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Plasma fibrinogen levels in type 2 diabetes mellitus: A case control study

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Abstract

Aim: The aim of the present study was to know the plasma fibrinogen levels in the patients of type 2 diabetes mellitus patients.

Material and method: The present study was conducted in the department of Medicine at Chattrapati Shivaji Subharti Hospital from February 2017 to January 2019. This study involved a total of 100 patients of which 50 were taken as cases (diabetics) and the other 50 as controls (non-diabetics) according to the inclusion and exclusion criteria. The various parameters that were studied included age of the patient (years), sex, body mass index (kg/m^2), smoking, blood pressure (mm Hg), plasma fibrinogen level (mg/dl), glycosylated hemoglobin (%), microalbuminuria, total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride level. Data were tabulated and examined using the Statistical Package for Social Sciences Version 22.0

Results: Mean HbA1c (%) level was found to be 7.89±1.40 in the case group while the same was reported to be 4.2±1.73 in the control group with statistically significant difference. Mean microalbuminuria level was found to be 436.8±94.32 in the case group while the same was reported to be 316.81±80.31 in the control group. Mean fibrinogen level in case and control group was 396.63±104.89 and 252.70±72.38 respectively.

Conclusion: In this study, the type 2 diabetic patients were found to have significantly higher levels of fibrinogen in comparison to non-diabetic subjects. This study shows that these newer risk factors like age, blood pressure, BMI, and blood glucose can be measured in diabetic patients for a better risk prediction.

Keywords: diabetes, fibrinogen, hba1c, microalbuminuria

Introduction

Diabetes Mellitus (DM) is a group of metabolic diseases which is characterized by hyperglycemia resulting from defect in insulin secretion, insulin action, or both [1]. In 1985 an estimated 30 million people around the world where diagnosed with diabetes; in 2000 that figure rose to over 150 million, and in 2012 the international diabetes federation estimate 371 million people with diabetes [2]. 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 [3, 4]. The major types of diabetes are type 1, type 2 and gestational diabetes (GDM) [5].

The metabolic syndrome is a cluster of various risk factors like hyperglycemia, hyperlipidemias and obesity. It is estimated that around a quarter of the world's adult population has metabolic syndrome and these people are twice as likely to die from, and three times a likely to have IHD or stroke compared with people without this syndrome [6]. Individuals with insulin resistance and type 2 diabetes mellitus have elevated levels of plasminogen activator inhibitors (especially PAI-1) and fibrinogen, which enhances the coagulation process and impairs fibrinolysis, thus favoring the development of thrombosis [7].

Fibrinogen, by virtue of its role in platelet crosslinking, thrombus formation, and increased blood viscosity, may enhance plaque progression, which in turn may lead to IHD[8]. Chronic complications of uncontrolled diabetes begins with loss of a small amount of albumin (Microalbuminuria) [9], this may lead to increase production of fibrinogen.

It has been reported that high fibrinogen concentration enhances the risk of cardiovascular disease in diabetic patients [10, 11]. Increased level of fibrinogen is a recognized risk factor for macrovascular disease through its variety of mechanisms including increased blood viscosity, increased size of fibrin clots, increased tissue deposition, stimulation of atherosclerosis and vascular thickening [12]. In view of above concepts and due to paucity of similar studies in Indian patients, this study had been undertaken to know the plasma fibrinogen levels in the patients of type 2 diabetes mellitus patients.

Materials and Method

The present study was conducted in the department of Medicine at Chattrapati Shivaji Subharti Hospital from February 2017 to January 2019. The present study involved a total of 100 patients of which 50 were taken as cases (diabetics) and the other 50 as controls (non-diabetics) according to the inclusion and exclusion criteria. Age and

gender were matched in cases and controls to avoid confounding bias. Patients were enrolled in the study after obtaining written informed consent from parents and approval from Institutional Ethical Committee. Data for the proposed study was collected in a pre-tested Proforma meeting the objectives of the study. A detailed history and clinical examination was done pertaining to various risk factors, and relevant laboratory investigations were done in both diabetic patients and in controls. The various parameters that were studied included age of the patient (years), sex, body mass index (kg/m^2), smoking, blood pressure (mm Hg), plasma fibrinogen level (mg/dl), glycosylated hemoglobin (%), microalbuminuria, total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride level. The patients were investigated further according to protocol to evaluate the risk factors.

Inclusion criteria

Patients with age >40 years, cases of Diabetes Mellitus based on the criteria taken in the study and patients on oral hypoglycemic agents were included in the study

Exclusion criteria

Type 1 diabetes mellitus subjects, patients with chronic infections, renal disease, endocrine disease, malignancy, patients with renal disease, patients with endocrine disease, patients with malignancy, patients on warfarin, patients on steroids, and patients on hormone replacement therapy.

