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Original Article

Serum Total Sialic Acid and Highly Sensitive C-reactive Protein: Prognostic Markers for the Diabetic Nephropathy

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ABSTRACT

Background: This study was undertaken to evaluate and establish the role of total sialic acid (TSA) and highly sensitive C-reactive protein (hs-CRP) in type 2 diabetes mellitus (T2DM) and its correlation with complications such as diabetic nephropathy.

Materials and Methods: One hundred fifty-seven patients with T2DM with nephropathy (DN) and 162 patients of T2DM without nephropathy (DM) along with 165 unrelated age and sex-matched healthy controls were included in the study. Serum glucose (fasting and postprandial) levels, renal profile, and lipid profile were done as per standard protocol. Serum TSA test levels and hs-CRP level were evaluated using thiobarbituric acid assay and immunoturbidimetric method respectively.

Results: We observed a higher concentration of serum TSA (82.67 ± 6.63 mg/dl) and hs-CRP (3.2 ± 1.44 mg/L) in diabetic nephropathy than the diabetes mellitus group (73.83 ± 6.90 mg/dl and 2.07 ± 1.32 mg/L, respectively). Both TSA and hs-CRP levels were found significantly correlated with fasting and postprandial blood sugar, hemoglobin A1c, and urine microalbumin levels in both DM and DN groups. Multinomial logistic regression analysis showed that both TSA and hs-CRP was independently associated with diabetic nephropathy.

Conclusion: High serum TSA and hs-CRP levels may increase the microangiopathic (diabetic nephropathy) complications of T2DM.

Key words: Diabetic nephropathy, highly sensitive C-reactive protein, total sialic acid, type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus (DM) has become a pandemic, most common noncommunicable disease, and around 347 million people worldwide have diabetes.[1] WHO projects that diabetes will be the 7th leading cause of death in 2030.[2]

Type 2 diabetes comprises 90% of people with diabetes around the world,[3] and is largely the result of excess body weight and physical inactivity. The long-term damage is caused by chronic hyperglycemia and results in dysfunction and failure of various organs especially the eyes, kidneys, nerves, heart, and blood vessels.[4] Diabetic nephropathy is one of the three major microangiopathies of

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diabetes mellitus and occurs in approximately 30% of type 2 DM (T2DM) patients.[5] Diabetic nephropathy is usually first recognized as proteinuria. Urinary albumin excretion may be then, an indicator of renal disease in T2DM patients and, in fact, may reflect a state of generalized vascular damage occurring throughout the body.

Acute inflammation is the immediate and early response to an injurious agent. T2DM is frequently associated with an inflammatory status; there is a cytokine associated acute phase reaction, part of the innate immune response.[6] Among several markers of inflammation, highly sensitive C-reactive protein (hs-CRP) is found to be significant in people with diabetes.[7] Several studies demonstrate that hs-CRP remained a significant predictor of diabetes risk even after adjusting with body mass index, family history of diabetes mellitus, smoking, and other factors.[8-10]

Sialic acid is a generic term for a family of acetylated derivatives of neuraminic acid. It is an essential component of glycoproteins and glycolipids. It is located in the terminal nonreducing ends of carbohydrate chains being linked to other sugars most commonly galactose and N-acetyl galactosamine. It acts as a cofactor of many cell surface receptors, e.g., insulin receptor and is positively associated with most of the serum acute phase reactants. In human plasma, large quantity of sialic acid is found as a component of orosomucoid, alpha-1-antitrypsin, haptoglobin, ceruloplasmin, fibrinogen, complement proteins, and transferrin.[11,12] Some of these sialylated glycoproteins are called acute phase reactants, and such substances rapidly increase in concentration after the onset of an inflammatory reaction or injury. Hypothesis has also been made that a cytokine-induced acute phase response is an integral part of the pathophysiology of T2DM, which leads to elevated serum sialic acid level.[12,13]

In diabetic nephropathy, there is a greater increase in sialic acid due to the damage of the vascular endothelial cells of the kidney and it is considered as a newly established potential risk factor for the development of diabetic nephropathy.[14]

Therefore, the aim of our study was to see the association of markers of acute phase response to serum total sialic acid (TSA) and hs-CRP levels in T2DM patients with and without nephropathy and compared them with controls.

