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Dr. Shilpa A
Assistant Professor, Department of Biochemistry, VIMS, Bellary, Karnataka, India

Dr. Ayyali Ambresh
Assistant Professor, Department of General Medicine, Bowring Medical College, Bangalore, Karnataka, India

Corresponding Author:
Dr. Ayyali Ambresh
Assistant Professor, Department of General Medicine, Bowring Medical College, Bangalore, Karnataka, India

Serum Sialic acid and plasma fibrinogen levels in type 2 diabetes and diabetic nephropathy

Dr. Shilpa A and Dr. Ayyali Ambresh

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Abstract
Background: Diabetes is one of the most common chronic hyperglycemic syndromes, affecting nearly 347 million people worldwide. Serum sialic acid levels correlate positively with albuminuria, hence serum levels are raised even before clinical nephropathy is diagnosed. Another biological marker of DN is fibrinogen. Hence, the present study was undertaken to estimate serum sialic acid & plasma fibrinogen levels in DM & DN and to know whether these levels could be used as early predictors of DN.
Objectives: To estimate Serum SA, Plasma Fibrinogen levels, FBS, HbA1c, Lipid profile, atherogenic ratios, Blood Urea, Serum Creatinine, eGFR & Urine albumin/Creatinine ratio in type 2 DM, DN patients and healthy controls. To correlate Serum SA and Plasma Fibrinogen levels with FBS, HbA1c, Lipid profile, Blood Urea, Serum Creatinine, eGFR and urine A/C ratio in type 2 DM and DN patients. To find out whether the levels of Serum SA and Plasma Fibrinogen levels can be used as markers for the early diagnosis of DN.
Methodology: A case control study includes total of 150 patients, of which 50 were diabetic without any complications, 50 were diabetic nephropathy patients and remaining 50 were age matched healthy controls.
Results: The duration of diabetes in DN patients was greater and statistically significant when compared to DM without nephropathy. The mean SA & fibrinogen levels in DN patients were increased & statistically significant when compared to DM without nephropathy.

Keywords: Diabetes Miletus, diabetic nephropathy, Salic acid, fibrinogen

Introduction
Diabetes is one of the most common chronic hyperglycemic syndromes, affecting nearly 347 million people worldwide. If unchecked, by 2025, it is expected that diabetes will reach epidemic proportions, affecting 333 million people globally. Much of this increase is expected to occur in developing countries including India [1, 2].
An elevation in the serum sialic acid (SA) concentration has been observed in DN [3]. Serum sialic acid levels correlate positively with albuminuria, hence serum levels are raised even before clinical nephropathy is diagnosed [4]. N-acetylneuraminic acid (referred to as sialic acid) is a negatively charged nine-carbon monosaccharide commonly attached to the carbohydrate chains of glycoproteins and glycolipids. The degree of sialylation is believed to be responsible for the negative charge of glycoproteins and for the pathogenesis of atherosclerosis. Another biological marker of DN is fibrinogen. Fibrinogen, is increased in diabetic patients [15].An increase in plasma fibrinogen levels is also considered an independent risk factor for diabetic nephropathy [6].
Hence, the present study was undertaken to estimate serum sialic acid & plasma fibrinogen levels in DM & DN and to know whether these levels could be used as early predictors of DN. Along with these, the other biochemical markers like fasting blood sugar, Glycated haemoglobin, lipid profile, blood urea, serum creatinine, atherogenic ratios, eGFR and urine albumin-creatinine ratio were also estimated & correlated with serum sialic acid & plasma fibrinogen levels in DM & DN.

Objectives
- To estimate Serum SA, Plasma Fibrinogen levels, FBS, HbA1c, Lipid profile, atherogenic ratios, Blood Urea, Serum Creatinine, eGFR & Urine albumin/Creatinine ratio in type 2 DM, DN patients and healthy controls.

