Original Research Article

Assessment of plasma fibrinogen as a marker of diabetic nephropathy

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ABSTRACT

Introduction: Diabetes is one of the most common chronic hyperglycemic syndrome. Diabetic Nephropathy is one of the major complications of DM characterized by persistent albuminuria, increased arterial blood pressure, a relentless decline in glomerular filtration rate (GFR) & a high risk of cardiovascular morbidity & mortality. The biological marker of DN is fibrinogen. Fibrinogen, is increased in diabetic patients. An increase in plasma fibrinogen levels is also considered an independent risk factor for diabetic nephropathy. Fibrinogen is the major coagulation protein in blood.

Objectives: To find out whether the levels of Plasma Fibrinogen levels can be used as markers for the early diagnosis of DN.

Materials and Methods-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.

Results: The mean plasma fibrinogen level in control group was 190.34 ± 72.83 mg/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. Hence above studies interpret that fibrinogen increases in diabetes with complications.

Conclusion: 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 fibrinogen could be used as early biomarkers for the diagnosis of DN.

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1. Introduction

Diabetes is one of the most common chronic hyperglycemic syndrome. Diabetic Nephropathy is one of the major complications of DM characterized by persistent albuminuria, increased arterial blood pressure, a relentless decline in glomerular filtration rate (GFR) & a high risk of cardiovascular morbidity & mortality.¹

The biological marker of DN is fibrinogen. Fibrinogen, is increased in diabetic patients.^{2,3} An increase in plasma fibrinogen levels is also considered an independent

risk factor for diabetic nephropathy.⁴ Fibrinogen is the major coagulation protein in blood. It is a glycoprotein and circulates as a dimer composed of three pairs of polypeptide chains.⁵ It is an acute phase protein & increases during the inflammatory process. Inflammation plays an important role in the development of atherosclerosis. Monocytes infiltrating the atherosclerosis differentiate into macrophages that release cytokines, such as interleukin-6, which increase plasma fibrinogen levels in serum.

Diabetes mellitus leads to dyslipidemia and this dyslipidemia is more in presence of DN.

Lipid ratios are better indicators of atherogenic risk in patients with DN as compared to lipids alone.⁶ Creatinine is

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not raised above the normal range until 60% of total kidney function is lost. Hence, the more accurate means of measuring renal function is eGFR. Microalbuminuria in DN is best investigated by the urinary albumin excretion in relation to creatinine, as assessed from the albumin-to-creatinine ratio.⁷

Hence, the present study was undertaken to estimate plasma fibrinogen levels in DM & DN and to know whether these levels could be used as early predictors of DN.

2. Objectives

To find out whether the levels of Plasma Fibrinogen levels can be used as markers for the early diagnosis of DN.

3. Materials and methods

3.1. Study design

3.1.1. 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.

3.1.2. Study period & duration

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

3.1.3. 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.

3.1.4. 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

3.1.5. Informed consent

All the patients enrolled in the study were explained about the purpose of the study in their own language and a written informed consent was taken as given in annexure II.

3.2. Selection criteria

3.2.1. 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.

3.2.2. Exclusion criteria

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

3.3. Methodology

Patients attending medicine and nephrology departments were examined.

Patients satisfying inclusion & exclusion criteria were included in the study

4. 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 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 70 years & majority of study subjects were in the age groups of 41-50 years as shown below in Table 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%

Table 3: 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 4.

Table 4: 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 5. Many of the study subjects included in this group were of 10-13years of diabetes.

Table 5: 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 6.

Table 6: 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 7 which showed majority of patients included in the study were suffering from nephropathy since 1-2 years.

The mean plasma fibrinogen level in control group was 190.34 ± 72.83 mg/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 8. There was statistically significant increase of fibrinogen levels in DM & DN groups with a p value of 0.0001.