MATERIALS AND METHODS

Subjects

Between July 2011 and Jun 2013, we recruited 157 patients with T2DM with nephropathy (DN) and 162 patients of T2DM without nephropathy (DM) from Outpatient Department of Medicine, M. Y. Hospital, Indore, Madhya Pradesh, India. This study was conducted with the approval of the Institutional Ethical Committee. Written informed consent was taken from the subjects prior to the study.

The inclusion criteria for patients were the onset of diabetes after the age of 35 years and no episodes of ketoacidosis. Patients on any kind of multivitamin, lipid lowering agents, anti-inflammatory drugs, analgesics, anticoagulants like aspirin, pregnant or lactating women, alcoholics, smokers and individuals with tobacco or drug addiction, past or present history of chronic illness like tuberculosis, rheumatoid arthritis other autoimmune disorders and patients of juvenile and type 1 DM were excluded from the study group.

Diabetic nephropathy is clinically defined by the presence of persistent microalbuminuria (>30 mg/day) in a diabetic patient in the absence of clinical or laboratory evidence of other kidney or urinary tract disease. For comparison, we recruited 165 unrelated age and sex matched healthy controls.

Fasting venous blood sample was drawn from all the subjects in EDTA tube (2 ml) and plain tube (5 ml), the serum was carefully separated and transferred to microtubes and stored at −20°C until analysis. Postprandial venous blood sample was collected 2 h after the meal. Total serum cholesterol, triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), fasting, and postprandial glucose were analyzed on the fully automated analyzer (Biosystems A25, Barcelona, Spain). Serum hs-CRP levels were analyzed by Turbidimetry Analysers (Quantimate, Tulip, India). Serum TSA levels were estimated using thiobarbituric acid assay method as described by Warren.[15]

Statistical analysis

All the data were entered in SPSS 20.0 (IBM Statistics, SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by Bonferroni test was applied to see the difference in means of various biochemical parameters in DM, DN, and control groups. Karl Pearson correlation test was applied to find out the linear correlation of different parameters.

The multinomial logistic analysis was performed with bootstrapping of 1000 samples to see the factors independently associated with diabetic nephropathy.

RESULTS

The mean age of DM and DN patients was 53.18 ± 10.4 and 54.59 ± 7.85 years, respectively. There was no significant difference in age and sex between all groups [Table 1].

We observed a significant higher fasting and postprandial blood glucose, as well as hemoglobin A1c (HbA1c) in DN group as compared to DM and control groups. The mean duration of diabetes was 5.00 ± 2.2 and 6.55 ± 2.7 years in DM and DN groups, respectively.

TG levels were found significantly different in all three groups [Table 1], and it was highest in diabetic nephropathy group (177.7 ± 112.36 mg/dl). Total cholesterol and LDL levels were similar in all three groups. However, HDL cholesterol levels were found significantly low in DM ($P < 0.0001$) and DN ($P < 0.0001$) group when compared from controls.

TSA was found significantly raised in DN group (82.67 ± 6.63 mg/dl) when compared from controls (60.68 ± 4.92 mg/dl). TSA was also found significantly higher in DM group as compared to controls ($P < 0.0001$) but it was within the normal limits.

TSA was found significantly correlated with fasting blood sugar (FBS), postprandial blood sugar (PPBS), HbA1c, urine microalbumin, and hs-CRP in both DM and DN groups [Table 2]. However, no correlation of serum TSA was observed with FBS, PPBS, and HbA1c in the control groups. Similarly, hs-CRP levels were also found significantly correlated with FBS, PPBS, HbA1c, urine