Parameters

The following parameters were studied in the present study:

a. Anthropometry: BMI, formerly called the Quetelet index, is a measure for indicating nutritional status in adults. It is defined as a person's weight in kilograms divided by the square of the person's height in metres (kg/m^2). Classification is given in figure 1.

BMI	Nutritional status
Below 18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Pre-obesity
30.0–34.9	Obesity class I
35.0–39.9	Obesity class II
Above 40	Obesity class III

Fig 1: BMI classification

b. Diabetes Mellitus
c. Dyslipidemia: Fasting blood sample collected for lipid profile, total cholesterol, HDL & triglycerides were directly assessed by standard enzymatic methods. LDL cholesterol was estimated using Freidwald's equation:

LDL cholesterol = Total cholesterol – HDL – triglycerides/5

According to National cholesterol education program (NCEP) ATP III guidelines patients were considered to have dyslipidemia when: Total cholesterol >200mg%, HDL <40mg%, LDL >130mg% and Triglycerides > 150mg%.

d. Plasma Fibrinogen level: measured by "Clauss method" using "Tulip Diagnostic (P) Ltd" fibrinogen kit. A calibration curve would be prepared by making serial dilutions of calibration plasma with Owren's veronal buffer (1 in 5, 1 10, 1 in 20, and 1 in 40). 0.2 ml of each dilution was warmed to 37°C for 3 minutes, then 0.1 ml of bovine thrombin solution added, and the clotting time was measured. Each test was performed in duplicate, the average would be calculated and a calibration curve would be constructed (The clotting time in seconds against the fibrinogen concentration in g/l) on log/log graph paper. The 1 in 10 dilution was considered to be 100% fibrinogen concentration. 1 in 10 dilutions was made from each patient's plasma, thrombin time would be measured as mentioned above, also in duplicate, and the fibrinogen level would be determined in g/l from the calibration curve[13-16].
e. HbA1C: was measured by "particle enhanced immunoturbidimetric test" using Erba Manheim Kit and reader II. Glycosylated Hemoglobin (GHb) is normal adult hemoglobin (HbA1) which is covalently bonded to a glucose molecule. GHb concentration is dependent on the average blood glucose concentration. Results were calculated automatically by the instrument[17].
f. Urine albumin excretion (UAE): UAE was measured by microalbuminuria which was detected by Micral test II strips and was considered positive if there was a colour change. This test is an immunological, semi quantitative determination of microalbuminuria. In this, a freshly voided early morning urine sample is collected. The strip is dipped in urine sample for 5 sec up to in between the two black strips, later withdrawn and the colour is read after 1 min.
g. Other investigations done were complete blood count, urea, serum creatinine, urine analysis, ECG and Chest X-ray

Statistical analysis

Data were tabulated and examined using the Statistical Package for Social Sciences Version 22.0 (IBM SPSS Statistics for Mac, Armonk, NY: IBM Corp, USA). Descriptive statistical analysis had been carried out in the present study. Results on continuous measurements are presented as Mean±SD. Categorical data has been presented as frequency distribution. P-value of <0.05 was considered as significant. Difference between two groups was determined using chi square test and student T test for categorical data and continuous data respectively.

Results

Table 1 shows the gender and age distribution of subjects among case and control group. Case group comprised of 58% males and 42% females while the control group consisted of 60% males and 40% females. The mean age of the subjects in case and control group was 56.02±8.71 years and 55.02±9.32 years respectively.

Mean HbA1c (%) level was found to be 7.89±1.40 in the case group while the same was reported to be 4.2±1.73 in the control group with statistically significant difference as

p<0.05. Mean BMI in case and control group was 25.91±3.02 and 24.02±1.98 respectively. Statistically significant difference was found when mean BMI was compared among case and control group (table 2).

Hypertension was present in 36% of the subjects in case group while it was absent in 64% of the subjects. In the control group, hypertension was present and absent in 4% and 96% of the subjects respectively. When hypertension was compared statistically among case and control group, it was found to be statistically significant as shown in table 3.

Mean microalbuminuria level was found to be 436.8±94.32 in the case group while the same was reported to be 316.81±80.31 in the control group with statistically significant difference. Mean fibrinogen level in case and control group was 396.63±104.89 and 252.70±72.38 respectively. Statistically significant difference was found when mean fibrinogen level was compared among case and control group (table 4).

The mean fibrinogen levels were higher in males (461.33±101.62) when compared to females (320.70±109.41), with statistically significant difference. It was observed that the diabetics with HbA1c >7% had a higher mean fibrinogen level (471.96) compared to diabetics with HbA1c <7% (308.22) which was found to be very highly significant. The mean fibrinogen levels in hypertensives and normotensive groups were 423.49±106.66 and 378.75±114.83 respectively. The mean fibrinogen level between hypertensives and normotensives was observed to be statistically significant as p<0.05. Mean fibrinogen level was found to be 404.46±99.91 in the smokers while the same was reported to be 390±103.40 in the non-smokers. Mean fibrinogen level in subjects with 18-25, 26-30, >30 BMI was 209.6±83.7, 395.99±119.3 and 403.7±109.6 respectively (table 5). When mean fibrinogen was compared according to the BMI categories using anova test, it was found to be statistically significant as p<0.05.