FBS was studied in all 3 groups. The mean FBS levels in control, DM & DN groups were 71.94 ± 15.6 mg/dl,

Table 7: 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

Table 8: Plasma Fibrinogen levels in study groups

	Controls	DM	DN
P. Fibrinogen (mg/dl)	190.34 ± 72.83	$522.76 \pm 115.79^*$	$657.64 \pm 124.61^{*\dagger}$

Statistical significance * $p < 0.0001$ compared to controls; $\dagger p < 0.0001$ compared to DM group

Fig. 1: Plasma Fibrinogen levels in study groups

123.38 ± 44.36 mg/dl & 178.3 ± 66.57 mg/dl respectively as shown in the Table 9. 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 9: FBS levels in study groups

	Controls	DM	DN
FBS (mg/dl)	71.94 ± 15.6	$123.38 \pm 44.36^*$	$178.3 \pm 66.57^{*\dagger}$

Statistical significance * $p < 0.0001$ compared to controls; $\dagger p < 0.0001$ 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} level 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 10. P value between groups was statistically significant.

Table 10: 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.

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 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 11. Blood Urea & Serum Creatinine levels were increased in DM group & DN group when compared to control group with further increase in DN group.

Table 11: 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

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 14.

Fig. 2: Correlation between Fibrinogen & TC in Diabetic Nephropathy patients

Blood Urea & serum creatinine had positive correlation coefficients of 0.04 & 0.11 in DM group and 0.31 (p value = 0.02) & 0.35 (p value = 0.01) in DN group respectively. Thus both parameters had linear correlation in DN group with fibrinogen as shown in Figure 6.

Atherogenic ratios- TC/HDL & LDL/HDL when correlated with fibrinogen had positive correlation with r

Fig. 3: Correlation between fibrinogen & triglyceride in diabetic nephropathy patients **Fig. 4:** Correlation between Fibrinogen & LDL in Diabetic Nephropathy patients **Fig. 5:** Correlation between Fibrinogen & HDL in Diabetic Nephropathy patients

values of 0.11 & 0.3 in DM group & 0.21 (p value = 0.05) & 0.5 (p value = 0.0002) in DN group respectively as shown in Figure 8.

Correlation coefficient between fibrinogen & eGFR was -0.25 & -0.2 (p value = 0.05) in DM & DN groups respectively as shown in Figure 10. Thus eGFR was negatively correlated with fibrinogen.

Urine A/C ratio had positive correlation with fibrinogen with r value of 0.33 & 0.48 (p value = 0.0004) in DM & DN

Table 12: Mean, standard deviation (SD) of all the parameters

S. No	Assay parameters	Controls		DM		DN	
		Mean	SD	Mean	SD	Mean	SD
1.	Plasma Fibrinogen	190.34	72.83	522.76	115.79	657.05	131.50
2.	FBS	71.94	15.66	123.38	44.36	174.75	65.83
3.	HbA _{1c}	5.954	0.29	7.606	0.512	7.86	0.49
4.	TC	101	20.13	192.46	49.57	231.525	53.19
5.	Triglyceride	121.78	17.16	194.64	25.95	249.38	86.92
6.	LDL	56.36	28.41	110.86	28.21	128.275	34.03
7.	HDL	30.2	4.90	24.48	3.97	19.675	2.99
8.	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 13: 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
HbA _{1c}	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

Table 14: Correlation coefficients of fibrinogen levels with other risk factors in DM & DN

Risk factors	Correlation coefficient (r) of Fibrinogen	
	DM	DN
FBS	0.2	0.2
HbA _{1c}	0.41	0.5
TC	0.1	0.3
Triglyceride	0.40	0.5
LDL	0.1	0.24
HDL	-0.1	-0.23
B Urea	0.04	0.31
S Creatinine	0.11	0.35
TC/HDL	0.11	0.21
LDL/HDL	0.3	0.5
eGFR	-0.25	-0.2
Urine A/C ratio	0.33	0.48

Pearson's correlation (r) between SA & FBS levels in DM & DN groups were 0.04 & 0.24 (p value = 0.05) respectively, thus had a positive correlation. There was also positive correlation between SA & HbA_{1c} with r values of 0.35 for DM & 0.38 for DN (p value = 0.006).

