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IP Indian Journal of Neurosciences

Journal homepage: https://www.ijnonline.org/

Original Research Article

# Assessment of plasma fibrinogen as a marker of diabetic nephropathy

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ARTICLE INFO

*Article history:*
Received 14-01-2021
Accepted 19-02-2021
Available online 12-03-2021

*Keywords:*
Conclusion: Fibrinogen correlated positively with FBS
HbA 1c
TC
triglyceride
LDL
Blood Urea
serum creatinine
Diabetes Miletus
Diabetic Nephropathy

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.



## 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 lipidsalone.[6] Creatinine is

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https://doi.org/10.18231/j.ijn.2021.008

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 1$^{st}$ January 2015 to 31$^{st}$ 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 ± 72.83mg/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 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, 123.38 ± 44.36mg/dl & 178.3 ± 66.57mg/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 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± 115.79* | 657.64 ± 124.61*† |

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

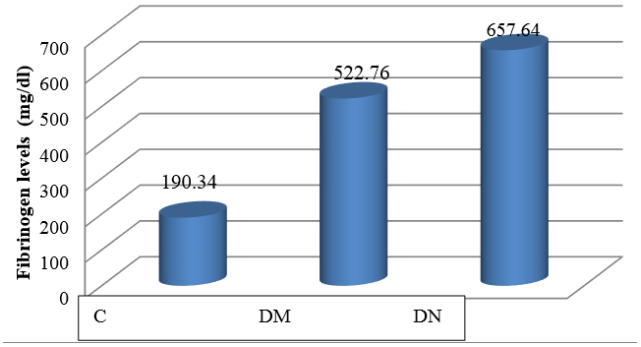



**Fig. 1:** Plasma Fibrinogen levels in study groups

**Table 9:** 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. $HbA_{1c}$ 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.

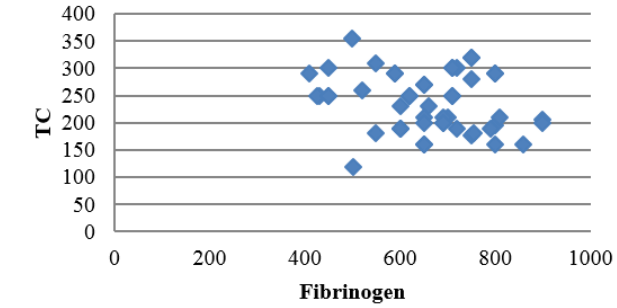
The mean Blood Urea level in control group was 21.48 ± 4.89 mg/dl. The mean B Urea levels in DM & DN groups were 34.04 ± 10.91 mg/dl & 75.86 ± 31.24 mg/dl respectively as shown in Table 14 . The mean Serum Creatinine levels of control, DM & DN groups were 0.82 ± 0.22mg/dl, 1.20 ± 0.21mg/dl & 5.39 ± 2.42mg/dl 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.




