Sialic acid: an early sensitive marker improves detection of nephropathy in type-2 diabetes mellitus patients

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Abstract

Introduction: Diabetic nephropathy has become the single most important cause of end stage renal disease worldwide. Detection of early stages of diabetic nephropathy with sensitive tests may be useful to prevent progression of nephropathy. The present study was undertaken to study the 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.

Materials and Methods: A total number of 100 subjects participated in present study including 50 clinically diagnosed cases of type-2 diabetic nephropathy patients. Fasting and post-prandial blood sugar, urea, creatinine, serum sialic acid and urinary microalbumin levels were estimated.

Results: The mean fasting blood sugar values in cases (181.40±45.82) as compared to controls (89.62±12.53) is statistically significant ($p<0.001$). The cases had higher HbA1c in comparison to controls (11.21±1.61 vs. 5.49±0.46%, $p<0.001$) and higher sialic acid levels as compared to control subjects (3.05±0.36 vs.1.87±0.33 m mol/L, $p<0.001$). In cases and controls, urinary microalbumin levels are 133.70±71.36 vs. 10.78 ± 2.8 µg/mg creatinine, $p<0.001$). The correlation study revealed a positive correlation between sialic acid and both fasting and post-prandial blood sugar in diabetic nephropathy cases ($r = 0.315$; $r = 0.47$). In cases serum sialic acid significantly correlated with HbA1c ($r = 0.421$). A positive correlation between sialic acid and urinary microalbumin ($r = 0.781$) in cases was observed. ROC curve analysis showed 98% sensitivity and 100% specificity in the measurements of sialic aicd, HbA1c, and Urine microalbumin.

Conclusion: Estimation of serum sialic acid prior to microalbumin in diabetic patients is helpful in assessing the glycemic control and identify the risk of nephropathy which is the main cause for mortality and morbidity among type-2 diabetes mellitus patients.

Keywords: Serum sialic acid, Microalbumin, HbA1c, Nephropathy, Type-2 diabetes mellitus.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Diabetes mellitus is associated with dyslipidaemia, atherosclerosis and predispose to certain specific micro vascular abnormalities like retinopathy, nephropathy and neuropathy. Diabetes mellitus with its chronic degenerative complications like micro and macrovascular angiopathy represents a major health and social problem. The prevalence of diabetes worldwide has extended the epidemic magnitudes and is expected to affect more than 350 million people by the year 2035.[1] Currently the countries with the largest number of diabetic patients are India, China and United States. In India alone, diabetes is expected to increase from 69.2 million in 2016 to 79.4 million by 2030.[2] India has a prevalence of 8.7%, higher than the world prevalence. Diabetes related deaths in India is dominated by infections and diabetic nephropathy unlike cardiovascular or cerebrovascular causes which dominate the developed countries.[3] Diabetic nephropathy, especially related to type 2 diabetes, has become the single most important cause of end stage renal disease (ESRD) worldwide. Nephropathy is the leading cause of chronic renal failure worldwide and is responsible for renal failure in about one third of patients who undergo dialysis.[4] It is suggested that patients with common risk factors including greater duration of diabetes, hypertension, poor metabolic control, smoking, obesity and hyperlipidaemia are more prone to develop diabetic complications.[5] Although type 1 and type 2 diabetes mellitus lead to ESRD, the great majority of patients are those with type-2 diabetes mellitus.

Serum sialic acid (SA), an acute phase reactant found to be increased in various conditions likes diabetes mellitus, cardiovascular diseases, cancer etc. SA level is increased in both type 1 and type 2 diabetes mellitus patients with albuminuria. SA is a newly established potential risk factor for the development of micro and macrovascular complications of diabetes.[6] It is formed by glucosamine in association with acetyl-L-carnitine. In human plasma a large quantity of SA is found in orosomucoid, alpha antitrypsin, haptoglobin, ceruloplasmin, fibrinogen, complement proteins and transferrin. Some of the sialyated glycoproteins are acute phase reactants and such substances rapidly increase in concentration after the onset of an inflammation reaction or injury like in nephropathy.[7] The raised SA is an acute phase reactant of inflammation resulting from elevated blood

glucose levels.[8] Increased SA levels were observed in patients with type-2 diabetes mellitus.[9]