Table 1: Demographic and biochemical profile in three groups

Parameter	Control	DM	DN	*P*		
				Control versus DM	Control versus DN	DM versus DN
Age (years)	54.35 (11.0)	53.18 (10.4)	54.59 (7.85)	0.851	1.00	0.612
Sex (male: female)	83:82	78:84	60:97	0.780	0.038	0.093
FBS (mg/dl)	85.97 (9.57)	152.05 (57.9)	7.50 (64.7)	<0.0001	<0.0001	<0.0001
PPBS (mg/dl)	114.56 (13.59)	239.25 (97.02)	287.49 (116.91)	<0.0001	<0.0001	<0.0001
HbA1c (%)	5.73 (0.35)	8.46 (1.5)	9.21 (1.84)	<0.0001	<0.0001	<0.0001
Creatinine (mg/dl)	0.88 (0.11)	0.93 (0.24)	1.46 (0.92)	1.000	<0.0001	<0.0001
Urine microalbumin (mg/day)	9.85 (3.4)	11.69 (4.09)	175.91 (234.84)	1.000	<0.0001	<0.0001
Urea (mg/dl)	23.5 (3.6)	24.93 (7.9)	45.95 (28.10)	1.000	<0.0001	<0.0001
Triglyceride (mg/dl)	128.30 (60.14)	154.91 (82.14)	177.7 (112.36)	0.018	<0.0001	0.058
Total cholesterol (mg/dl)	185.22 (36.31)	182.29 (44.78)	187.41 (52.93)	1.000	1.000	0.926
LDL (mg/dl)	116.31 (27.44)	113.40 (33.26)	113.84 (40.91)	1.000	1.000	1.000
HDL (mg/dl)	44.97 (6.66)	40.91 (8.17)	39.96 (7.7)	<0.0001	<0.0001	0.078
VLDL (mg/dl)	25.66 (12.02)	30.98 (16.42)	35.56 (22.47)	0.018	0.0000	0.058
TSA (mg/dl)	60.68 (4.92)	73.83 (6.9)	82.67 (6.63)	<0.0001	<0.0001	<0.0001
hs-CRP (mg/L)	1.16 (0.50)	2.07 (1.32)	3.20 (1.44)	<0.0001	<0.0001	<0.0001

DM: Diabetes mellitus, DN: Diabetic nephropathy, FBS: Fasting blood sugar, PPBS: Postprandial blood sugar, HbA1c: Hemoglobin A1c, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, hs-CRP: Highly sensitive C-reactive protein, TSA: Total sialic acid

Table 2: Correlation of hs-CRP and TSA with other parameters

	hs-CRP			TSA		
	Control	DM	DN	Control	DM	DN
FBS	0.225 (0.004)	0.183 (0.020)	0.371 (<0.0001)	−0.017 (0.830)	0.198 (0.011)	0.289 (<0.001)
PPBS	0.505 (0.181)	0.165 (0.036)	0.382 (<0.0001)	−0.024 (0.760)	0.206 (0.009)	0.245 (0.002)
HbA1c	0.08 (0.287)	0.335 (<0.0001)	0.375 (<0.0001)	−0.021 (0.792)	0.333 (<0.0001)	0.218 (0.006)
Micro-albumin	0.965 (<0.0001)	0.053 (<0.0001)	0.801 (<0.0001)	0.340 (<0.0001)	0.972 (0.000)	0.792 (0.000)
Triglyceride	0.085 (0.279)	0.109 (0.169)	0.220 (0.006)	−0.009 (0.906)	0.130 (0.092)	0.246 (0.002)
Cholesterol	0.045 (0.565)	0.067 (0.396)	0.182 (0.023)	0.006 (0.944)	0.092 (0.246)	0.252 (0.001)
HDL	−0.035 (0.654)	0.020 (0.805)	0.028 (0.725)	0.037 (0.641)	0.075 (0.343)	0.082 (0.307)
LDL	0.066 (0.0403)	0.018 (0.805)	0.129 (0.107)	0.150 (0.846)	0.033 (0.680)	0.184 (0.021)
VLDL	0.085 (0.279)	0.109 (0.169)	0.220 (0.006)	−0.009 (0.906)	0.130 (0.092)	0.246 (0.002)
hs-CRP	-	-	-	0.350 (<0.0001)	0.621 (<0.0001)	0.900 (<0.0001)
Sialic acid	0.350 (<0.0001)	0.621 (<0.0001)	0.900 (<0.0001)	-	-	-

Values represent the correlation coefficient. Numbers in () represents the *P* value. hs-CRP: Highly sensitive C-reactive protein, TSA: Total sialic acid, DM: Diabetes mellitus, DN: Diabetic nephropathy, FBS: Fasting blood sugar, PPBS: Postprandial blood sugar, HbA1c: Hemoglobin A1c, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein

microalbumin and total sialic acid in both DM and DN groups [Table 2].