r=0.3, p value =0.03

**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 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

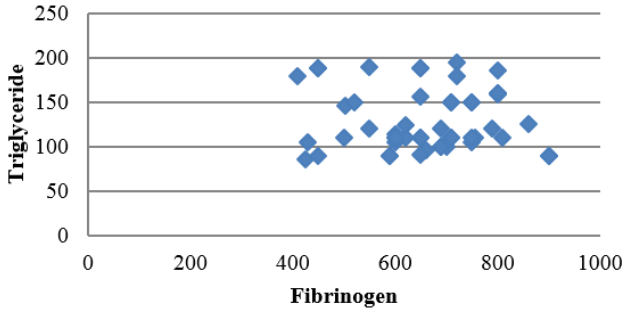



**Fig. 3:** Correlation between fibrinogen &triglyceride in diabetic nephropathy patients

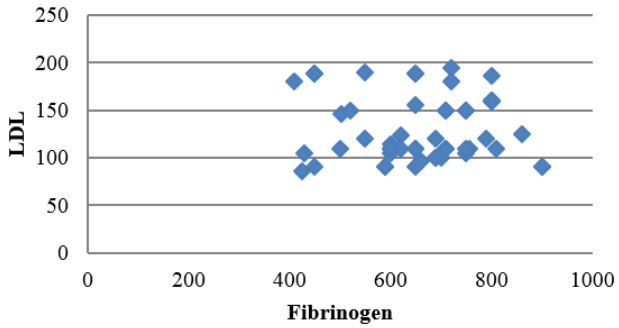
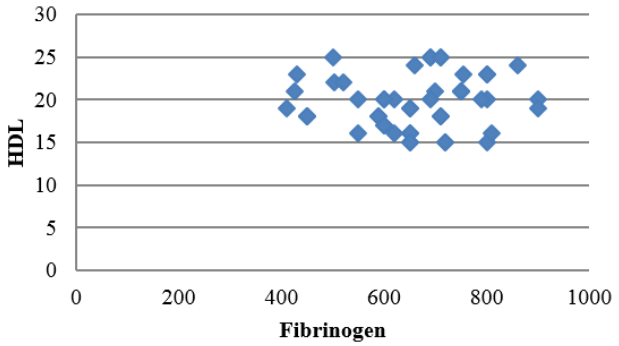



**Fig. 4:** Correlation between Fibrinogen & LDL in Diabetic Nephropathy patients




r=-0.23, p value =0.05

**Fig. 5:** Correlation between Fibrinogen & HDL in Diabetic Nephropathy patients

**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 |
| 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 |

**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).

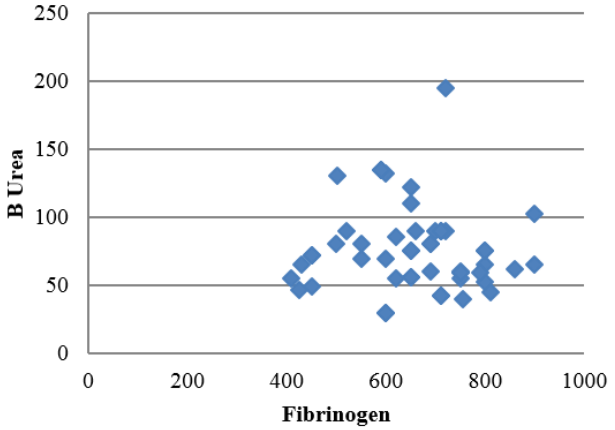



**Fig. 6:** Correlation between Fibrinogen & Blood Urea in Diabetic Nephropathy patients

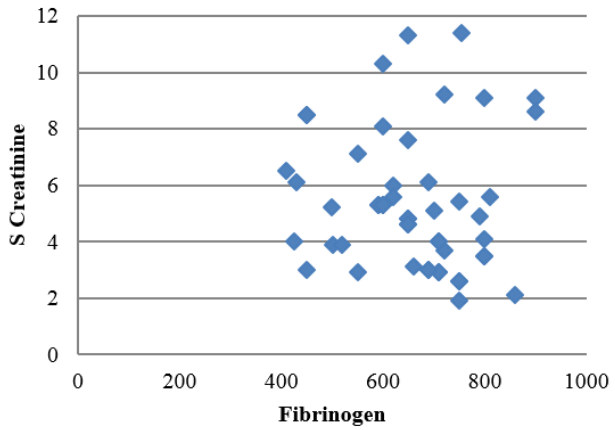



**Fig. 7:** Correlation between Fibrinogen & S Creatinine in Diabetic Nephropathy patients

groups respectively as shown in Figure 11.

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,[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, Schmidtz 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