$r=0.31$, p value =0.02

Fig. 6: Correlation between Fibrinogen & Blood Urea in Diabetic Nephropathy patients

$r=0.21$, p value =0.05

Fig. 8: Correlation between Fibrinogen & TC/HDL in Diabetic Nephropathy patients

$r=0.35$, p value =0.01

Fig. 7: Correlation between Fibrinogen & S Creatinine in Diabetic Nephropathy patients

$r=0.5$, p value =0.0002

Fig. 9: Correlation between Fibrinogen & LDL/HDL in Diabetic Nephropathy patients

groups respectively as shown in Figure 11.

The mean plasma fibrinogen level in control group was 190.34 ± 72.83 mg/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,⁸ Laurell et al,⁹ Alper et al.¹⁰ Hence above studies interpret that fibrinogen increases in diabetes with complications. Our findings were also similar to studies done by Killingsworth et al, Ganda et al, Collier et al, Schmidt et al & Eraslan M et al.^{11–14} The cause of increased fibrinogen production in type 2 DM are insulin resistance, hyperglucagonemia acting as stimulators

$r=-0.2$, p value =0.05

Fig. 10: Correlation between Fibrinogen & eGFR in Diabetic Nephropathy patients

Fig. 11: Correlation between Fibrinogen & Urine A/C ratio in Diabetic Nephropathy patients

of fibrinogen production in the liver, and possibly, also a subclinical inflammatory state. Thus diabetic patients should be followed up with fibrinogen levels to prevent complications like diabetic nephropathy.

The mean FBS levels of control, DM & DN groups were 71.94 ± 15.6 mg/dl, 123.38 ± 44.36 mg/dl & 178.3 ± 66.57 mg/dl respectively. FBS levels were increased in DM group & DN group when compared to control group which was statistically significant.

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} level was increased in DM & DN groups when compared to controls with a ($p=0.0001$) which was statistically significant. There was only a slight increase of HbA_{1c} levels in DN group when compared to DM group.

We also observed that plasma fibrinogen concentrations were significantly increased and positively co-related with several known risk factors, notably FBS, glycemic control (HbA_{1c}), lipid profile, Blood urea, Serum creatinine & UAC whereas negatively correlated with HDL & eGFR in DM & DN groups. At present microalbuminuria is considered as the earliest marker for DN. In our study fibrinogen correlated positively with UAC. These findings strengthen the hypothesis that an increase in circulating inflammatory biomarkers like fibrinogen levels are early manifestation of diabetic renal disease. Hence, plasma fibrinogen levels could be used for early diagnosis of DN. The results of our study are completely in accordance with recent studies with the same concept.¹⁵

5. Conclusion

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 fibrinogen could be used as early biomarkers for the diagnosis of DN.

6. Summary

The present study consists of total 150 patients, studied in 3 groups, group I- 50 were age & sex matched healthy controls, group II- 50 were diabetic patients and group III- 50 were diabetic nephropathy patients. The aim of the study was to fibrinogen in type 2 DM, DN patients and healthy controls, to correlate fibrinogen levels with FBS, HbA_{1c}, Lipid profile, Blood Urea, Serum Creatinine, eGFR and urine A/C ratio in type 2 DM and DN patients and to find out whether fibrinogen can be used as markers for the early diagnosis of DN. The mean plasma fibrinogen level in control group was 190.34 ± 72.83 mg/dl. The mean plasma fibrinogen levels in DM & DN groups were 522.76 ± 115.79 mg/dl & 657.64 ± 124.61 mg/dl respectively. There was progressive statistically significant increase of fibrinogen levels in DM & DN groups with a p value of 0.0001. Correlation coefficient between fibrinogen & eGFR was -0.25 & -0.2 in DM & DN groups respectively. Thus eGFR was negatively correlated with fibrinogen. Urine A/C ratio had positive correlation with fibrinogen with a r value of 0.33 & 0.48 in DM & DN groups respectively.

7. Source of Funding

No financial support was received for the work within this manuscript.

8. Conflict of Interest

The authors declare that they have no conflict of interest.

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