- To correlate Serum SA and Plasma Fibrinogen levels with FBS, HbA1c, Lipid profile, Blood Urea, Serum Creatinine, eGFR and urine A/C ratio in type 2 DM and DN patients.
- To find out whether the levels of Serum SA and Plasma Fibrinogen levels can be used as markers for the early diagnosis of DN.

Materials and methodology

Study design

A case control study.

The study includes total of 150 patients, of which 50 were diabetic without any complications, 50 were diabetic nephropathy patients and remaining 50 were age matched healthy controls.

Study period & duration

The study was conducted from 1st January 2015 to 31st December 2015

Study site

Study was conducted in Department of Biochemistry of a tertiary care hospital. Patients were recruited from out-patient department (OPD) and inpatient department (IPD) of medicine and nephrology of tertiary care hospital.

Ethical committee approval

The permission of Institutional Ethics Committee (IEC) was taken before starting the study. Ethical Committee Approval No-VIMS/PG/IEC/14/2014-15 dated 07.11.2014

Selection criteria

Inclusion criteria

Patients of both gender aged above 30 years, diagnosed as type 2 diabetes mellitus by clinicians according to American Diabetes Association (ADA) guidelines and

Patients diagnosed as diabetic nephropathy by clinicians.

Exclusion criteria

- Type 1 diabetes mellitus
- Patients with severe complications of diabetes mellitus other than nephropathy
- Pregnant women
- Patients with history of - acute febrile illness, current episode of urinary tract infection, pyelonephritis, urinary tract obstruction, congestive heart failure or acute coronary syndrome
- Patients with gout& patients on anti-inflammatory drug or allopurinol
- History of kidney transplant
- Albuminuria documented due to causes that are other than diabetes

Methodology

Patients attending medicine and nephrology departments were examined. Patients satisfying inclusion & exclusion criteria were included in the study.

Results

The study includes total of 150 patients, studied in 3 groups. Group I- 50, age & sex matched healthy controls; group II- 50, diabetic patients without any complications and group III- 50, diabetic nephropathy patients. The mean age of subjects in 3 groups - control, DM & DN were 40.5 ± 10.9 years, 51.46± 11.3 years& 51.9 ± 8.38 years respectively as shown in table no 1.

Table 1: Mean Age in Study groups

Particulars	Control	DM	DN
Age (in yrs)	40.5 ± 10.9	51.46 ± 11.3	51.9 ± 8.38

The age group of all study subjects ranged from 25 to 70years & majority of study subjects were in the age groups of 41-50 years as shown below in table no 2.

Table 2: Age Distribution in Study groups

Age (yrs)	Control	DM	DN	Percentage
25-30	11	2	-	2%
31-40	15	11	5	12%
41-50	17	18	17	34%
51-60	6	8	18	30%
61-70	1	11	10	22%
Total	50	50	50	100%

The gender distribution in study groups was almost similar. The number of male patients included in study groups-control, DM & DN groups was 35, 36 & 37 respectively. The number of female patients included in study groups-control, DM & DN groups was 15, 14 & 13 respectively as shown in table no 3.

Table 3: Gender distribution in study groups

Gender	Control	Percentage	DM	Percentage	DN	Percentage
Males	35	70	36	72	37	74
Females	15	30	14	28	13	36
Total	50	100	50	100	50	100

The duration of diabetes in DM group was 3.58 ± 3.13years whereas in DN group it increased to 10.14 ± 3.07 years which was statistically significant with a p value of 0.0001 as shown in table no 4.

Table 4: Mean Duration of diabetes in study groups

	DM	DN	P value
Duration of Diabetes (years)	3.58 ± 3.13	10.14 ± 3.07	0.0001

Based on the duration of diabetes, the subjects in DM group were divided as shown in table no 5.

Table 5: Distribution based on Duration of diabetes in DM group

Duration of DM (years)	No of patients in DM group
< 1	3
1-2	9
2-3	13
3-4	10
4-5	8
5-10	4
10-15	3
Total	50

The subjects in DN group were studied according to the duration of diabetes as shown in table no 6. Many of the study subjects included in this group were of 10-13 years of study subjects included in this group were of 10-13years of diabetes.

