

## Role of sialic acid in prediction of diabetic nephropathy

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**Abstract:** *Background:* Diabetic nephropathy is a major microvascular complication of diabetes mellitus and the most common cause of end stage renal disease worldwide. It has been proposed that inflammatory process seems to play an important role in the development of diabetes and its late complications. Serum sialic acid, an acute phase reactant and acute phase reactants are considered as the indicators of microvascular angiopathy. Microalbuminuria is a predictor of incipient nephropathy in diabetic patients. *Aim:* The study was undertaken to evaluate serum sialic acid and microalbuminuria levels and to assess the correlation of serum sialic acid and microalbuminuria in diabetic nephropathy patients. *Method:* The diabetic subjects were divided into two groups. Group I – 120 patients with diabetes mellitus with early stage of nephropathy based on urinary albumin excretion per day and Group II – 120 newly diagnosed or known diabetics on treatment but without any complication. The age and sex matched 120 controls were selected from the healthy persons from same region. Both the cases and controls were selected by a simple random method. Blood samples were analyzed for fasting and post prandial blood sugar, blood urea, serum creatinine, serum sialic acid, glycated hemoglobin (HbA1c) and urine sample for microalbumin levels and systolic, diastolic blood pressure was recorded in both cases and controls. *Result:* Serum sialic acid levels were found to be significantly increased in diabetes with or without nephropathy compared to controls. Urinary microalbumin in diabetic nephropathy patients was significantly higher compared with diabetics and healthy individuals. In diabetes without any complications, serum sialic acid level had no statistically significant positive correlation with serum urea, creatinine and urinary microalbumin but in diabetes with nephropathy, serum sialic acid level statistically was well correlated with serum urea, creatinine and urinary microalbumin. *Conclusion:* Elevated serum sialic acid and urinary microalbumin levels are strongly associated with the presence of nephropathy. Serum sialic acid may be a predictor of renal dysfunction in diabetic nephropathy.

**Keywords:** Diabetic nephropathy, Microalbuminuria, Sialic acid.

### Introduction

Diabetes mellitus is a metabolic disorders with hyperglycemia either due to absolute or relative deficiency of insulin secretion or reduction in the biologic effectiveness of insulin or both [1]. This causes decreased uptake of glucose into muscle and adipose tissue and it leads to chronic extra cellular hyperglycemia which results in significant long-term sequelae, particularly damage and/or dysfunction and failure of various organs throughout the body, [2-3] especially the kidneys, eyes, nerves, heart and blood vessels. In India alone, diabetes is expected to increase from 40.6 million in 2006 to 79.4 million by 2030 [4].

Diabetic nephropathy is a major microvascular complication of diabetes mellitus and the most common cause of end stage renal disease worldwide. It remains a major cause of morbidity

and mortality in persons with diabetes mellitus. Diabetic nephropathy is characterized by a progressive increase in the excretion of protein, particularly albumin, an early and continuing rise in blood pressure and a late decline in glomerular filtration rate (GFR) leading eventually to End Stage Renal Disease (ESRD) [5].

Microalbuminuria is an important risk factor for cardiovascular disease and progressive renal impairment. Microalbuminuria arises from the increased passage of albumin through the glomerular filtration barrier which results from ultra-structural changes [6]. Increase in urine albumin seen with diabetic nephropathy can be attributed to degradation of the glomerular basement membranes and hypertension, both characteristic of diabetic nephropathy. It is estimated that death due to

renal disease is 17 times more common in diabetics than in non diabetics [5]. Importantly, microalbuminuria in the diabetics can be interpreted as an early sign of renal involvement in diabetes [7]. Once overt nephropathy is present, progression cannot be halted, only slowed. It is more effective to screen for early nephropathy with sensitive tests for microalbuminuria and to prevent the earliest stages of damage by vigorous control of hyperglycemia and hypertension.

