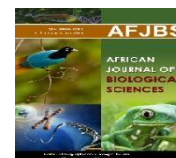


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### **A study of the Relationship between Serum Sialic Acid and urine microalbumin in Patients of Type 2 Diabetes Mellitus Nephropathy**

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#### **Introduction:**

Diabetes represents a spectrum of metabolic disorders, which has become a major health challenge worldwide <sup>[1]</sup>. Diabetes mellitus is the most common endocrine disorder, the prevalence of which is rising alarmingly in India <sup>[2]</sup>. Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances in carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. <sup>[3]</sup> Diabetes mellitus progressively causes macro and microvascular complications. Diabetic nephropathy is a major microvascular complication of diabetes mellitus and the most common cause of end-stage renal disease

worldwide.<sup>[4]</sup> Microalbuminuria, the dominant feature of diabetic nephropathy is defined as an albumin excretion rate of 30–300 mg/24 hrs.<sup>[5]</sup>

Sialic acid is a component of glycoprotein found on cell membranes. Elevated levels may indicate excessive cell membrane damage, but more specifically for cells of vascular tissue. Damage to vascular tissue leads to ischemia which affects the smallest blood vessels, particularly in the retina, kidneys, heart, and brain.<sup>[6]</sup> Sialic acid can be used as a measurement of the acute phase response because many proteins of the immune response are glycoproteins, and these glycoproteins have sialic acid as the terminal sugar on their oligosaccharide chain and an acute phase reactant and urinary albumin excretion are found to be increased in diabetic nephropathy patients.<sup>[7]</sup> The mechanism associated with the role of sialic acid is in maintaining 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 into circulation.<sup>[8]</sup>

Therefore, laboratory assessment of sialic acid as a marker of microvascular complications can be a diagnostic tool for diabetes mellitus. Microalbumin is an established predictor of diabetic nephropathy.<sup>[9]</sup> Sialic acid is also increased in serum because of vascular damage.<sup>[10]</sup> In doing a literature survey it was found that increased level of sialic acid is associated with progressive diabetic microvascular complications.<sup>[9, 10]</sup>

Hence the study was undertaken to evaluate serum sialic acid and microalbumin levels and to correlate it with glycemic control in diabetic nephropathy patients.

### **Material and Method:**

This was a cross-sectional study that was conducted in the Department of Biochemistry, GAIMS of GK General Hospital, Bhuj. Prior Ethical approval was taken from the Institutional Ethical Committee. Clinically diagnosed diabetic nephropathy patients visiting the medicine OPD (outdoor patient department) and NCD (Non-Communicable Disease) were selected for the study. Before the onset of the study, the ethical consent form was taken from enrolled subjects in the study.

The selection of participants was based on selection criteria which are as follows:

### **Inclusion criteria:**

Clinically diagnosed diabetic nephropathy patients visiting medicine OPD and NCD.

### **Exclusion criteria:**

Patients suffering from acute and chronic inflammatory conditions, other metabolic conditions like ketoacidosis, cerebrovascular accidents, preeclamptic patients, preexisting chronic kidney disease, chronic renal failure, chronic glomerulonephritis, nephrotic syndrome, preexisting autoimmune disease and primary hypertensive were excluded from the study.

This study included 75 subjects with diabetes type 2 of which 44 were males and 31 were females. Control includes a total of 75 out of which 49 Males and 26 females. Diabetic patients were defined as those who had known diabetes according to World Health Organization (WHO) criteria.

### **Sample collection:**

In a plain tube, blood was collected for serum sialic acid estimation, and another aliquot of whole blood sample for HbA1C was collected in an EDTA tube by venipuncture of the antecubital vein from every individual. After overnight fasting of 10-12 hours, a venous blood sample for fasting blood sugar was collected in Fluoride Vacuette. Serum and plasma were obtained by centrifugation at 3000 rpm for 10 minutes in plain tubes.

### **Biochemical investigations:**

#### **1. Fasting blood sugar and post-prandial blood sugar:**

A plasma glucose level of fasting and PPBS was estimated by the Glucose oxidase peroxidase method using a kit of Vitros dry test system. The analysis was carried out on Vitros 5600 DTS auto analyzer. Quality control and calibration are done before sample analysis regularly to ensure quality performance. Intraassay CV% was 1.3% and interassay CV% was 1.4%.

#### **2. Glycated Hemoglobin (HbA1c):**

HbA1C was measured in whole blood by using HPLC techniques on NANO H110 of LABSYSTEMS. Prior analysis standardization and calibration of the instrument were done.

#### **3. Serum total sialic acid:**

Biochemical analysis of the serum sialic acid is based on the reaction between Sialic acid and ninhydrin in the presence of an acidic medium (according to Yao *et al.*). This leads to the formation of a colored product which can be measured by using a spectrophotometer at 470 nm. The method consists following steps.