Table 6: Distribution based on Duration of diabetes in DN group

Duration of DM (years)	DN group
< 5	1
5-8	8
8-10	11
10-13	17
13-15	13
Total	50

The study subjects of 2 groups (DM & DN) were compared based on duration of diabetes, which is shown below; as the duration of diabetes increases the incidence of nephropathy also increased as shown in the table no 7.

Table 7: Comparison of Distribution of Duration of diabetes between 2 groups

Duration of DM (Yrs)	DM group	DN group
1-3	25	-
3-5	18	1
5-8	2	8
8-10	2	11
10-13	3	17
13-15	-	13
Total	50	50

The study subjects in DN group was distributed based on the duration of nephropathy as shown in table no 8, which showed majority of patients included in the study were suffering from nephropathy since 1-2 years.

Table 8: Distribution of DN patients based on duration of nephropathy

Duration of nephropathy (years)	No of patients
<1	6
1-2	22
2-3	15
3-4	4
4-5	1
5-6	2

The mean sialic acid level in control group was 37.24 ± 10.33 mg/dl, whereas in DM & DN groups were 94.06 ± 26.64 mg/dl and 107.25 ± 35.28 mg/dl respectively as shown in table no 9. The comparison of SA levels between groups (C-DM, C-DN & DM –DN) was statistically significant with p value of <0.001

Table No 9: Serum Sialic acid levels in study groups

	Controls	DM	DN
S. Sialic acid (mg/dl)	37.24 ± 10.33	94.06 ± 26.64*	107.25 ± 35.28*†

Statistical significance * p< 0.0001 compared to controls; †p< 0.05 compared to DM group

The mean plasma fibrinogen level in control group was 190.34 ± 72.83mg/dl. The mean plasma fibrinogen levels in DM & DN groups were 522.76 ± 115.79 mg/dl & 657.64 ± 124.61 mg/dl respectively as shown in table no 10. There was statistically significant increase of fibrinogen levels in DM & DN groups with a p value of 0.0001.

Table 10: Plasma Fibrinogen levels in study groups

	Controls	DM	DN
P. Fibrinogen (mg/dl)	190.34 ± 72.83	522.76±115.79*	657.64±124.61*†

Statistical significance * p<0.0001 compared to controls; †p< 0.0001 compared to DM group.

FBS was studied in all 3 groups. The mean FBS levels in control, DM & DN groups were 71.94 ± 15.6 mg/dl, 123.38 ± 44.36mg/dl & 178.3 ± 66.57mg/dl respectively as shown in the table no 11. FBS levels were increased in DM group & DN group when compared to control group which was statistically significant with a p value of 0.0001. The increased FBS levels in DN group when compared to DM group was also statistically very significant.

Table 11: FBS levels in study groups

	Controls	DM	DN
FBS (mg/dl)	71.94 ± 15.6	123.38 ± 44.36*	178.3 ± 66.57*†

Statistical significance * p< 0.0001 compared to controls; †p<0.0001 compared to DM patients.

The mean HbA_{1c} level in control group was 5.95 ± 0.29%. The mean HbA_{1c} levels in DM & DN groups were 7.60 ± 0.51%& 7.83 ± 0.48% respectively. HbA_{1c} lsevel was increased in DM & DN groups when compared to controls with a p value of 0.0001. There was only a slight increase of HbA_{1c} levels in DN group when compared to DM group as shown in table no 12. P value between groups was statistically significant.

Table 12: HbA_{1c} levels in study groups

	Controls	DM	DN
HbA_{1c} (%)	5.95 ± 0.29	7.60 ± 0.51*	7.83 ± 0.48*†

Statistical significance * p< 0.0001 compared to controls; †p< 0.05 compared to DM patients.