It has been proposed that elevated glucose levels promotes inflammatory process that seems to play an important role in the development of diabetes and its late complications [8]. In diabetes, acute phase reactants are considered as the indicators of microvascular angiopathy. Sialic acid is a component of cell membranes [9] and elevated levels may indicate excessive cell membrane damage, but more specifically to the cells of vascular tissue. If there is damage to vascular tissue, this leads to ischaemia and this ischaemia is most visible in the smallest blood vessels, including those of the retina of the eyes, kidneys, heart and brain.

It is this ischaemia that leads to conditions including, but not limited to retinopathy, nephropathy and neuropathy. In addition, serum sialic acid is one of the markers for inflammation [10] and sialic acid can be used as a measurement of the acute phase response because many of the proteins of the immune response are actually glycoproteins and these glycoproteins have sialic acid as the terminal sugar on their oligosaccharide chain [11].

The present study was conducted to investigate any correlation between serum inflammatory marker and urinary microalbumin in patients suffering from diabetic nephropathy. If there is any correlation risk among diabetics, this would allow medical practitioners to better manage their diabetic patients with regards to halt the renal complications in early stage and improve not only the life expectancy, but the quality of life of these patients.

### Material and Methods

*Study area:* This hospital based cross-sectional study <sup>8</sup>(4) was conducted in the Diabetic and Nephrology clinics with the collaboration of

Department of Biochemistry of Burdwan Medical College, Burdwan, West Bengal, India.

*Ethics Statement:* The study was approved and permitted by the institutional ethics committee for care and use of laboratory and started after obtaining the written consent from the concerned ethics committee [Memo No.BMC/2179/1 (16)].

*Selection of subjects:* The diabetic subjects were divided into two groups. Group I – 120 patients with diabetes mellitus with early stage of nephropathy based on urinary albumin excretion per day and Group II – 120 newly diagnosed or known diabetics on treatment but without any complication. Diagnosis of diabetes mellitus was made according to American Diabetic Association (ADA) [12]. The selected patients were from Burdwan district and adjoining areas.

The study was conducted between March 2012 and April 2015. The age and sex matched 120 controls were selected from the healthy persons from same region. Both the cases and controls were selected by a simple random method. Every patient was informed about the details of the study through individual interviews and all the provided written informed consent. Subjects with history of cardiac diseases, smoking, alcohol intake, pregnancy, malignancy or any acute and chronic inflammatory disorders are excluded from the study group. Patients suffering from metabolic conditions like ketoacidosis, cerebrovascular accidents, preeclamptic patients, pre-existing chronic kidney disease, chronic renal failure, chronic glomerulonephritis, nephrotic syndrome, and primary hypertensives were excluded from the study.

*Collection of samples:* All the subjects were reported fasting in the morning after 10–12 hr overnight fast. Peripheral venous blood was drawn under aseptic precautions from all participants and the samples were divided into three aliquots. The first one was collected in oxalate and fluoride vial for obtaining plasma for fasting as well as postprandial (two hours after meal) glucose estimation on the same

day, second one in EDTA containing vial for HbA<sub>1C</sub> assays and blood collected in plain vacutainer was processed to obtain serum. All serum samples were stored at (-70°C) and kept under these conditions until chemical analysis was performed. 24 hours urine sample was collected under aseptic precautions for the estimation of urinary microalbumin. All parameter assays should be done as soon as possible.

*Parameters assay:* Plasma glucose level was estimated by glucose oxidase-peroxidase enzymatic method using span diagnostic kit as per the manufacturer's instructions [13] by completely automated clinical chemistry analyzers – ERBA XL-600 after usual daily calibration and ensuring quality performance before starting analysis and the samples were analyzed along with the other routine samples. Intraassay CV% was 1.2% and interassay CV% was 2.1%. Using commercially available Hemoglobin A<sub>1C</sub> kit supplied by Siemens Company did Hemoglobin A1C test. It implies the principle of turbidimetric inhibition immunoassay (TINIA) [14-15]. This company also supplied total Hb kit for estimation total Hb by alkaline hematin method.

