systolic BP (mmHg)	123.77±8.13	125.33±6.33	149.03±16.74*
Diastolic BP (mmHg)	79.37±6.79	78.73±4.11	92.6±7.99*
BMI (kg m <sup>-2</sup> )	21.35±2.56	27.18±2.59*	36.65±2.15*
Smoking (%)	00	41	54

Values are Mean±SD, \* p<0.01 as compared to control subjects

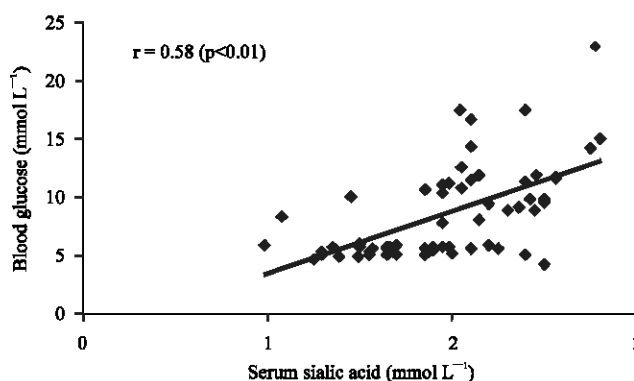


Fig. 1: Correlation between serum sialic acid and blood glucose in diabetic patients with nephropathy

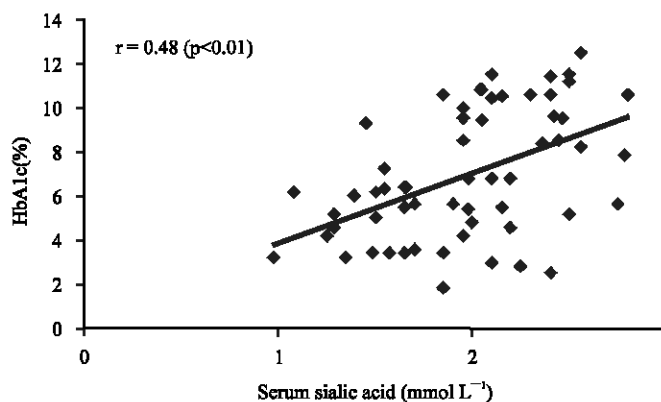


Fig. 2: Correlation between serum sialic acid and HbA1c in diabetic patients with nephropathy

Systolic and diastolic blood pressures were raised significantly ( $p < 0.01$ ) in patients with diabetic nephropathy where as no significant change was observed in diabetic patients as compared to control subjects (Table 2). BMI was significantly ( $p < 0.01$ ) increased in both diabetic nephropathy patients and diabetic patients without nephropathy as compared to control subjects (Table 2).

Regression and correlation analysis showed a significant positive correlation between serum sialic acid and blood glucose ( $r = 0.58$ ,  $p < 0.01$ ), HbA1c ( $r = 0.48$ ,  $p < 0.01$ ), serum urea ( $r = 0.37$ ,  $p < 0.05$ ) and serum creatinine ( $r = 0.34$ ,  $p < 0.05$ ) in diabetic nephropathy patients (Fig. 1-4). Systolic ( $r = 0.36$ ,  $p < 0.05$ ) and diastolic ( $r = 0.31$ ,  $p < 0.05$ ) blood pressures were also positively correlated with serum sialic acid in diabetic nephropathy patients (Fig. 5 and 6). A significant positive correlation was also observed between serum sialic acid and BMI ( $r = 0.52$ ,  $p < 0.01$ ) (Fig. 7).

No significant correlation between serum sialic acid and blood glucose, HbA1c, serum urea, serum creatinine, systolic and diastolic blood pressure and BMI was observed in diabetic patients without nephropathy (Table 3).