Lipid profile was estimated in all 3 groups. The mean total Cholesterol levels of control, DM & DN groups were 101 ± 20.13 mg/dl, 192.46 ± 49.5 mg/dl & 235.62 ± 53.23 mg/dl respectively as shown in the table no 13.

The mean triglyceride levels of control, DM & DN groups were 121.78 ± 17.16mg/dl, 194.64 ± 25.95 mg/dl &249.38 ± 8.92 mg/dl respectively as shown in the table no 13.

The mean LDL levels of control, DM & DN groups were 56.36 ± 28.41mg/dl, 110.86 ± 28.21mg/dl & 129.78 ± 34.30 mg/dl respectively as shown in the table no 13. TC, triglyceride levels&LDL levels were increased in DM group & DN group when compared to control group, further increased in DN group when compared to DM group which was statistically significant with a p value of <0.001.

The mean HDL levels in control group was 30.2 ± 4.9 mg/dl whereas in DM & DN groups were 24.48 ± 3.97 mg/dl & 19.67 ± 2.99 mg/dl respectively as shown in table no 13. The HDL levels were decreased in DM & DN group when compared to controls which was statistically significant. HDL level was higher in DM group than compared to DN group.

Table 13: Lipid profile levels in study groups

	Controls	DM	DN
TC (mg/dl)	101 ± 20.13	192.46 ± 49.5*	235.62 ± 53.23*†
Triglyceride (mg/dl)	121.78 ± 17.16	194.64 ± 25.95*	249.38 ± 8.92*†
LDL (mg/dl)	56.36 ± 28.41	110.86 ± 28.21*	129.78 ± 34.30*†
HDL(mg/dl)	30.2 ± 4.9	24.48 ± 3.97*	19.67 ± 2.99*†

Statistical significance * $p< 0.0001$ compared to controls; †$p< 0.001$ compared to DM patients

The mean Blood Urea level in control group was 21.48 ± 4.89 mg/dl. The mean B Urea levels in DM & DN groups were 34.04 ± 10.91 mg/dl & 75.86 ± 31.24 mg/dl respectively as shown in table no 14. The mean Serum Creatinine levels of control, DM & DN groups were 0.82 ± 0.22mg/dl, 1.20 ± 0.21mg/dl & 5.39 ± 2.42mg/dl respectively as shown in the table no 14. Blood Urea &Serum Creatinine levels were increased in DM group & DN group when compared to control group with further increase in DN group.

Table 14: Blood Urea & Serum Creatinine levels in study groups

	Controls	DM	DN
B Urea (mg/dl)	21.48 ± 4.89	34.04 ± 10.91*	75.86 ± 31.24*†
S Creatinine (mg/dl)	0.82 ± 0.22	1.20 ± 0.21*	5.39 ± 2.42*†

Statistical significance * $p< 0.0001$ compared to controls; †$p< 0.0001$ compared to DM patients.

The mean TC/HDL ratios in controls, DM & DN groups was 3.41± 0.85, 8.03 ± 2.40 & 12.20 ± 3.24 respectively which was statistically significant as shown in table no 15. The mean LDL/HDL ratio in controls was 1.94 ± 1.10, whereas in DM & DN group was 4.63 ± 1.42 & 5.39 ± 1.29 respectively with a significant p value as shown in table no 18. There was significant increase in both the ratios in DM & DN group when compared to controls. Further, increase was observed in DN group.

Mean eGFR in controls was 111.36 ± 38.81ml/min, which was decreased in DM group (62.20 ± 14.83ml/min), further reduced in DN group (13.97 ± 7.12) as shown in table no 15. This reduced eGFR values were statistically significant when compared between groups.