#### **Preparation of Acid ninhydrin method:**

The acid ninhydrin reagent was freshly prepared. About 250 mg ninhydrin was dissolved in 6 ml glacial acetic acid and 4 ml concentrated HCL, by thorough vortexing for 30 min.

#### **Procedure**

A volume of 0.1 ml of serum is mixed with 0.9 ml of saline. To this solution, 4 ml of ethanol is added and the precipitate is obtained, followed by centrifugation. To the precipitate 1.0 ml of distilled water and 1.0 ml glacial acetic acid were added, followed by 1.0 ml of acid ninhydrin reagent. The reaction mixture was vortexed and then heated for 10 min in a boiling water bath. After cooling, the mixture under tap water, absorbance was measured at 470 nm using a spectrophotometer. The standard graph was obtained and based on the standard curve the concentration of serum total sialic acid was found.

**Serum Urea:**

Blood urea was measured by the Urease method on a Vitros 5600 DTS auto analyzer. The method involves a reaction between serum urea and urease enzyme, the intermediate ammonia produced is reacted with dye and produces a colored compound which was measured at 670 nm.

**Serum Creatinine:**

The creatinine is converted to sarcosine and urea by creatine amidinohydrolase. The sarcosine, in the presence of sarcosine oxidase, is oxidized to glycine, formaldehyde, and hydrogen peroxide. The final reaction involves the peroxidase-catalyzed oxidation of a leuco dye to produce a colored product.

**Urine Microalbumin**

A spot urine sample was collected for urine microalbumin estimation. Immuno turbidimetric assay on Vitros 5600 DTS analyzer was used to estimate urinary microalbumin levels. Antialbumin antibodies react with the antigen in the sample to form antigen/antibody complexes which, following agglutination, are measured turbidimetrically.

**Table 1: Comparison of study variables between Subjects and controls**

Variables	Case	Control	t-value	P-value
FBS (mg/dl)	161±53.2	81±5.2	26.5	<0.0001
S.Creatinine (mg/dl)	8.75±2.05	0.8±0.3	32.6	<0.0001
S. Urea (mg/dl)	93.5±15.4	30.8±7.1	31.9	<0.0001
UrineMicroalbumin (mg/dl)	96.5±17.2	6.4±1.6	45.17	<0.0001
HbA1C (%)	9.2±1.8	5.3±0.3	28.06	<0.0001
Sialic acid (mg/dl)	105±21	51±6	34.8	<0.0001

**Table 2: Correlations of study variables with sialic acids in subjects.**

Study Variables	t value	P-value	r-value
Sialicacid (mg/dl) v/s FBS (mg/dl)	8.9	<0.0001	0.259
Sialicacid (mg/dl) V/S HbA1C (mg/dl)	38.06	<0.0001	0.542

Sialic acid(mg/dl) V/S Urine Microalbumin(mg/dl)	5.06	<0.0001	0.891
Sialicacid(mg/dl) V/S Creatinine (mg/dl)	37.78	<0.0001	0.328
Sialicacid(mg/dl) V/S Urea (mg/dl)	6.092	<0.0001	0.194

### Results:

The present study was conducted to evaluate and find out the correlation between serum sialic acid and diabetic nephropathy indicators which included serum creatinine, serum urea, and Urine microalbumin. Table 1 shows the relationship between serum sialic acid and study variables in case and control groups. In subjects, the mean value of FBS was  $161 \pm 53.2$  mg/dl which was significantly higher than controls  $81 \pm 5.2$ . The Sialic acid in subjects was  $105 \pm 21$  mg/dl which is higher than controls  $51 \pm 6$  mg/dl. The urine microalbumin level was found to be  $96.5 \pm 17.2$  in subjects, whereas  $6.4 \pm 1.6$  mg/dl was in controls. Serum Creatinine level in subjects was  $8.75 \pm 2.05$  mg/dl which was higher than subjects  $0.8 \pm 0.3$  mg/dl. Serum Urea in diabetic nephropathy subjects was  $93.5 \pm 15.4$  mg/dl, which is positively higher than controls  $30.8 \pm 7.1$  mg/dl. The Correlation of Serum sialic acid with study variables was carried out by applying a student t-test and Pearson correlation coefficient r-value which found a significant correlation. The level and correlation of serum sialic acid and Urine microalbumin were found to be significantly positive where the p-value was  $<0.0001$ . There was also observed a significant positive correlation between the level of sialic acid, serum creatinine, and serum urea.

