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Research Article

CORRELATION OF SERUM SIALIC ACID WITH GLYCEMIC STATUS IN DIABETIC NEPHROPATHY

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Abstract: Diabetes mellitus is a major public health problem, the prevalence of which is rising continuously in both developed and developing countries. Diabetes mellitus is a chronic metabolic disorder that leads to severe cardiovascular, renal, neurologic and retinal complications. Diabetic nephropathy is the most common cause of end-stage renal disease requiring dialysis. In diabetes mellitus, acute phase reactants are considered as the indicators of microvascular angiopathy. Serum sialic acid, an acute phase reactant found to be increased in various conditions likes diabetes mellitus, cardiovascular diseases, cancer etc. Screening for the earliest stages of renal damage and aggressive control of blood glucose and blood pressure can help prevent more severe renal involvement. Hence the study was conducted to evaluate serum sialic acid and glycated hemoglobin (HbA1c) levels and to assess the correlation of serum sialic acid with glycemic control in diabetic nephropathy patients. In the present study 100 participants were involved of which 50 were diagnosed to have diabetic nephropathy and 50 were age and sex matched healthy controls. Blood samples were analyzed to estimate fasting blood sugar (FBS), postprandial blood sugar (PPBS), blood urea, serum creatinine, serum sialic acid and glycated hemoglobin levels and blood pressure (both systolic and diastolic) 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 was performed to establish the relationship between the study variables. In diabetic nephropathy patients statistically significant increase in levels of FBS, PPBS, blood urea, serum creatinine, serum sialic acid, HbA1c, systolic and diastolic blood pressure was observed when compared to controls ($p<0.001$). A positive correlation was found between glycemic status and serum sialic acid levels in diabetic nephropathy patients.

Keywords: Diabetic nephropathy, Sialic acid, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorder of carbohydrate metabolism in which glucose is underutilized producing hyperglycemia. Type 1 diabetes mellitus results from cellular mediated autoimmune destruction of pancreatic β-cells of islets of langerhans and results in loss of insulin production. Type 2 diabetes mellitus is the most common form of diabetes accounting for 90% of cases. One of the more debilitating aspects of diabetes is that of numerous complications that can arise from the disease, which includes diabetic retinopathy, diabetic nephropathy and peripheral neuropathy. It also increases the risk of stroke, myocardial infarction and peripheral vascular diseases. The development and severity of these complications are dependent on the duration of the disease and how well it is managed. Prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and is expected to rise to 5.4% by the year 2025.[1,2] The worldwide prevalence of diabetes mellitus has risen dramatically from an estimated 30 million cases in 1985 to 177 million in 2000. Based on the current trends, >360 million individuals will have diabetes by the year 2030.[3] In India alone, diabetes is expected to increase from 40.6 million in 2006 to 79.4 million in 2030.[4] Diabetic nephropathy has been the leading cause of deaths due to end stage renal disease (ESRD) in diabetes that affects more than 40% of diabetic patients.[5] Diabetic nephropathy is characterized by proteinuria >300mg/24h, increased blood pressure and progressive decline in renal function eventually leading to end stage renal disease.[6] Hyperglycemia by causing increased mitochondrial production of reactive oxygen species (ROS) causes strand breaks in nuclear DNA, which activates Poly ADP-ribose polymerase (PARP). PARP then modifies glyceraldhyde - 3 - phosphate dehydrogenase (GAPDH), and reduces its activity.[3] This decreased GAPDH causes increased hexosamine pathway flux, increased polyol pathway flux, increased intracellular advanced glycation end product formation, (AGEs) and activation of protein kinase C.[3,7] These pathways in combination, lead to extracellular matrix accumulation and increased renal albumin permeability resulting in proteinuria, glomerulosclerosis and tubulointerstitial fibrosis.[8] Serum sialic acid is a marker of the acute phase response. Sialic acid is released from the terminal oligosaccharide chains of some

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glycoproteins and glycolipids in the acute phase of inflammation.[9] Sialic acid acts as a cofactor of many cell surface receptors and positively associated with most of the serum acute phase reactants. Serum sialic acid and several serum acute phase proteins are elevated in diabetes. 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.[10] 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, 16-17,20-23] Both glycemic control and rigorous control of blood pressure have significant impact on prevention and progression of diabetic nephropathy. Hence the study was undertaken to estimate serum sialic acid and glycated hemoglobin levels and to correlate serum sialic acid with glycated hemoglobin in diabetic nephropathy cases.

MATERIALS AND METHODS

With the approval of institutional ethical and research committee 50 clinically diagnosed diabetic nephropathy cases attending medicine department in Adichunchanagiri hospitals 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 from patients. Patients suffering from acute and chronic inflammatory conditions, other metabolic conditions like cerebrovascular accidents, ketoacidosis, preeclamptic patients, pre-existing chronic kidney disease, chronic glomerulonephritis, nephrotic syndrome, chronic renal failure and primary hypertensives were excluded from the study. Blood sample was drawn under aseptic precautions from controls and clinically diagnosed diabetic nephropathy cases. 2ml of blood was collected in both fasting and post prandial state with an anticoagulant sodium fluoride for the estimation of blood glucose (Glucose Oxidase method). Whole blood was taken in heparin-coated tube for the estimation of glycated hemoglobin (Affinity chromatography). 2 ml of blood was taken without anticoagulant and serum was separated and used for the estimation of serum sialic acid (Modified Thiobarbituric acid assay of Warren), blood urea (Glutamate dehydrogenase (GLDH) Urease method) and serum creatinine (Jaffe's method). Systolic and diastolic blood pressure was recorded in both cases and controls. Statistical analysis was done and statistical significance was compared between cases and controls using student "t" test. Pearson correlation was performed to establish the relationship between the study variables using 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 for the analysis of the data. Results were represented as mean±SD. 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.