Serum sialic acid were measured by a spectrophotometric assay [16]. 150 µl serum was mixed with 3.6 ml of 5% trichloroacetic acid (TCA) and the tubes were covered with marbles and kept in a boiling water bath for 15 minutes. The tubes were cooled and centrifuged for 10 minutes at 2000Xg. 1ml of supernatant was mixed with 2ml each of acid reagent and diphenylamine reagent. Standard and blank samples were treated in the same way. The contents were mixed using a vertex mixer, covered with marbles and placed in a boiling water bath for 30 minutes. Development of a purple color was measured on a dual beam spectrophotometer (UV5704SS) at 540 nm. Intra-assay and inter-assay CV% were 2.9 and 4.4 respectively.

Serum creatinine was estimated by modified Jaffe's method [17] and Blood urea (Glutamate dehydrogenase-Urease method) in auto analyser (Transasia, XL- 600) using commercially available kit. Intra-assay CV% was 1.8 and inter-assay CV% was 2.9. Urine microalbumin was measured by immunoturbidimetric method using goat anti-human albumin antiserum and human albumin standards on a timed 24-h urine collection [18-19] using semiautoanalyser (Chem 5v2 plus) after exclusion of urinary tract infection. Intra assay CV% was 2.3 and intra assay CV was 3.1 for this method. Microalbuminuria was defined as a urinary albumin excretion of 20–200 mg/day.

Both diastolic and systolic blood pressure was measured according to the standard procedure [20] and the mean of two readings was used for analysis.. Hypertension was defined as a blood pressure >140/90 mmHg or if the subject was taking antihypertensive medication. Weight and height measurements were obtained, using the Rosscraft Tom Kit Anthropometric Instrument Set, Canada [21]. BMI was calculated as the weight in kilograms divided by the square of height in meters. Body mass index (BMI) was calculated as the weight (kg) divided by the square of height (m<sup>2</sup>).

*Statistical analysis:* The data for biochemical analysis was subjected to standard statistical analysis such as Student's t test using the Statistical Package for Social Science (SPSS) 11.5 software. For all tests 'p' value was considered to be significant if it was less than 0.05 at a confidence level of 95 %.

## Results

*The characteristics and their comparison among different groups of study population – Unpaired t test:* Baseline personal profile and clinical details of the study population are shown in Table 1.

**Table-1: Biochemical and anthropometric variables and their comparison between the controls, diabetics, and diabetics without nephropathy**

Characteristics	Control	Diabetes mellitus without complication	Diabetic nephropathy
Number of subjects in each group (n)	130	130	130
Age	46.87 ± 10.46	45.28 ± 9.11	45.12 ± 11.04
Sex			
Males	62 (47.7)	61 (47)	63 (48.4)
Females	68 (52.3)	69 (53)	67 (51.6)
BMI (Kg/m <sup>2</sup> )	23.8±2.8	23.4±2.6	24.3 ± 1.9
Fasting plasma glucose (mg/dl)	91.2 ± 10.5	134.9 ± 47.8 □ □ □	162.6 ± 81.7 □ □
PPBS (mg/dl)	121.27 ± 11.57	197 ± 56.38 □ □	305.36 ± 76.20 □ □
Diabetes duration (years)		3.7 ± 1.1	15.4 ± 6.2
HbA1c (%)	5.50 ± 0.45	11.17±1.63 □ □	11.85 ± 1.39 □ □
Serum urea (mg/dl)	29.79 ± 5.64	27.18 ± 6.88	59.25 ± 17.62 □
Serum creatinine (mg/dl)	1.06 ± 0.20	1.13 ± 0.68	1.17 ± 0.32 □
Systolic blood pressure (mm Hg)	117.88 ± 5.62	121.38 ± 10.58	155.27 ± 13.93 □ □
Diastolic blood pressure (mm Hg)	73.72 ± 5.71	76.29 ± 6.94	96.12 ± 6.93 □ □

Data are expressed as numbers (group percentages in parentheses) for categorical variables and mean values ± SD for continuous variables; p values were compared to Group I (Control);  
 □ □ indicates highly statistical significant compare to control; □ indicates statistical significant compare to control; p < 0.05 consider statistically significant; p < 0.001 consider highly statistically significant

*Comparison between concentrations of serum sialic acid and urinary micro-albumin among study groups – Unpaired t test:* Serum sialic acid levels were found to be significantly increased in diabetes with or without nephropathy compared

to controls. Urinary microalbumin in diabetic nephropathy patients was significantly higher compared with diabetics and healthy individuals (Table1).

