Evaluation of Serum Sialic Acid and Microalbuminuria in Diabetic Nephropathy

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Research Article

Abstract: Diabetes mellitus is the most common endocrine disorder, the prevalence of which is rising alarmingly in India. Diabetic nephropathy is a major microvascular complication of diabetes mellitus and the most common cause of end stage renal disease worldwide. Serum sialic acid, an acute phase reactant and urinary albumin excretion are found to be increased in diabetic nephropathy patients. In diabetes, acute phase reactants are considered as the indicators of microvascular angiopathy. Microalbuminuria is a predictor of incipient nephropathy in diabetic patients. Hence the study was undertaken to evaluate serum sialic acid and microalbuminuria levels and to assess the correlation of serum sialic acid and microalbuminuria with glycemic control in diabetic nephropathy patients. Present study involved 100 participants of which 50 were diagnosed to have diabetic nephropathy and 50 were age and sex matched healthy controls. Blood samples were analyzed for fasting and post prandial blood sugar (FBS, PPBS), 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. Statistical analysis was done using Student't' test (two tailed, independent) to find the significance of study parameters on continuous scale between two groups (Inter group analysis) on metric parameters. Pearson correlation between the study variables is performed to establish the relationship. Statistically significant increase in levels of FBS, PPBS, blood urea, serum creatinine, serum sialic acid, HbA1c, systolic, diastolic blood pressure and urinary microalbumin was observed in cases compared to controls ($p<0.001$). A positive correlation was found between glycemic status, serum sialic acid and urinary microalbumin levels in diabetic nephropathy patients. Elevated serum sialic acid and urinary microalbumin levels are strongly associated with the presence of nephropathy. Serum sialic acid can be used as a marker of renal dysfunction in diabetic nephropathy.

Keywords: Diabetic nephropathy, Sialic acid, Microalbuminuria.

1. Introduction

Diabetes mellitus is the major healthcare problem worldwide. 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 [1]. Diabetes is a chronic disease and sustained hyperglycemia attacks both micro vessels and macro vessels throughout the body. It is the leading cause of retinopathy, nephropathy, end-stage renal disease, non traumatic lower extremity amputations and cardiovascular disease. The prevalence of diabetes mellitus is continuously rising in both developed and developing countries which has reached epidemic proportion and has become one of the most challenging health problems of the 21st century. The global prevalence of diabetes is expected to increase from 4% in 1995 to 5.4% by the year 2025[2]. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030[3]. Currently the countries with the largest number of diabetic patients are India, China and United States[2]. In India alone, diabetes is expected to increase from 40.6 million in 2006 to 79.4 million by 2030[4]. Diabetic nephropathy, leading cause of ESRD remains a major cause of morbidity and mortality in persons with either type 1 or type 2 DM. 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[5]. Hyperglycemia causes increased mitochondrial production of reactive oxygen species (ROS). The ROS causes strand breaks in nuclear DNA, which activates Poly ADP-ribose polymerase (PARP). PARP then modifies glyceraldhyde-3-phosphate dehydrogenase (GAPDH), thereby reducing its activity[6]. Finally decreased GAPDH causes increased polyol pathway flux, increased intracellular advanced glycation end product formation, activation of protein kinase C and increased hexosamine pathway flux[6,7]. These pathways in combination, ultimately lead to increased renal albumin permeability and extracellular matrix accumulation, resulting in proteinuria, glomerulosclerosis and ultimately tubulointerstitial fibrosis[8]. Serum sialic acid is a newly established potential risk factor for the development of micro and macrovascular complications of diabetes[9]. Serum sialic acid is a component of glycoprotein such as acute

phase proteins and several serum acute phase proteins are elevated in diabetes. Serum sialic acid level is increased in both type 1 and type 2 diabetes mellitus patients with albuminuria. 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[10]. 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 ultrastructural changes rather than alterations in glomerular pressure or filtration rate alone[11]. It is estimated that death due to renal disease is 17 times more common in diabetics than in non diabetics[5]. 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. Several studies have demonstrated increased serum sialic acid levels in diabetic nephropathy patients when compared to controls and demonstrated the increasing trends of sialic acid in diabetic patients with the progression of complications such as nephropathy[9],[15]-[21] Hence the study was undertaken to estimate serum sialic acid and microalbuminuria and to correlate serum sialic acid and microalbuminuria with glycated hemoglobin in diabetic nephropathy cases.