RESULTS

In the present study 50 diabetic nephropathy cases and 50 healthy controls were included to evaluate FBS, PPBS, blood urea, serum creatinine, HbA1c, serum sialic acid, systolic and diastolic blood pressure. There was a statistically highly significant increase ($p<0.001$) in levels of FBS, PPBS, blood urea, serum creatinine, serum sialic acid, HbA1c, systolic and diastolic blood pressure in cases when compared to controls as shown in the Table1. Correlation study as shown in the Table2 revealed a significant positive correlation between serum sialic acid with glycated hemoglobin and FBS, PPBS, blood urea, serum creatinine, systolic and diastolic blood pressure in cases indicating that as sialic acid increases, glycated hemoglobin and other parameters also increases.

DISCUSSION

Diabetes mellitus, a lifelong progressive disorder is the result of body's inability to produce insulin or use insulin to its full potential, and is characterized by high circulating glucose.[7] Pathophysiologic event in diabetic nephropathy is basement membrane damage leading to progressive thickening of basement membrane, change in mesangial and vascular cells, formation of AGEs, accumulation of polyols via aldose reductase pathway and activation of protein kinase C.[11,12,13] Passage of macromolecules through the basement membrane activate inflammatory pathways which contributes to damage secondarily.[14] Between 20 and 40% of patients with diabetes ultimately develop nephropathy.[15] In the present study the mean values of FBS and PPBS 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 ($P<0.001$). 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. The complication of diabetes depends not only by the duration of diabetes mellitus but also by the mean average level of chronic glycemia. 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. An elevated HbA1c indicates

poor control of diabetes mellitus. The risk of retinopathy and renal complications are proportionately increased with elevated HbA1c levels. In the present study the mean HbA1c values were 5.50±0.45 and 11.17±1.63 in controls and in cases which is statistically highly significant (P<0.001) and correlated well with a clinical diagnosis. This is in accordance with the several studies done by Shahid SM, Mahaboob T[9], Chen JW, Gall MA[16] and Melidonis A, Tournis S[17] who found increased HbA1c levels in diabetic patients with and without nephropathy compared to controls. Blood urea measurement has been widely used as an indicator of kidney function. In this study the mean blood urea values were 23.79±4.44 and 61.63±12.37 in controls and 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 and 2.83±0.68 in controls and in cases, which is statistically highly significant (P<0.001). This is in accordance with several studies, which have shown increase in serum creatinine levels in diabetic nephropathy patients compared to healthy controls.[9,17] Sialic acid, an acetylated derivative of neuraminic acid, is an essential component of glycoproteins and glycolipids. Serum sialic acid is a marker of the acute phase response. 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.[18] The diabetic process stimulates cytokine production from cells throughout the body and these cytokines play a direct role in the development of vascular complications. The vascular endothelium carries a high concentration of sialic acid hence extensive microvascular damage associated with diabetes results in its shedding into the circulation. This leads to an increase in vascular permeability and increased serum sialic acid concentration.[19] Present study revealed an increase in serum sialic acid levels (3.06±0.35) in cases compared to controls (1.90±0.30) which is statistically highly significant (P<0.001) and correlated well with a clinical diagnosis. This is in accordance with the several study done by Shahid SM and Mahaboob T,[9] Chen JW, Gall MA,[16] Melidonis A, Tournis S[17] Shahid SM and Shaik R,[20] Shivananda Nayak B and Geetha Bhaktha,[21] Krishnamurthy U, Halyal SS[22], M Usaman K and Mansoor A[23] who have shown increased serum sialic acid levels in diabetic patients with the progression of complications such as nephropathy. Hypertension can aggravate progression of diabetic nephropathy. In our study the mean Systolic BP values were 116.00±6.32 and 155.36±14.78 in controls and in cases and mean diastolic BP values were 76.92±4.56 and 92.88±4.92 in controls and in cases, which is statistically highly significant. Our study is in consistent with several studies.[16,17]

Correlation between sialic acid and other study parameters: Correlation study revealed that 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). 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. There is 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. 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 study is in accordance with the study done by Shahid SM and Mahaboob T who showed significant positive correlation between serum sialic acid and FBS, PPBS, blood urea, serum creatinine, HbA1c levels, systolic and diastolic blood pressure in diabetic nephropathy patients compared to controls.[9]

SUMMARY AND CONCLUSION

'Prevention is better than cure' holds good for diabetic nephropathy, as the best way of treatment for this disease is to control the risk factors such as increase in blood glucose and blood pressure level. Hence the study was undertaken to estimate serum sialic acid and glycated hemoglobin levels and to assess the correlation of serum sialic acid levels with glycemic control and other parameters in diabetic nephropathy patients. There was a statistically significant increase in levels of FBS, PPBS, blood urea, serum creatinine, HbA1c, serum sialic acid and both systolic and diastolic blood pressure in cases compared to controls. Correlation study revealed a large positive correlation between serum sialic acid and HbA1c in diabetic nephropathy cases. It is evident from the present study that serum sialic acid concentrations were strongly associated with development of diabetic nephropathy, a microvascular complication of diabetes. The markers of renal dysfunction (urea and creatinine), glycemic control (blood sugar and HbA1c) and hypertension were clinically correlated with increasing concentration of sialic acid hence sialic acid can be used as a marker of renal dysfunction in diabetic nephropathy.

Table 1: 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**
Blood 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**

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

Table 2: 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*

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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