Original Article

Evaluation of Plasma Fibrinogen Levels and Its Association with Microalbuminuria and Glycemic Control in Type 2 Diabetes Mellitus

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Increased level of Fibrinogen is supposed to be a risk factor for Macrovascular disease. Insulin acutely increases Fibrinogen production in an individual with Type 2 Diabetes. There is a correlation between fibrinogen level and duration of Diabetes.

Aim : To evaluate the levels of Plasma Fibrinogen and its association with Microalbuminuria and glycemic control in patients of Type 2 Diabetes Mellitus (T2DM).

Materials and Methods : A hospital-based Cross-Sectional Study was conducted at the Department of Medicine, Bharati Hospital and Research Centre. The study aimed to evaluate the levels of plasma fibrinogen and its association with microalbuminuria and glycemic control in patients with T2DM. A total of 100 subjects (males and females) presenting with Diabetes Mellitus to our hospital were included in the study after informed consent. A detailed clinical history and relevant laboratory investigations were done.

Statistical Analysis : The quantitative data was represented as their Mean±SD. Categorical and nominal data were expressed in percentage. The t-test was used for analysing quantitative data, or else non-parametric data were analysed by Mann Whitney test. All analysis was carried out by using SPSS software version 21.

Results : Mean Fibrinogen level in study cases was 507.8 mg/dl with 26% had Fibrinogen levels of more than 500 mg/dl. Micro and Macro-albuminuria were seen in 25% and 9% cases. Mean Fibrinogen level was significantly more in cases with a duration of Diabetes 5 years, poor glycemic control and Microalbuminuria.

Conclusion : Microalbuminuric Diabetic patients and poor Glycemic control patients showed higher fibrinogen levels. It can be concluded that Hyperfibrinogenaemia may precede the onset of Clinical Vascular Complications.

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Key words **: Diabetes Mellitus, Fibrinogen, Microalbuminuria, Cardiovascular complications.**

Type 2 Diabetes Mellitus (T2DM) is a non-autoimmune, complex, heterogeneous and polygenic metabolic disease condition in which the body cannot produce enough insulin and is hence characterized by abnormal Glucose Homeostasis[1].

Chronic complication can be vascular (Microvascular and Macrovascular) and non-vascular (foot ulcer, infections, and dermatological manifestations). Microvascular complications include Diabetic Nephropathy including Microalbuminuria, Diabetic Retinopathy and Diabetic Neuropathy. Studies on Diabetes-related complications are, therefore, vital to assess the burden of Diabetes.

The magnitude of the impact of T2DM on the kidney is such that nearly 25-40% of patients develop kidney damage and Chronic Kidney Disease (CKD)[2]. Additionally, among these patients, the risk of Cardiovascular Disease (CVD) morbidity, and premature mortality associated with DM and CKD is greatest[3].

It has been reported that high Fibrinogen concentration increases the risk of Cardiovascular disease in diabetic patients. There is a significant correlation between Fibrinogen[3] level and duration of Diabetes, FBS, PPBS & HbA1C[5].

The increase in Urine Albumin excretion rate is also a marker of poor control of Diabetes. Microalbuminuria has been recognized as an important biomarker to

Editor's Comment :

- Fibrinogen levels were significantly higher in microalbuminuric diabetic patients and cases with poor glycemic control.
- It can be concluded that hyperfibrinogenemia may precede the onset of clinical vascular complications.It has been shown that diabetes itself is a procoagulant state.
- Additional hypercoagulability as evidenced by increased fibrinogen levels may contribute to this state.
- This procoagulant state may contribute to atherosclerosis, which is the major cause of cardiovascular related morbidity and mortality.

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predict Microvascular and Macrovascular diabetic complications[6]. Very few studies have been carried out to demonstrate the association of high plasma fibrinogen levels with glycemic control and albuminuria in T2DM. In the present study, we aimed to evaluate the levels of Plasma Fibrinogen and its association with Microalbuminuria and glycemic control in patients of Diabetes Mellitus.

Aims

To evaluate the levels of Plasma Fibrinogen and its association with Microalbuminuria and glycemic control in patients of T2DM.

Objectives

(1) To study the Plasma Fibrinogen levels in T2DM.

(2) To determine the prevalence of microalbuminuria in T2DM[1].

(3) To correlate the association of high Fibrinogen levels and Microalbuminuria with poor glycemic control.