### Discussion:

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both, and insulin resistance. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of hyperglycemia.<sup>[11]</sup> Diabetes is often progressively observed with the development of microvascular complications. Among these complications, diabetic nephropathy is commonly observed which later on results in kidney dysfunctions.<sup>[12]</sup> The progressive course of diabetic nephropathy is also the leading cause of glomerulosclerosis and end-stage renal disease worldwide.<sup>[13]</sup> Evaluation at an early stage is primarily important to restrict organ damage, therefore early indicators of diabetic nephropathy such as urine microalbumin have a clinically important role.<sup>[14]</sup> The early phase of diabetic renal disease called incipient diabetic nephropathy characterized by increased albumin excretion in the range of 30-200 mg/l (microalbuminuria).<sup>[15]</sup> At this stage, urine is negative for the standard dipstick method and renal function is normal by standard clinical tests but can be detectable only by using a sensitive assay. While doing a literature survey there were many studies found that show a positive correlation between serum sialic acid and urine microalbumin. In the present study, it was found significant positive correlation between serum sialic acid, urine microalbumin, and

serum creatinine. Recently, a study conducted by Sheshukumar et al. observed the association between sialic acid and urine microalbumin in diabetic nephropathy

Subjects. <sup>[16]</sup> Serum sialic acid is an important component of cell membranes and vascular permeability. The vascular endothelium carries a high concentration of SA, hence extensive microvascular damage accounts for its release into the circulation leading to increased vascular permeability and serum SA concentration. The main mechanism associated with the role of Serum sialic acid is in maintaining the negative charge of renal glomerular basement membrane which is one of the main regulators of membrane permeability. <sup>[17]</sup> Thus, in diabetes, elevated levels of serum sialic acid indicate excessive damage to the vascular cells of the kidneys leading to diabetic nephropathy. <sup>[17]</sup> Therefore, SA is established as a potential risk factor for the development of macro and microvascular complications of diabetes. Increased level of serum sialic acid is found to be elevated in NIDDM patients with increased levels of urine microalbumin. <sup>[14]</sup> A study by Jawaid et al. found increased serum sialic acid and Urine microalbumin. <sup>[18]</sup> A study by Ritah and Simon et al. observed the positive significance of Sialic acid, glucose, and urine microalbumin. <sup>[19]</sup> A study conducted by Melidonis et al. showed that SA levels were higher in type 2 DM patients and those with signs of nephropathy had higher levels of Sialic acid than those without. <sup>[20]</sup> The study by Crook M et.al also found that serum Sialic acid was significantly higher in patients with diabetic complications than in those without complications. <sup>[14]</sup> The prospective study (10 years follow-up) conducted by Hiroki Yokoyama et al. found a significant association between Serum sialic acid and Urine microalbumin in IDDM diabetic nephropathy. <sup>[21]</sup> In that study, they concluded an increased serum sialic acid concentration is predictive for the onset of microalbuminuria independent of age, sex, diabetes duration, smoking, blood pressure, and total and HDL cholesterol. <sup>[21]</sup> The present study is the study conducted by Dwija et al. and Shivandand Nayak et al. who demonstrated the significance of sialic acid and Urine Microalbumin in diabetic nephropathy cases. <sup>[12,22]</sup> Tournis S and Chen JW, Gall MA in their study, demonstrated an increase in urinary albumin levels in diabetic nephropathy patients compared to controls. <sup>[23]</sup> Our study shows similar results to the study done by Shahid SM and Mahaboob T who also showed a significant positive correlation between serum sialic acid and FBS, blood urea, serum creatinine, and HbA1c levels. <sup>[24]</sup> In another study, Krishnamurthy U and Halyal SS also showed a progressive rise in serum sialic acid levels with increasing microalbumin excretion and a significant positive correlation between them in diabetic patients with microalbuminuria. <sup>[25]</sup>

### **Conclusion:**

The major concern with diabetes mellitus is the progressive development of microvascular complications which ultimately result in damage to multi-organ derangement. Prior diagnosis can be useful to prevent further advancement in complications. Urine microalbumin is established as a potential marker for diabetic nephropathy. The correlation of serum sialic acid, urine microalbumin, and serum creatinine proposed serum sialic acid as an accessory diagnostic marker for diabetic nephropathy. Estimation of sialic acid before microalbumin in diabetic patients helps

assess glycemic control and identify the risk for nephropathy and other secondary complications of diabetes mellitus, which are the main causes of mortality and morbidity among type-2 diabetes mellitus patients. Further large prospective cohort studies in patients with type-2 diabetes mellitus are required to identify the role of Sialic acid as a biomarker in early detection of diabetic nephropathy.

**Declarations:**

There are no conflicts of interest in this study. No source of funding support was received for the work.

**Consent to Participate:**

Written informed consent was obtained from the participant.

**Confidentiality:**

They are maintained as per the Indian Council of Medical Research (ICMR) guidelines.

**Ethical Consideration:**

Ethical permission was obtained from the institutional ethics committee (IEC) of Gujarat Adani Institute of Medical Science, Bhuj, Gujarat, India.

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