2. Materials and methods

Clinically diagnosed 50 diabetic nephropathy cases attending medicine department in Adichunchanagiri hospital and research centre, B G Nagar, Mandya were included in the study. Age and sex matched 50 healthy individuals were taken as control group. Informed consent was taken and the study was approved by ethical and research committee of the institution. Patients suffering from acute and chronic inflammatory conditions, other 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. Blood sample was drawn under aseptic precautions from clinically diagnosed cases of diabetic nephropathy and controls. 2ml of blood in both fasting state and 2 hours after meals was collected with an anticoagulant sodium fluoride for the estimation of blood glucose (Glucose Oxidase method). Whole blood in heparin coated tube for the estimation of glycated hemoglobin (Affinity chromatography). 2 ml of blood with no anticoagulant was taken and allowed to clot and serum was separated and used for the measurement of serum sialic acid (Modified Thiobarbituric acid assay of Warren), blood urea (Glutamate dehydrogenase(GLDH) Urease method) and serum creatinine (Jaffe's method). Urine sample was collected under aseptic precautions for the estimation of urinary microalbumin (Immunoturbidimetry). Both systolic and diastolic blood pressure was recorded in cases and controls. Statistical analysis was done using student "t" test and statistical significance was compared between the cases and the controls. Pearson correlation between the study variables is performed to establish the relationship. The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 , Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data. Results are represented 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, systolic and diastolic pressure.

3. Results

Present study included 50 diabetic nephropathy cases and 50 healthy controls to evaluate FBS, PPBS, blood urea, serum creatinine, HbA1c, serum sialic acid, systolic and diastolic blood pressure, urinary microalbumin levels. The age distribution pattern of cases and controls under study as shown in the Table1 ranged from 40 to 90 years with mean age of 63.70±9.69 in cases and 63.48±10.33 in controls. There was an increase in FBS, PPBS, blood urea, serum creatinine, serum sialic acid, HbA1c, systolic and diastolic blood pressure and urinary microalbumin levels in patients as compared to controls which was statistically highly significant ($p<0.001$) as shown in the Table2. In the present study as shown in the Table3 and Fig1 there was a significant positive correlation between serum sialic acid and FBS, PPBS, blood urea, serum creatinine, HbA1c, urinary microalbumin levels, systolic and diastolic blood pressure in cases indicating that as these parameters increases sialic acid also increases.

4. Discussion

Diabetes mellitus is a group of metabolic disorder of carbohydrate metabolism in which glucose is underutilized producing hyperglycemia. Diabetic nephropathy is the most common cause of end-stage renal disease requiring dialysis. Advanced diabetic nephropathy is also the leading cause of glomerulosclerosis and end-stage renal disease worldwide. Between 20% and 40% of patients with