Material and Methods

A hospital-based Cross-sectional Study was conducted at the Department of Medicine, Bharati hospital and Research Centre. The study aimed to evaluate the levels of Plasma Fibrinogen and its association with Microalbuminuria and glycemic control in patients with T2DM. A total of 100 subjects (Males and Females) presenting with Diabetes Mellitus to our hospital were included in the study after informed consent. One standard questionnaire for each subject[4] included: Personal data, drug usage, disease history and physical examination. Demographic information was taken along with history and physical examination. Clinical features and Blood Biochemistry investigations were also noted. Laboratory investigations required were: Plasma Fibrinogen Levels, Urine Protein: Creatinine Ratio (UPCR) and HbA1c. Following observations were made during the study :

Study Duration : 18 months.

Inclusion Criteria : (1) Either gender with age of >18 years and confirmed diagnosis of Diabetes [ADA 2017]. (2) Patients willing to give written informed consent and follow study related procedures. Diabetes Mellitus was diagnosed by fasting Plasma Glucose ≥126 mg/dl (7 mmol/l), random plasma glucose ≥200 mg/dl(11.1 mmol/l), along with other symptoms of Diabetes and with HbA1c ≥6.5%, 2-hour plasma glucose ≥200 mg/dl(11.1 mmol/l) during OGTT(75 g).

Exclusion Criteria : (1) Previous history of myocardial infarction. (2) Previous history of renal disease (Acute kidney injury or Chronic Kidney disease).

Methodology : The study was carried out amongst 100 patients attending Bharati Hospital, after Ethical Clearance from review board and informed consent taken from the patients. One standard questionnaire for each subject included: Personal data, drug usage, disease history and physical examination[5]. Demographic information was taken along with history and physical examination. Clinical features and Blood Biochemistry investigations were also noted. Laboratory investigations required were: (1) Plasma Fibrinogen levels. (2) Urinary Protein: Creatinine Ratio (UPCR) 3. HbA1c Normal values of lab investigations: (1) Plasma Fibrinogen levels- 180-360 mg/dl. (2) HbA1c- up to 6.4%. (3) UPCR- 0.2 gm proteins per gram of Creatinine.

Statistical Analysis : The quantitative data was represented as their Mean ± SD. Categorical and nominal data was expressed in percentage. The T-test was used for analysing quantitative data, or else non-parametric data was analysed by Mann Whitney test and categorical data was analysed by using chi-square test. Pearson's correlation coefficient was used to compute correlation between quantitative variables. The significance threshold of p-value was set at 0.05. All analysis was carried out by using SPSS software version 21.

Results

Mean age of the study cases was 63.24 years with 50% of the cases being above 60 years of age. Male pre-dominance was seen in the study group with 59% males to 41% females.

The mean fibrinogen level in study cases was 507.8 mg/dl with 26% had Fibrinogen levels of more than 500 mg/dl (Table 1)[1]. The mean duration of Diabetes was 6.9 years with 49% of cases being diagnosed as Diabetes from over 5 years (Table 2). Micro and Macro-albuminuria were seen in 25% and 9% cases (Fig 1). Poor glycemic control was noted in 39% cases (HbA1c>7%) while in 8% cases glycated haemoglobin was more than 10%. Mean Fibrinogen level was significantly more in cases with a duration of Diabetes >5 years (511.29 *versus* 303.24 mg/dl), poor glycemic

Table 1 —*Distribution of study group as per fibrinogen levels*

Fibrinogen levels mg/dl	N	%
100-200	12	12
201-300	15	15
301-400	21	21
401-500	26	26
501-600	15	15
601-700	6	6
701-800	5	5
Total	100	100

Table 2 —*Distribution of study group as per duration of Diabetes Mellitus*

Duration of DM	N	%
<1 years	18	18
1-5years	33	33
6-10 years	30	30
>10 years	19	19
Total	100	100

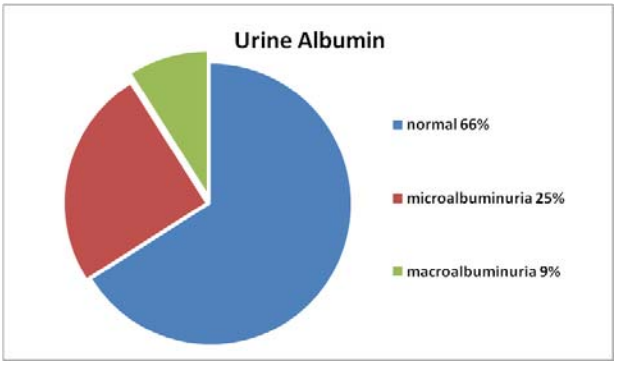

Fig 1 — Distribution of of study group as per urine albumin levels

control (482.8 *versus* 308.7mg/dl) and Microalbuminuria (520.56 *versus* 272.73 mg/dl) (Table 3). Out of the 39 cases with poor glycemic control, 59% had albuminuria as compared to 18% cases with good glycemic control (Table 4). On multivariate analysis, micro-albuminuria (OR-1.21; 1.01-1.59) and poor glycemic[1] control (OR-1.67; 1.17-2.43) was observed to be significantly correlated with higher Fibrinogen levels (p<0.01) (Table 5).