Multinomial logistic regression analysis showed that both TSA and hs-CRP was independently associated with diabetic nephropathy [Table 3].

DISCUSSION

Inflammation plays a major role in the pathogenesis of T2DM and its complications. Hence inflammatory markers or acute phase markers have gained the importance as indicators and predictors of the diabetic process.

It is perceived that chronic low-grade inflammation as evidenced by elevated hs-CRP might potentially be a cause underlying the etiology and manifestation of T2DM, although the exact mechanisms are still not well understood. Martha and Fernando[7] in their study found that hyperglycemia is an associated factor to the increase of serum CRP levels; in uncontrolled type 2 diabetes subjects. Lima *et al*.[16] and Amanullah *et al*.[17] in their study found that hypertensive patients with T2DM had higher levels of hs-CRP than normal subjects. This finding suggests that patients with two associated diseases have a more active inflammatory state. Several studies demonstrate that hs-CRP remained a significant predictor of diabetes risk even after adjusting with BMI, family history of DM, smoking, and other factors.[8] Chiriboga *et al*.,[18] Jager *et al*.[19] and Pfützner and Forst[20] in their studies showed that in people with diabetes, CRP levels in highest tertile (>0.28 mg/dl) were associated with a two-fold increase in cardiovascular mortality after adjusting for age, sex, and glucose tolerance tests. Similar to these previous studies hs-CRP levels were found raised in diabetic mellitus with or without nephropathy. Patients with diabetic nephropathy have significant higher hs-CRP than diabetic mellitus.

Table 3: Multinomial logistic regression analysis

Parameter	B	*P*	95% CI
Age	−0.067	0.251	−0.134-0.201
Sex (male)	−0.211	0.885	−4.530-3.882
FBS (mg/dl)	0.005	0.862	−0.35-0.088
PPBS (mg/dl)	0.006	0.660	−0.053-0.022
HbA1c (%)	0.157	0.833	−1.808-2.044
Triglyceride (mg/dl)	63.984	0.994	0.00-66.62
Cholesterol (mg/dl)	−0.047	0.344	−0.145-0.154
HDL (mg/dl)	0.148	0.362	−0.362-0.306
LDL (mg/dl)	−0.34	0.558	−0.183-0.151
VLDL (mg/dl)	−319.9	0.378	−333.15-0.001
Urine micro-albumin	1.759	0.001	1.460-1.799
hs-CRP (mg/L)	−32.887	0.002	−52.071-25.265
Sialic acid (mg/dl)	1.990	0.017	−1.496-4.729

CI: Confidence interval, FBS: Fasting blood sugar, PPBS: Postprandial blood sugar, HbA1c: Hemoglobin A1c, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very-low-density lipoprotein, hs-CRP: Highly sensitive C-reactive protein

TSA was also found raised in both diabetes mellitus with or without nephropathy. Similar to our study K Prajna *et al*. observed significantly increased TSA levels in diabetes mellitus without any complication and in diabetes mellitus with nephropathy as compared to control.[21] It was also fond positively correlated with blood glucose levels in both the groups. Similar to our study Masuda *et al*.[22] have shown that serum TSA reflects the status of blood glucose control and the progression of the ischemic disease of the lower extremities in T2DM. Crook *et al*. shows that serum TSA is a newly established potential risk factor for the development of macrovascular and microvascular complications of diabetes.[23]

Similar findings have been stated by Chen *et al*.,[13] Nayak and Bhaktha[14] and Krishnamurthy *et al*.[24] Shahid and Mahboob in their study found increased serum TSA as a potential risk factor for the development of macro and microvascular complications of diabetes.[25] In conclusion serum hs-CRP and TSA may be used as a predictive biomarkers of diabetic nephropathy.

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Conflicts of interest

There are no conflicts of interest.

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