Microalbuminuria is the earliest manifestation of diabetic nephropathy and a predictor of incipient nephropathy in diabetic patients. Diabetic nephropathy is characterized by increased albumin excretion in the range of 30 - 300 mg/l (Microalbuminuria). Urine is negative for standard dipsticks in this stage of Renal disease.[10] Detection of early stages of diabetic nephropathy with sensitive tests like SA may be useful to prevent progression of nephropathy. The aim of the present study was to estimate SA and micro albuminuria and to correlate SA and micro albuminuria with glycated haemoglobin (HbA1c) and other diabetic indicators in diabetic nephropathy cases.

Materials and Methods

A total number of 100 subjects participated in present study. 50 clinically diagnosed cases of diabetic nephropathy patients in type 2 Diabetes Mellitus were selected for cases. Patients suffering from acute and chronic inflammatory conditions, other metabolic conditions like ketoacidosis, cerebrovascular accidents, preeclampsia patients, pre-existing chronic kidney disease, chronic renal failure, chronic glomerulonephritis, nephrotic syndrome, smokers, alcoholics, patients with psychiatric disorders and primary hypertensives were excluded from study. Age and sex matched healthy individuals were taken as control group. Informed consent was obtained from all study subjects. Study was approved by institutional ethical committee.

Peripheral venous blood was drawn under aseptic precautions from all participants and the samples were analyzed immediately after collection.. For SA assay, one serum sample was aliquoted and frozen at -20^{o}C until analysis. Random urine sample was collected under aseptic precautions for the estimation of urinary microalbumin. Fasting blood sugar (FBS), post-prandial blood sugar (PPBS), urea and creatinine levels were estimated by using Roche cobas c501 fully automated chemistry analyzer (Roche Diagnostics, U.S.A.). HbA_{1c} levels were estimated by using Bio-Rad D-10™ Dual Program intended for the percent determination of hemoglobin A_{1c} in human whole blood using ion-exchange high-performance liquid chromatography (HPLC). Immunoturbidimetric assay on Roche Cobas c501analyzer 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. SA levels were estimated by using modified thiobarbituric acid assay of Warren.[11]

The data was analyzed using GraphPad Prism software version 6.0. Descriptive results are expressed as mean and SD of various parameters in different groups ($p<0.05$ taken as significant) and correlation was found among the parameters. Scatter plot and ROC curve analysis was done in cases and control groups.

Results

A Comparative study with 50 diabetic nephropathy cases and 50 controls was undertaken and FBS, PPBS, blood urea, serum creatinine, HbA1c, SA and urinary microalbumin levels were studied. The age distribution pattern of controls and diabetic nephropathy cases under study which ranges from 41 years to 80 years with mean age of 63.14±9.459 in controls and 62.08± 9.357 in cases (p=0.314). The mean FBS values in cases (181.40±45.82) as compared to controls (89.62±12.53) is statistically significant ($p<0.001$). The Mean PPBS values in cases are 286.1± 68.32 as compared to controls 120±10.95 which is statistically significant ($p<0.001$). The mean blood urea and serum creatinine values in cases are 63.92 ± 12.98 and 2.87 ±0.76 vs controls 25.76 ± 4.33 and 1.0 7± 0.19 which are statistically significant ($p<0.001$). The diabetic nephropathy subjects had higher HbA1c in comparison to controls (11.21±1.61 vs. 5.49±0.46%, $p<0.001$). Also, the diabetic nephropathy patients have higher SA levels as compared to control subjects (3.05±0.36 vs.1.87±0.33 mmol/L, $p<0.001$). In cases and controls, urinary microalbumin levels are 133.70±71.36 vs. 10.78 ± 2.8 mg/g creatinine, $p<0.001$). Comparison of study variables in controls and diabetic nephropathy subjects are given in Table 1.