Table 3 — *Mean fibrinogen level comparison with duration of Diabetes mellitus,glycaemic control and microalbuminuria*

Variable	N	Mean fibrinogen	SD	pvalue
Duration of DM :				
≤ 5 years	51	303.24	120.2	<0.01
>5 years	49	511.29	129.4	
Glycaemic control (HbA1C) :				
Good	61	308.77	131.21	<0.01
Poor	39	482.8	154.37	
Microalbuminuria :				
Absent	66	272.73	99.87	<0.01
Present	33	520.56	116.7	

Table 4 — *Association of Albuminuria with glycaemic control*

Linear regression analysis : Fibrinogen asdependent variable			
Variable	OR	p-value	95%CI
UPCR	1.21	<0.05	1.01-1.59
Duration	1.12	0.06	0.68-1.33
HbA1C	1.67	<0.01	1.17-2.43

Table 5 — *Linear regression analysis for prediction of fibrinogen levels.*

Albuminuria	Glycaemic control				Total
	Good	%	Poor	%	
Absent	50	82	16	41	66
Present	11	18	23	59	34
Total	61	100	39	100	100
P-value <0.01					

Discussion

Mean age of the study cases was 63.24 years with 50% of the cases being above 60 years of age. Male predominance was seen in the study group with 59% males to 41% females. The mean duration[1] of Diabetes was 6.9 years with 49% of cases being diagnosed as Diabetics from over 5 years. Poor glycaemic control (HbA1c>7%) was noted in 39% cases while in 8% cases, glycated haemoglobin was more than 10%. Global report on Diabetics by WHO mentions that type 2 Diabetes rises after the age of 40 and males are affected more than females. Similar demographic details have also been observed by other authors. Venishetty S, *et al*. We observed that the mean for age in subjects were 59.04 (±13.47) years predominated by age group of 61-70 years (31.67%), followed by 51-60 years (30%). There were 71-80 years (13.33%), 41-50 years (11.67%) 31-40 years was (7.5%) and <30 years (3.33%). There were 69 males (57.5%) and 51 females (42.5%) in this study. They also observed that maximum of their patients had uncontrolled diabetes, they had mean HbA1c levels of 8.15 ± 1.7 majority number of subjects (51.67%) had levels of more than 8.1, some (20%) had controlled sugars with levels of less than 6.5, while others had levels of 7.1-7.5 (11.67%), 7.6 - 8 (9.16%) and 6.5 - 7 (7.50%). Fibrinogen is the major Plasma Protein coagulation factor in the hepatocyte. Its plasma concentration is 1.5-4.5 g/L when measured as cuttable Protein and approximately 8.8mmol/L Total Protein. It is a heterodimeric molecule, with each half containing three different polypeptide chains (Aa, Bb, and g) which are linked by disulphide bridges. The mean Fibrinogen level in the present study cases was 507.8 mg/dl with 26% had Fibrinogen levels of more than 500 mg/dl. Micro and macro-albuminuria were seen in 34% of the cases and the Mean Fibrinogen level was significantly more in cases with a duration of diabetes >5 years (511.29 *versus* 303.24 mg/dl), poor glycemic control (482.8 *versus* 308.7 mg/dl), and Microalbuminuria (520.56 *versus* 272.73 mg%). On multivariate analysis, micro-albuminuria (OR- 1.21; 1.01-1.59) and poor glycaemic control (OR-1.67; 1.17-2.43) was observed to be significantly correlated with higher fibrinogen levels (p<0.01)

Similar results have been reported by Das US, *et al*[8]. In their study, 60% were Males & 40% Females. The majority of the patients belonged to the age group of 41 to 50 years both in the case of Males and