diabetes ultimately develop nephropathy[12]. In our study the mean FBS and PPBS values were 90.86±13.29 and 119.72±10.57 in controls and 187.60±58.14 and 295.22±81.87 in cases which is statistically highly significant (P<0.001). Hyperglycemia is a causative factor in the pathogenesis of diabetic nephropathy. Glucose reacts non enzymatically with primary amines of proteins forming glycated compounds. Hyperglycemia exerts toxic effects and results in kidney damage by directly altering intracellular signaling pathways and via many biochemical pathways[13]. Measurement of blood urea has been widely used as an indicator of kidney function[14]. In our study the mean blood urea values were 23.79±4.44 in controls and 61.63±12.37 in cases which is statistically highly significant (P<0.001). Serum creatinine is the most important indicator of renal function. In our study the mean serum creatinine values were 1.06±0.20 in controls and 2.83±0.68 in cases which is statistically highly significant (P<0.001). Our study is in accordance with several studies[15],[16],[17]. Glycated hemoglobin is a marker for both severity and long term control of disease which reflects the average blood glucose concentration over the preceding 6-8 weeks and is unaffected by diet, exercise, insulin therapy and other drugs. The mean HbA1c values were directly proportional to the risk of developing retinopathy and nephropathy. In our study the mean HbA1c values were 5.50±0.45 in controls and 11.17±1.63 in cases which is statistically highly significant (P<0.001). Our study is in accordance with the study done by Melidonis A, Tournis S[16] and Chen JW, Gall MA[17] who found increased HbA1c levels in diabetic patients with and without nephropathy compared to controls. Sialic acid acts as a cofactor of many cell surface receptors and positively associated with most of the serum acute phase reactants. Sialic acid regulates vascular permeability. The vascular endothelium carries a high concentration of sialic acid hence extensive microvascular damage associated with diabetes result in its shedding into the circulation leading to an increase in vascular permeability and increased serum sialic acid concentration[1]. Tissue injury caused by diabetic vascular complications stimulates local cytokine secretions from cells involved in the complications such as macrophages and endothelium. This induces an acute phase response which involves the release of acute phase glycoproteins with sialic acid from the liver into the general circulation again leading to increased serum sialic acid concentrations[1]. In our study the mean serum sialic acid values were 1.90±0.30 in controls and 3.06±0.35 in cases which is statistically highly significant (P<0.001). Our study is in accordance with the several study done which have shown increased serum sialic acid levels in diabetic nephropathy patients when compared to controls and demonstrated the increasing trends of sialic acid in diabetic patients with the progression of complications such as nephropathy[9],[15]-[21]. Hypertension plays a critical role in the progression of diabetic nephropathy. In our study the mean Systolic BP values were 116.00±6.32 in controls and 155.36±14.78 in cases and mean diastolic BP values were 76.92±4.56 in controls and 92.88±4.92 in cases which is statistically highly significant. Our study is in accordance with several studies[16],[17]. Microalbuminuria is defined as the excretion of 30 to 300 mg of albumin per day in urine. It is a predictor of progressive renal damage. It is a clinically important indicator of deteriorating renal function in diabetic patients. Microalbuminuria 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[22]. 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[22]. In our study the mean values of urinary microalbumin were 10.35±2.77 in controls and 153.38±69.64 in cases which is statistically highly significant (P<0.001) and correlated well with the clinical diagnosis. Our study is in accordance with Shivananda Nayak B and Geetha Bhaktha, who demonstrated significantly increased urinary microalbumin levels in diabetic nephropathy patients compared to healthy controls[15]. Melidonis A, Tournis S and Chen JW, Gall MA in their study, demonstrated increase in urinary albumin levels in diabetic nephropathy patients compared to controls[16],[17].

Correlation between sialic acid and other study parameters: Correlation study revealed a small positive correlation between serum sialic acid and both FBS and PPBS in diabetic nephropathy cases (r=0.262; r=0.383), indicating the role of hyperglycemia towards renal damage. 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. 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. In diabetic nephropathy cases there is a significantly large positive correlation between serum sialic acid and HbA1c

(r=0.567) indicating that as HbA1c increases, serum sialic acid also increases. This correlation is not distorted when cases were compared with controls as controls showed very small positive correlation between serum sialic acid and HbA1c (r=0.031). There is a large positive correlation between serum sialic acid and systolic BP in cases (r=0.596) where as control group showed moderate correlation (r = 0.429). Correlation study also revealed moderate positive correlation between serum sialic acid and diastolic BP in cases (r=0.331). 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. Our study is in accordance with the study done by Shahid SM and Mahaboob T who also showed significant positive correlation between serum sialic acid and FBS, blood urea, serum creatinine, HbA1c levels, systolic and diastolic blood pressure in diabetic nephropathy patients compared to controls[9]. In another study, Krishnamurthy U and Halyal SS also showed progressive rise in serum sialic acid levels with increasing microalbumin excretion and significant positive correlation between them in diabetic patients with microalbuminurics[19].

5. Summary and Conclusion

The leading cause of mortality in diabetic patients is through kidney damage caused by diabetic nephropathy. The pathophysiology of the disease has to be well known, in order to prevent a disease. Present study was undertaken to study serum sialic acid levels and microalbuminuria and to assess whether there is a relationship between these two with glycemic control and other parameters in diabetic nephropathy patients. Statistically significant increase was observed in values of FBS, PPBS, blood urea, serum creatinine, HbA1c, serum sialic acid, urinary microalbumin levels and both systolic and diastolic blood pressure in cases compared to controls. In our study a very large positive correlation was observed between serum sialic acid and urinary microalbumin in cases. It was also observed that serum sialic acid concentrations were strongly associated with several risk factors like glycemic control (HbA1c), renal dysfunction (urea and creatinine) and hypertension for the development of micro and macrovascular complications. These markers were clinically correlated with increasing concentration of sialic acid. It is concluded that increase in circulating serum sialic acid is an early manifestation of diabetic renal disease and hence sialic acid can be used as a marker of renal dysfunction in diabetic nephropathy. Further studies would help to clarify its role in pathogenesis of diabetic renal disease.