Table 15: TC/HDL, LDL/HDL ratios &e GFR in study groups

	Controls	DM	DN
TC/HDL	3.41 ± 0.85	8.03 ± 2.40*	12.20 ± 3.24*†
LDL/HDL	1.94 ± 1.10	4.63 ± 1.42*	5.39 ± 1.29*†
eGFR (ml/min)	111.36 ± 38.81	62.20 ± 14.83*	13.97 ± 7.12*†

Statistical significance * $p< 0.0001$ compared to controls; †$p< 0.01$ compared to DM patients

The mean urine A/C ratio was increased in DN group (0.41 ± 0.16 mg/g Cr) when compared to DM group (0.12 ± 0.07 mg/g Cr) and controls (0.072 ± 0.06 mg/g Cr) as shown in table no 16.

Table 16: Urine A/C ratio in study groups

	Controls	DM	DN
Urine A/C mg/g Cr	0.072 ± 0.06	0.12 ± 0.07*	0.41 ± 0.16*†

Statistical significance * $p< 0.001$ compared to controls; †$p< 0.0001$ compared to DM patients. Mean, standard deviation (SD) of all parameters is shown in table no 17.

Table 17: Mean, standard deviation (SD) of all the parameters

Sl. No	Assay parameters	Controls		DM		DN	
		Mean	SD	Mean	SD	Mean	SD
1.	Serum Sialic acid	37.24	10.33	94.06	26.64	107.25	35.28
2.	Plasma Fibrinogen	190.34	72.83	522.76	115.79	657.05	131.50
3.	FBS	71.94	15.66	123.38	44.36	174.75	65.83
4.	HbA_{1c}	5.954	0.29	7.606	0.512	7.86	0.49
5.	TC	101	20.13	192.46	49.57	231.525	53.19
6.	Triglyceride	121.78	17.16	194.64	25.95	249.38	86.92
7.	LDL	56.36	28.41	110.86	28.21	128.275	34.03
8.	HDL	30.2	4.90	24.48	3.97	19.675	2.99
9.	Blood Urea	21.48	4.89	34.04	10.917	77	32.03
10.	Serum Creatinine	0.82	0.22	1.204	0.210	5.63	2.53
11.	TC/HDL	3.41	0.85	8.03	2.404	12.01	3.18
12.	LDL/HDL	1.94	1.106	4.63	1.421	5.28	1.35
13.	eGFR	111.36	38.81	62.20	14.83	13.52	7.26
14.	Urine A/C ratio	0.072	0.060	0.12	0.072	0.42	0.16

Table 18: P values of all parameters between groups

	Control & Dm	C & DN	DM & DN
Sialic acid	0.0001	0.0001	0.0463
Firbinogen	0.0001	0.0001	0.0001
FBS	0.0001	0.0001	0.0001
HbA1c	0.0001	0.0001	0.0179
TC	0.0001	0.0001	0.0005
Triglyceride	0.0001	0.001	0.0004
LDL	0.0001	0.0001	0.0094
HDL	0.0001	0.0001	0.0001
B Urea	0.0001	0.0001	0.0001

S Creat	0.0001	0.0001	0.0001
TC/HDL	0.0001	0.0001	0.0001
LDL/HDL	0.0001	0.0001	0.0293
eGFR	0.0001	0.0001	0.0001
U A/C ratio	0.0004	0.0001	0.0001

SA & fibrinogen levels were correlated with levels of FBS, HBA_{1C}, LDL, HDL, TC, Blood Urea, serum creatinine, TC/HDL, LDL/HDL, eGFR& Urine A/C ratio in DM &DN group as shown in table no 19.

Table 19: Correlation coefficients of SA & fibrinogen levels with other risk factors in DM & DN

Risk factors	Correlation coefficient (r) of SA		Correlation coefficient (r) of Fibrinogen	
	DM	DN	DM	DN
FBS	0.04	0.24	0.2	0.2
HbA_{1C}	0.35	0.38	0.41	0.5
TC	0.36	0.42	0.1	0.3
Triglyceride	0.37	0.46	0.40	0.5
LDL	0.05	0.45	0.1	0.24
HDL	-0.12	-0.26	-0.1	-0.23
B Urea	0.16	0.38	0.04	0.31
S Creatinine	0.15	0.5	0.11	0.35
TC/HDL	0.2	0.34	0.11	0.21
LDL/HDL	0.1	0.4	0.3	0.5
eGFR	-0.09	-0.3	-0.25	-0.2
Urine A/C ratio	0.2	0.29	0.33	0.48