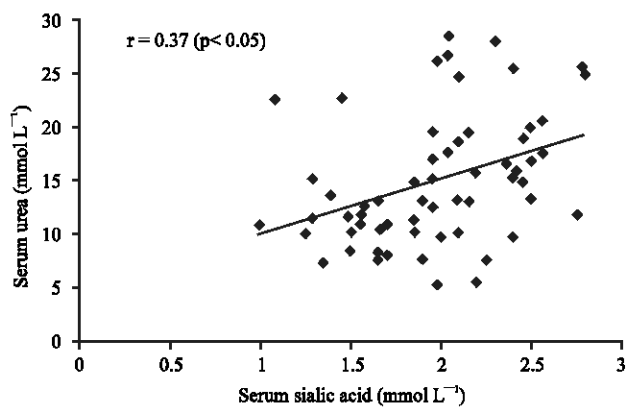


Fig. 3: Correlation between serum sialic acid and serum urea in diabetic patients with nephropathy

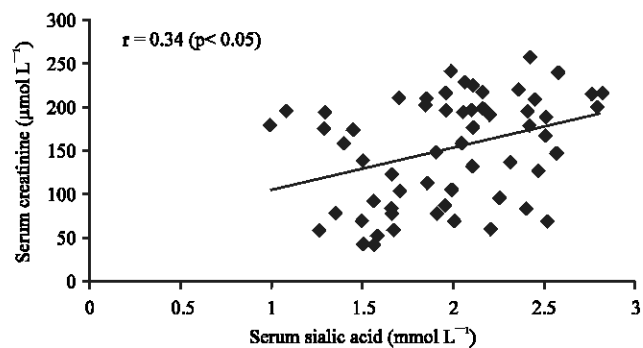


Fig. 4: Correlation between serum sialic acid and serum creatinine in diabetic patients with nephropathy

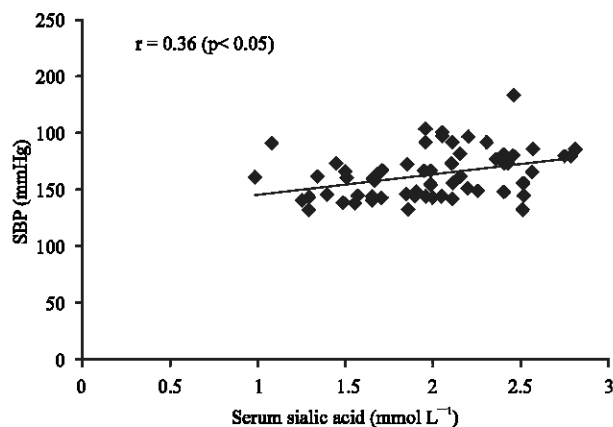


Fig. 5: Correlation between serum sialic acid and SBP in diabetic patients with nephropathy

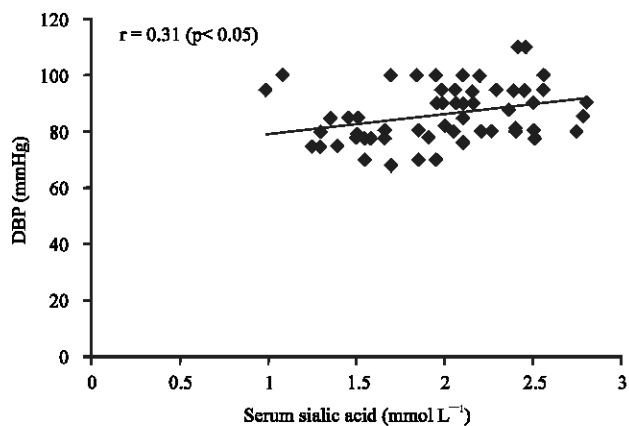


Fig. 6: Correlation between serum sialic acid and DBP in diabetic patients with nephropathy