Females. The values of Serum Fibrinogen were 352.49±123.31, Urinary Albumin Creatinine ratio was 468.76±613.95. Their study also revealed a statistically significant positive correlation between log ACR and Serum Fibrinogen level with a Pearson correlation value of 0.613 and significance (2-tailed) of 0.000. These results are also corroborative of previous studies. Also observed that increased coagulability may impair endothelial function thus 9 promoting macrovascular and microvascular diseases. Plasma PAI-1(Plasminogen Activator Inhibitor-1) concentrations were significantly higher in obese than lean Diabetic patients (P<0.0001). In conclusion, both coagulation and Fibrinolytic Systems are enhanced in lean and obese Type 2 Diabetic patients compared with healthy subjects. The coagulation system activation was found to be similar in both lean and obese diabetic individuals, but the fibrinolytic activity was significantly lower in obese individuals. VM Dalla, *et al*[10] also supported the associations between plasma fibrinogen and diabetic Nephropathy in the form of albumin to Creatinine ratio, reduced glomerular function or increased glomerular basement membrane width in their studies. Fibrinogen may have some association with GBM thickening not only via inflammatory mechanism but also through endothelial damage, coagulant activity, and platelet activation. Casale Monferrato's study by Bruno G, *et al*[11]. demonstrated that Fibrinogen acts as an independent predictor of progression to overt Nephropathy in Type 2 diabetes. In this study, metabolic syndrome was studied as a risk factor for cardiovascular mortality in T2DM. Other studies have also shown higher levels of Fibrinogen in diabetics compared to the non-diabetic population and have stated that Fibrinogen levels showed an increasing trend with the duration of Diabetes.

In our study, we also found an association between the duration of DM and Fibrinogen levels. Our results were also by Chikkamath V, *et al*[12] their study In also, there was the association between the two. Patients with more than 5 years of Diabetes history had hyperfibrinogenemia.

Association of Fibrinogen with HbA1c (Glycemic Control) :

HbA1c generally denotes the overall control of BSL's over the past 3 months. It has been reported that Fibrinopeptide A is positively related to blood Glucose. The present study observed that poor glycemic control (OR-1.67; 1.17-2.43) was significantly correlated with higher Fibrinogen levels (p<0.01). In a study done by Bruno G, *et al*[13], Fibrinogen level was significantly associated with HBA1c value. Another study by Ceriello A, *et al*[14] suggested that Hyperfibrinogenemia is one way by which Hyperglycemia activates coagulation. Therefore, both epidemiologic and clinical findings support the hypothesis that poor glycemic control may lead to Thrombophilia, a condition that might be involved in the increased Cardiovascular risk in patients with Diabetes.

Microalbuminuria and Fibrinogen Levels :

This study determines Micro-albuminuria (OR-1.21;1.01-1.59) to be significantly correlated with higher Fibrinogen levels. Fibrinogen levels were much higher in patients who had Microalbuminuria compared to those with no proteinuria (p<0.05). This is in comparison to several studies listed below. Vestra D, *et al*[15] found higher fibrinogen levels in patients with in Microalbuminuria and overt proteinuria compared to those with no Proteinuria. In this study they studied the association of nephropathy and Glomerular Basement Membrane (GBM) thickening with acute phase markers of inflammation. Similar results were also obtained in other studies, which suggested that the positive association seen between albumin excretion rate and Fibrinogen level could explain the increased cardiovascular-related morbidity and mortality in diabetic patients with Microalbuminuria and Macroalbuminuria.

The relation between Fibrinogen and mild to moderate renal dysfunction was evaluated in the Diabetic Control and Complications Trial (DCCT) study by Klein, *et al*[18]. Elevated levels of fibrinogen have been associated with progression to overt nephropathy and higher 5-year mortality. Similar results were obtained in studies by Gomes MB, *et al*[16] and Festa, *et al*[17] which suggested that the positive association seen between Albumin excretion rate and Fibrinogen level could explain the increased Cardiovascular-related morbidity and mortality in Diabetic patients with Microalbuminuria and Macroalbuminuria.

Conclusion

Fibrinogen level was associated with haemoglobin A1C value and Albumin excretion rate measured by Microalbuminuria. Clinic-based studies state that Plasma Fibrinogen levels were higher in diabetic patients with Microalbuminuria than in Diabetic patients with normal albuminuria. Because microalbuminuria has been recognized as a powerful predictor of cardiovascular-related illness and death, high Fibrinogen levels may be considered a potential additional risk factor in patients with Diabetes. Based on the present study findings, it can be concluded that Hyperfibrinogenemia is a mechanism of the

increased Cardiovascular risk faced by patients with T2DM. It has been shown that Diabetes itself is a procoagulant state. Additional hypercoagulability determined by increased Fibrinogen levels may contribute to a procoagulant state. This procoagulant state may contribute to Atherosclerosis. Fibrinogen levels were significantly higher in Microalbuminuric Diabetic patients and cases with poor Glycemic control. It can be concluded that Hyperfibrinogenemia precedes the onset of Clinical Vascular Complications and therefore, it could be a possible mechanism of the increased cardio-vascular risk in patients with T2DM.

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