Table 1: Age distribution of patients studied

Age in years	Cases		Controls	
	No	%	No	%
41-50	6	12.0	9	18.0
51-60	12	24.0	13	26.0
61-70	21	42.0	19	38.0
71-80	10	20.0	7	14.0
81-90	1	2.0	2	4.0
Total	50	100.0	50	100.0
Mean ± SD	63.70±9.69		63.48±10.33	

Table 2: Comparison of study variables in two groups studied

Study variables	Cases	Controls	P value
FBS (mg/dl)	187.60±58.14	90.86±13.29	<0.001**
PPBS (mg/dl)	295.22±81.87	119.72±10.57	<0.001**
Urea (mg/dl)	61.63±12.37	23.79±4.44	<0.001**
S.Creatinine(mg/dl)	2.83±0.68	1.06±0.20	<0.001**
HbA1c (%)	11.17±1.63	5.50±0.45	<0.001**
S.Sialic acid (mmol/l)	3.06±0.35	1.90±0.30	<0.001**
Systolic BP (mm Hg)	155.36±14.78	116.00±6.32	<0.001**
Diastolic BP (mm Hg)	92.88±4.92	76.92±4.56	<0.001**
Microalbumin (mg/l)	153.38±69.64	10.35±2.77	<0.001**

FBS-Fasting blood glucose, PPBS-Post prandial blood glucose , HbA1c-Glycated hemoglobin (** Highly significant)

Table 3: Pearson correlation of Serum Sialic acid and other study variables in cases and controls

Pair	Cases		Controls	
	r value	p value	r value	p value
S.Sialic acid (mmol/l) vs FBS (mg/dl)	0.262	0.066+	0.107	0.460
S.Sialic acid (mmol/l) vs PPBS (mg/dl)	0.383	0.006**	-0.020	0.892
S.Sialic acid (mmol/l) vs Urea (mg/dl)	0.209	0.145	-0.174	0.231
S.Sialic acid (mmol/l) vs S.Creatinine(mg/dl)	0.413	0.003**	0.036	0.804
S.Sialic acid (mmol/l) vs HbA1c (%)	0.567	<0.001**	0.031	0.829
S.Sialic acid (mmol/l) vs Systolic BP (mm Hg)	0.596	<0.001**	0.429	0.002**
S.Sialic acid (mmol/l) vs Diastolic BP (mm Hg)	0.331	0.019*	0.330	0.019*
S.Sialic acid (mmol/l) vs Microalbumin (mg/l)	0.733	<0.001**	0.054	0.712

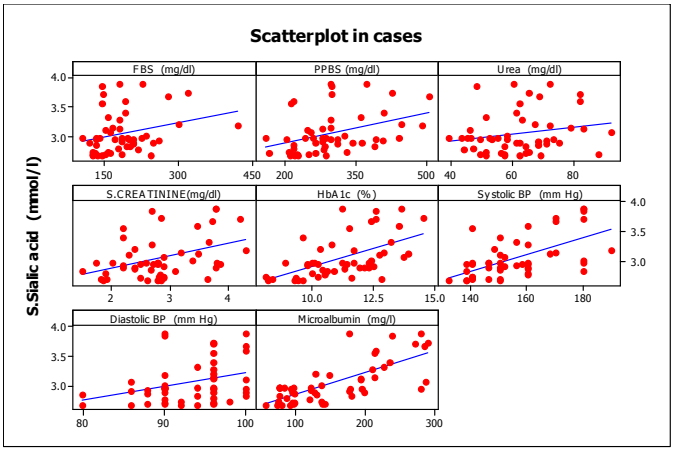

Figure 1: Scatter plot for the correlation of serum sialic acid with other parameters

Classification of Correlation Co-efficient (r)

Up to 0.1	Trivial Correlations
0.1-0.3	Small Correlation
0.3-0.5	Moderate Correlation
0.5-0.7	Large Correlation
0.7-0.9	Very Large Correlation
0.9- 1.0	Nearly Perfect correlation
1.0	Perfect correlation

Significant figures

+ Suggestive significance (P value: 0.05<P<0.10)

* Moderately significant (P value:0.01<P ≤ 0.05)

** Strongly significant (P value : P≤0.01)

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