Table 1: Comparison of study variables in controls and cases

Study variables	Controls	Cases	p value
FBS (mg/dl)	89.62 ± 12.53	181.40 ± 45.82*	<0.001
PPBS (mg/dl)	120.00±10.95	291.7± 67.75*	<0.001
Blood urea (mg/dl)	25.76±4.33	63.92 ± 12.98*	<0.001
Serum creatinine (mg/dl)	1.07± 0.19	2.87 ± 0.76*	<0.001
HbA1c (%)	5.49 ± 0.46	11.21 ± 1.61*	<0.001
Serum sialic acid (mmol/L)	1.87 ± 0.33	3.05 ± 0.36*	<0.001
Urinary microalbumin (mg/g crt)	10.78 ± 2.80	133.70 ± 71.36*	<0.001

*p<0.001 as compared to healthy controls

The correlation study revealed a positive correlation between SA and both FBS and PPBS in diabetic nephropathy cases (r = 0.315; r = 0.47), whereas there is a negative correlation between SA and FBS (r = - 0.066) in control group and also a negative correlation between serum SA and PPBS in controls (r = -0.058) (Table 2).

Correlation study revealed a positive correlation between SA and blood urea (r = 0.405) in cases where as control group

Table 2: Pearson correlation of serum Sialic acid and other study variables in controls and cases.

S.No.	Sialic acid vs other parameters	Controls R VALUE	P VALUE	Cases R VALUE	P VALUE
1	Sialic acid (mmol/l) vs FBS (mg/dl)	–0.066	0.648	0.315	0.026
2	Sialic acid (mmol/l) vs PPBS (mg/dl)	–0.058	0.69	0.47	<0.001
3	Sialic acid (mmol/l) vs Urea (mg/dl)	–0.155	0.281	0.405	0.003
4	Sialic acid (mmol/l) vs Creatinine (mg/dl)	–0.068	0.637	0.406	0.003
5	Sialic acid (mmol/l) vs HbA1c(%)	–0.041	0.779	0.421	0.002
6	Sialic acid (mmol/l) vs Microalbumin (mg/gm crt)	–0.077	0.597	0.781	<0.001

shows negative correlation between serum SA and blood urea (r=-0.155). There is a positive correlation between serum SA and serum creatinine in cases (r = 0.406) and a negative correlation in controls (r = -0.068). In diabetic nephropathy cases serum SA significantly correlated with HbA1c (r = 0.421) in cases and negatively in controls (r = -0.041). There was a positive correlation between SA and urinary microalbumin (r = 0.781) in cases and negative correlation in controls (r = -0.077). (Table 2).

In order to assess the maximum sensitivity, specificity and diagnostic efficiency of various parameters in identifying abnormality, the best cut off values are calculated using Receiver Operator Characteristics (ROC) analysis. Best cut off values are established by selecting a point closer to the left hand curve that provides greatest sum of sensitivity and specificity. (Table 3).

Receiver Operator Characteristics curve analysis showed 98% sensitivity and 100 % specificity in the measurements of SA, HbA1c, and Urine microalbumin. ROC curve showed 100 % specificity in all the parameters of the study. As the study parameters were measured in already established cases of diabetic nephropathy, there was almost no overlapping in the two distributions of true positives and false positives, resulting in a high specificity.

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 the hyperglycemia.[12] Several pathogenic processes are involved in the development of diabetes. Long term complications of diabetes include retinopathy, nephropathy, peripheral neuropathy with risk of foot ulcers, Charcot joints and autonomic neuropathy causing gastrointestinal, genitourinary, cardiovascular symptoms and sexual dysfunction. Diabetic nephropathy affects more than 30% of diabetic patients and it is a leading cause of