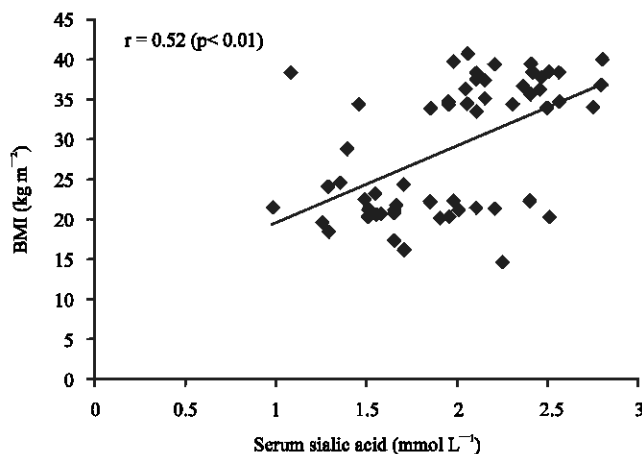


Fig. 7: Correlation between serum sialic acid and BMI in diabetic patients with nephropathy

Table 3: Correlation between serum sialic acid and frequent risk factors in diabetes

	r =	p > value
Blood glucose	0.22	Non significant
HbA1c	0.28	Non significant
Serum urea	0.09	Non significant
Serum creatinine	0.15	Non significant
SBP	0.03	Non significant
DBP	0.20	Non significant
BMI	0.25	Non significant

## Discussion

The present study demonstrates the increasing trends of serum sialic acid in diabetic patients with the progression of complications such as nephropathy. We also found that serum sialic acid concentration was significantly associated with several known risk factors for the development of diabetic micro and macrovascular complications i.e. glycemic control (HbA1c), renal dysfunction (urea and creatinine), hypertension and smoking. The results are completely in accordance with the

recent studies in same area (Crook *et al.*, 2000; Gavella *et al.*, 2003). A relationship between serum sialic acid and microvascular complication has been observed before in small scale studies for type 1 and type 2 diabetes (Chen *et al.*, 1996; Powerie *et al.*, 1996).

Serum sialic acid is a marker of acute phase response (Pickup *et al.*, 1997). Acute phase glycoproteins with sialic acid as a component of the oligosaccharide side chain being produced by liver, stimulated by proinflammatory cytokines. Therefore the two most likely explanations for the present findings are either or both of the following:

- Tissue injury caused by diabetic vascular complications stimulates local cytokine secretion from cells involved in the complications such as endothelium and macrophages, which are known to be the major sources of cytokine productions (Boumann and Gauldie, 1994) and this induces an acute phase response.
- The diabetic process stimulates cytokine production from cells throughout the body and these cytokines play a direct role in the causation of vascular complication. The latter is supported by evidence that proinflammatory cytokines cause endothelial dysfunction by increasing capillary permeability, inducing prothrombotic properties and promoting leukocyte recruitment by synthesis of adhesion molecules and chemoattractants (Mantovani and Bussolino, 1997).

The realization that microalbuminuria is a non specific marker of inflammation in the general population further suggests that cytokinemia from a variety of causes leads to microvascular abnormalities. The need for early predictors of diabetic vascular complications such as nephropathy has recently been reviewed (Caramori *et al.*, 2000). Some patients with microalbuminuria have quite advanced renal structure changes and microalbuminuria may here be a marker of microvascular damage that has already been occurred (Yokoyama *et al.*, 1996). If circulating sialic acid increases before microangiopathy develops, it may be an early signal of processes such as hypercytokinemia that cause or drastically increase the risk of renal failure.

In conclusion, present suggests that increased serum sialic acid levels are strongly associated with the development of diabetic nephropathy. The markers of glycemic control (blood sugar and HbA1c) and renal insufficiency (serum urea and creatinine), hypertension and obesity are clinically correlated with the increasing concentrations of sialic acid. These findings strengthen the hypothesis that increase in circulating serum sialic acid is an early manifestation of the diabetic renal disease. Further research would be of help to clarify the temporality and pathophysiologic explanation of the associations found.

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