**Table-2: Comparison between concentrations of serum sialic acid and urinary micro-albumin of group I and II with control (Group III) of study population**

Parameters	Control (Group III)	Diabetes mellitus without complication (Group II)	Diabetic nephropathy (Group I)
Serum sialic acid (mg/dl)	56.8 ± 8.77	64.0 ± 9.76 □	70.9 ± 14.1 □ □
Urinary micro-albumin (mg/day)	9.9 ± 6.12	8.17 ± 4.11	144.8 ± 23.94 □ □

Values are in mean ± SD; p values were compared to Group-I (Control); □ □ indicates highly statistical significant compare to control; □ indicates statistical significant compare to control; p < 0.05 consider statistically significant; p < 0.001 consider highly statistically significant.

*Correlation of serum sialic acid with serum urea, creatinine and urinary micro-albumin – Bivariant correlation:* It was shown in Table 3 that in diabetes without any complications, serum sialic acid level had no statistically significant positive

correlation with serum urea, creatinine and urinary microalbumin but in diabetes with nephropathy (Table 4), serum sialic acid level statistically was well correlated with serum urea, creatinine and urinary microalbumin.

<b>Table-3: Correlation of serum sialic acid with serum urea, creatinine and urinary microalbumin in diabetes without complication</b>		
<b>Categories</b>	<b>Pearson correlation (r)</b>	<b>Significance (p value)</b>
Serum sialic acid vs Urine microalbumin	0.265	0.110
Serum sialic acid Serum Creatinine	0.112	0.128
Serum sialic acid Serum urea	0.103	0.158

<b>Table-4: Correlation of serum sialic acid with serum urea, creatinine and urinary microalbumin in diabetes with nephropathy</b>		
<b>Categories</b>	<b>Pearson correlation (r)</b>	<b>Significance (p value)</b>
Serum sialic acid vs Urine microalbumin	0.692	0.01
Serum sialic acid Serum Creatinine	0.572	0.01
Serum sialic acid Serum urea	0.482	0.03

### Discussion

Diabetes mellitus is the major health problem in India and also worldwide. Diabetes is a chronic metabolic disorder which is characterized by chronic hyperglycemia resulting from disturbances in metabolism of carbohydrate, protein and lipid. This sustained hyperglycemia affects both micro and macrovascular systems throughout the body and results from prospective studies suggest that inflammation involved in the pathogenesis of diabetes [22]. These are the leading cause of diabetic complications such as diabetic nephropathy. Pathophysiologic event of diabetic nephropathy is wide-spread endothelial dysfunction and activates inflammatory pathway [23]. As sialic acid is an important acute phase reactant and also maintains the negative charge of renal glomerular basement membrane that is one of the main regulators of membrane permeability, so the present study was performed to compare serum sialic acid between healthy controls and diabetes with or without renal complications and their relation with urinary microalbumin in these subjects groups.

First, it was found that serum sialic acid levels were found to be significantly increased in diabetes with or without nephropathy compared to controls. The vascular endothelium carries a high concentration of sialic acid. Research studies have shown that the concentration of sialic acid in serum is elevated in pathological states when there is tissue damage, tissue proliferation and inflammation. Hence extensive microvascular damage associated with diabetes mellitus, could account for its shedding into the circulation leading to an increase in vascular permeability and overall increased SSA concentrations [24]. Tissue injury caused by diabetic vascular complications stimulates local cytokine secretion from cellular infiltrates such as macrophages and endothelial cells. This induces an acute phase response with release of acute phase glycoproteins with sialic acid from the liver into the general circulation again leading to increased SSA concentrations [24]. Another possible explanation for the increased SSA is that there may be a difference in the ratio between the two forms of erythrocyte sialidases which are important in maintaining the viability of the erythrocyte and its survival in the circulating blood [25].