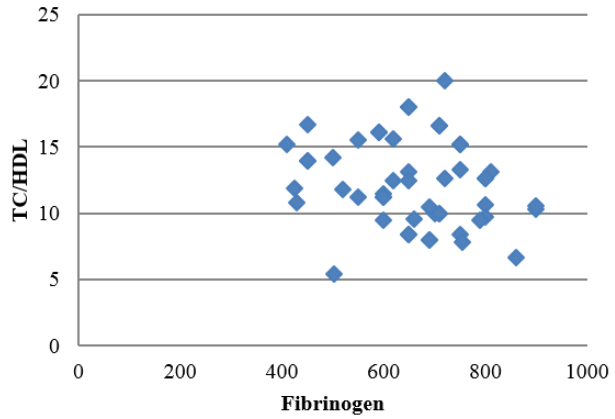



**Fig. 8:** Correlation between Fibrinogen & TC/HDL in Diabetic Nephropathy patients

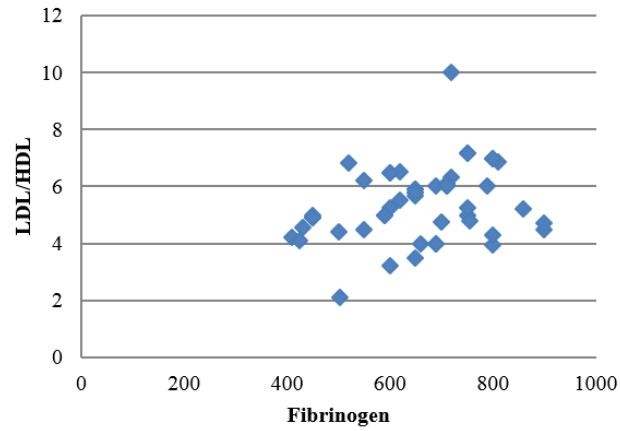



**Fig. 9:** Correlation between Fibrinogen & LDL/HDL in Diabetic Nephropathy patients

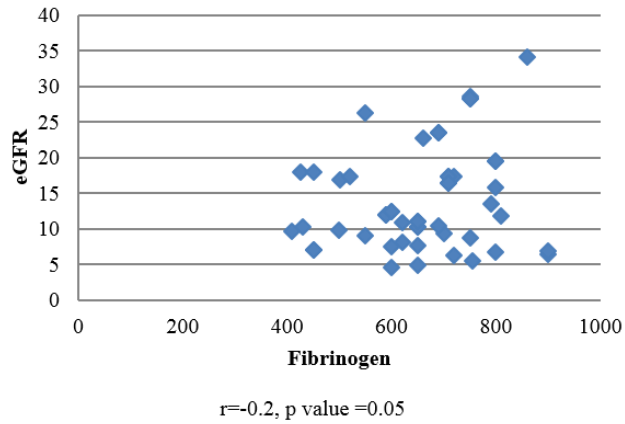



**Fig. 10:** Correlation between Fibrinogen & eGFR in Diabetic Nephropathy patients

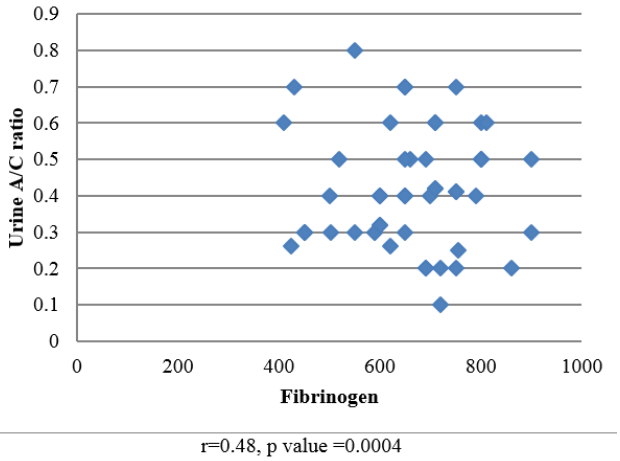



**Fig. 11:** Correlation between Fibrinogen & Urine A/C ratio in Diabetic Nephropathy patients

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 ± 44.36mg/dl & 178.3 ± 66.57mg/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 (HbA1c), lipid profile, Blood urea, Serum creatinine& UAC whereas negatively correlated with HDL & eGFR in DM & DN groups. At present microalbuminria 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 fibrinogenin type 2 DM, DN patients and healthy controls, to correlate fibrinogen levels with FBS, HBA1c, 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 ± 72.83mg/dl. The mean plasma fibrinogen levels in DM & DN groups were 522.76 ± 115.79 mg/dl & 657.64 ± 124.61 mg/dl respectively. 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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**Cite this article:** Ambresh A, Shilpa A. Assessment of plasma fibrinogen as a marker of diabetic nephropathy. *IP Indian J Neurosci* 2021;7(1):44-51.