Discussion

In the present study, the mean sialic acid level in control group was 37.24 ± 10.33 mg/dl, whereas in DM & DN groups were 94.06 ± 26.64 mg/dl and 107.25 ± 35.28 mg/dl respectively. There was statistically significant increase in SA levels in DM when compared to controls, with further significant increase in DN group. Shivanand *et al* [7] & Crook M *et al.* [8] studies have found that serum SA was significantly higher in patients with diabetic complications than in those without any of the complications which were similar to the findings in our study. The finding of our study is also in accordance with Syed Muhammad Shahid *et al* [9], Crook *et al*, Gavella *et al*, Chan *et al* & Powerie *et al* studies [10, 11-12]. Hyperglycemia and insulin resistance promote inflammation by increased oxidative stress leading to tissue injury which stimulates local cytokine secretion from cellular infiltrates, such as macrophages and endothelial cells. This induces an acute phase response with release of acute phase proteins. The vascular endothelium carries a high concentration of sialic acid, which undergo desialylation leading to increased SA levels in DM & DN.

The mean plasma fibrinogen level in control group was 190.34 ± 72.83mg/dl. The mean plasma fibrinogen levels in DM & DN groups were 522.76 ± 115.79 mg/dl & 657.64 ± 124.61 mg/dl respectively. In our study, fibrinogen levels were increased significantly in DM group compared to controls which was further increased in DN group which is in accordance to study done by Venkataramana G *et al* [14], Laurell *et al* [15], Alper *et al* [16]. Hence above studies interpret that fibrinogen increases in diabetes with complications. Our findings were also similar to studies done by Killingsworth *et al*, Ganda e al, Collier *et al*, Schmidtz *et al* & Eraslan M *et al* [17-20]. The cause of increased fibrinogen production in type 2 DM are insulin resistance, hyperglucagonemia acting as stimulators of fibrinogen production in the liver, and possibly, also a subclinical inflammatory state

Thus diabetic patients should be followed up with two inflammatory early biomarkers- sialic acid & fibrinogen to prevent complications like diabetic nephropathy.

Mean eGFR in controls was 111.36 ± 38.81ml/min, which was decreased in DM group (62.20 ± 14.83ml/min) & further reduced in DN group (13.97 ± 7.12). This reduced eGFR values was statistically significant when compared between groups which was similar to Steven *et al* study. There is increase in vascular permeability & vasoconstriction of afferent arteriole in DM leading to decrease in eGFR.

Conclusion

- The duration of diabetes in DN patients was greater and statistically significant when compared to DM without nephropathy.
- The mean SA & fibrinogen levels in DN patients were increased &statistically significant when compared to DM without nephropathy.
- The mean FBS, HbA_{1c}, TC, triglyceride, LDL, Blood Urea, serum creatinine, TC/HDL, LDL/HDL& urine A/C ratio were significantly increased whereas HDL & eGFR was deceased in diabetic nephropathy patients when compared to DM patients.
- FBS, HbA_{1c}, TC, triglyceride, LDL, Blood Urea, serum creatinine, TC/HDL, LDL/HDL& urine A/C ratio were correlated positively with SA levels in both DM & DN group, whereas there was negative correlation of SA with HDL & eGFR.
- Fibrinogen correlated positively with FBS, HbA_{1c}, TC, triglyceride, LDL, Blood Urea, serum creatinine, TC/HDL, LDL/HDL& urine A/C ratio in both DM & DN group, whereas there was negative correlation of fibrinogen with HDL & eGFR.
- Thus SA & fibrinogen could be used as early biomarkers for the diagnosis of DN.

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Conflict of Interest: None

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