Pathophysiologic event of diabetic nephropathy is basement membrane damage leading to progressive thickening of basement membrane, changes in mesangial and vascular cells, formation of AGEs, accumulation of polyol via aldose reductase pathway and activation of protein kinase C [26-28]. This wide-spread endothelial dysfunction and or vascular damage leads to progressive increase in excretion of protein particularly albumin, decline in GFR and ultimately leads to ESRD [29]. Passage of macromolecules through the basement membrane activates inflammatory pathway [30]. And results from prospective studies suggest that inflammation involved in the pathogenesis of diabetes [31]. Sialic acid maintains the negative charge of renal glomerular basement membrane, which is one of the main regulators of membrane permeability. Due to increased vascular permeability there is shedding of vascular endothelial sialic acid leading to its increased levels in circulation [32].

Urinary albumin excretion in diabetic nephropathy patients was significantly higher compared with diabetics and healthy individuals. The increase in urine albumin in the diabetics can be interpreted as an early sign of nephropathic changes in those individuals. Increase in urine albumin seen with diabetic nephropathy can be attributed to degradation of the glomerular basement membranes characteristic of diabetic nephropathy. The presence of microalbuminuria is a marker of endothelial dysfunction, and is due to widespread endothelial dysfunction arising from the effects of cytokines and other inflammatory mediators which are released during the intense inflammatory responses that are associated with critical illness [33]. The effects of disruption of the integrity of the endothelial barriers is manifested as altered glomerular endothelial permeability in the kidneys, allowing increased amounts of albumin to escape into the glomerular ultrafiltrate. The tubular reabsorptive mechanism for albumin from the ultrafiltrate is exceeded beyond its threshold capacity, leading to increased excretion of albumin in the urine in diabetic nephropathy [33].

Our correlation study revealed very large positive correlation between serum sialic acid and urinary microalbumin ( $r=0.733$ ) in cases showing that as microalbumin excretion increases, serum sialic acid also increases pointing contributory role of serum sialic acid towards renal damage. This correlation is not distorted in cases when compared to controls as there is a very small positive correlation between serum sialic acid and urinary microalbumin ( $r=0.054$ ) in controls. Blood urea measurement has been widely used as an indicator of kidney function and correlation study revealed a small positive correlation between serum sialic acid and blood urea ( $r=0.209$ ) indicating that as blood urea increases, serum sialic acid also increases. Serum creatinine is the most important indicator of renal function and there is a moderate positive correlation between serum sialic acid and serum creatinine in

cases ( $r=0.413$ ) showing that as serum creatinine increases serum sialic acid also increases. These findings indicate that serum sialic acid increases with severity of diabetic renal complication.

Limitations of this study are that the sample size could have been larger. Patients with diabetic nephropathy could also have had early signs of other diabetic complications such as retinopathy and neuropathy. There was no definite way of determining this and no way of assessing the impact of these complications on our study. Future researchers were recommended that a larger sample size should be observed over a longer time period, multiple blood and urine samples from the same patient should be collected and 24 hr.urine samples should be collected instead of one time urine samples. Finally, we recommend that researchers take into account other diabetic complications such as diabetic retinopathy and diabetic neuropathy to allow for a more complete study.

### Conclusion

From this study it is concluded that serum sialic acid concentrations were increased in diabetes with and without renal complications. It is also concluded that elevated serum sialic acid and urinary microalbumin concentration were strongly related in diabetic nephropathy. Therefore, estimation of sialic acid levels may help in early prediction and prevention of microvascular complications occurring due to diabetes mellitus, thereby sialic acid can be used as a marker of renal dysfunction in diabetic nephropathy thus decreasing the mortality and morbidity.

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