Table 3: ROC curve analysis of study parameters

Parameter	AUC	Standard error	P value	Cut off value	Sensitivity	Specificity
FBS	1	0	< 0.0001	110.5	100	100
PPBS	1	0	< 0.0001	148.5	100	100
Urea	1	0	< 0.0001	36.7	100	100
Creatinine	1	0	< 0.0001	1.490	100	100
HbA1c	1	0	< 0.0001	8.5	98	100
Sialic acid	0.998	0.0023	< 0.0001	2.65	98	100
Microalbumin	1	0	< 0.0001	45	98	100

end stage renal disease. Advanced diabetic nephropathy is also the leading cause of glomerulosclerosis and end-stage renal disease worldwide. However, there is an early phase of diabetic renal disease called incipient diabetic nephropathy characterized by increased albumin excretion in the range of 30-200 mg/l (microalbuminuria). At this stage urine is negative for standard dipstick method and renal function is normal by standard clinical tests but can be detectable only by the use of sensitive assay. Therefore, we intended to correlate SA with microalbuminuria which is a marker of early renal damage to establish the role of estimation of serum SA in diabetes.

SA is an important component of cell membranes and vascular permeability. Disturbances in the metabolism of sialic acid, either due to genetic defect or at the post-translational level, may impair physiological function and lead to disease. 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 overall increased serum SA concentration. 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 SA from liver into general circulation leading to their increased levels.[13]

The main mechanism associated with the role of SA is in maintaining the negative charge of renal glomerular basement membrane which is one of the main regulators of membrane permeability. Thus, in diabetes, elevated levels indicate excessive damage of the vascular cells of kidneys leads to diabetic nephropathy. Therefore, SA is established as a potential risk factor for the development of macro- and microvascular complications of diabetes. Elevated plasma SA concentration is strongly related to the presence of microvascular complications in type I diabetes.[14] Also, links between SA and risk factors for vascular disease, such as blood lipids, smoking, hyperfibrinogenemia, and lipoprotein have been reported earlier.

SA levels found to be elevated in NIDDM patients with microalbuminuric when compared to controls and demonstrated that there is a progressive rise in SA levels with increase in urinary albumin excretion in NIDDM patients.[15] SA levels were significantly increased in diabetics with severity of nephropathy and with the degree of urinary albumin excretion.[16] 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 SA than those without nephropathy.[17] In another study, done by Shahid SM and Mahaboob T, it was shown that SA levels were significantly increased in both diabetic and diabetic nephropathy patients compared to controls.[18] Crook M et.al also found that serum SA was significantly higher in patients with diabetic complications than in those without complications.[19]

The present study is in accordance with the study done by Shahid SM and Shaik R, who found that increased serum SA levels in diabetic hypertensive patients as well as in diabetic nephropathy patients when compared to controls and demonstrated the increasing trends of SA in diabetic patients with the progression of complications such as nephropathy.[18] In diabetes without any complications, SA level had no statistically significant positive correlation with serum urea, creatinine and urinary microalbumin but in diabetes with nephropathy, SA level was well correlated with serum urea, creatinine and urinary microalbumin.[20] Prajna et al., observed similar increase in serum SA levels, in type-2 diabetics without any complications and type-2 diabetics with nephropathy, when compared to controls.[21] Similar to the present study Masuda *et al*., have shown that serum total SA reflects the status of blood glucose control and the progression of the ischemic disease of the lower extremities in Type-2 Diabetes.[22] Both SA and hs-CRP levels were found significantly correlated with fasting and post-prandial blood sugar, HbA1c, and urine microalbumin levels in both diabetes mellitus and diabetic nephropathy groups.[23] Our results are also in consistent with study conducted by Roozbeh and his colleagues who demonstrated that there is a strong association between elevated serum and urine neuraminidase activity and serum and urine SA levels with the presence of diabetic nephropathy in type diabetic patients.[24] Furthermore, Jafri et al., showed that SA is more closely related with diabetic complications than other parameters which show acute stage and therefore elevated level of SA may be taken as an important indicator of diabetic nephropathy.[25]

Conclusion

In conclusion, estimation of SA prior to microalbumin in diabetic patients is helpful in assessing the glycemic control and to identify the risk for nephropathy and other secondary complications of diabetes mellitus, which are the main causes for 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 SA as a biomarker in early detection of diabetic nephropathy.

Conflict of Interest: None.

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