Table 1: Gender and mean age distribution of the study subjects

Variables	Case		Control	
	N	%	N	%
Gender				
Male	29	58	30	60
Female	21	42	20	40
Total	50	100	50	100
Age				
	Mean		SD	
Mean	56.02		55.02	
SD	8.71		9.32	

Table 2: Mean HbA1c (%) AND BMI level among the study subjects

Groups	HbA1c (%)	
	Mean	SD
Case	7.89	1.40
Control	4.2	1.73
t test	9.87	
p value	<0.01*	
BMI		
	Mean	SD
Case	25.91	3.02
Control	24.02	1.98
t test	4.59	
p value	0.001*	

*: statistically significant

Table 3: Hypertension and smoking status among the study subjects

Variables	Case		Control	
	N	%	N	%
Hypertension				
Present	18	36	2	4
Absent	32	64	48	96
Chi square	14.03			
p value	<0.01*			
Smoking				
Present	22	44	19	38
Absent	28	56	31	62
Chi square	0.37			
p value	0.54#			

*: statistically significant, #: statistically insignificant

Table 4: Microalbuminuria and fibrinogen level among the study subjects

Groups	Microalbuminuria	
	Mean	SD
Case	436.8	94.32
Control	316.81	80.31
t test	10.92	
p value	<0.01*	
Fibrinogen Levels		
	Mean	SD
Case	396.63	104.89
Control	252.70	72.38
t test	14.51	
p value	<0.01*	

*: statistically significant

Table 5: Mean and standard deviation of fibrinogen levels according to various parameters

Variables	Fibrinogen levels	
	Mean	Std. Deviation
Gender		
Male	461.33	101.62
Female	320.70	109.41
t test	16.22	
p value	<0.01*	
HBA1C		
6-7%	308.22	101.24
>7%	471.96	104.39
t test	11.46	
p value	<0.01*	
Blood pressure		
Hypertensives	423.49	106.66
Normotensives	378.75	114.83
t test	18.91	
p value	<0.01*	
Smoking status		
Smokers	404.46	99.81
Non smokers	390.00	103.40
t test	1.91	
p value	0.14	
BMI		
18-25	209.6	83.7
26-30	395.99	119.3
>30	403.7	109.6
Anova test	8.98	
p value	<0.01*	

*: statistically significant

Discussion

Increased level of fibrinogen is a recognized risk factor for macrovascular disease through its variety of mechanisms

including increased blood viscosity, increased size of fibrin clots, increased tissue deposition, stimulation of atherosclerosis and vascular thickening. Insulin acutely increases fibrinogen production in an individual with type-2 diabetes but not in individual without diabetes. There is significant correlation between fibrinogen level and duration of diabetes, FBS, PPBS & HbA1C [18]. In view of above concepts and due to paucity of similar studies in Indian patients, this study had been undertaken to know the plasma fibrinogen levels in the patients of type 2 diabetes mellitus patients.

Gender and Fibrinogen

In a study done by Bruno G *et al* [19] showed slightly different fibrinogen levels between men (3.7± 0.9 g/l) and women (3.6 ± 0.9 g/l). Study done by Lee AJ *et al* [20] also showed a similar difference. In the present study it was found that the mean fibrinogen levels were higher in males (461.33 mg/dl) when compared to females (320.70mg/dl), with statistically significant difference. Pankaj Kumar Saini [21] found no significant association between fibrinogen and sex. Study done by Jain *et al*. also did not find any association between fibrinogen and sex [22].

Association of fibrinogen with HbA1c (glycemic control)

Pankaj Kumar Saini *et al* [21] in his study reported that mean fibrinogen level was higher in patients with poor glycemic control. The correlation between glycemic control and fibrinogen levels could be due to the following reasons: (a) glycosylated fibrinogen is less susceptible to plasmin degradation and (b) relative insulin deficiency in diabetics results in differential protein synthesis, i.e., 29% decrease in albumin synthesis and 50% increase in fibrinogen synthesis. The current research showed that mean HbA1c (%) level was found to be 7.89±1.40 in the case group while the same was reported to be 4.2±1.73 in the control group with statistically significant difference. In a study done by Bruno G *et al* [19], fibrinogen level was significantly associated with HBA1c value. Another study by Ceriello A [23] suggested that hyperfibrinogenemia is one way by which hyperglycemia activates coagulation. Sanjay Dhawale *et al* [24] revealed that serum fibrinogen level was significantly higher in all diabetic patients compared to non-diabetic control.

Microalbuminuria and fibrinogen levels

In the current research, fibrinogen levels were significantly higher in patients who had microalbuminuria compared to those with no proteinuria ($p<0.05$). Similar results were reported by Saini PK *et al* [21]. Dalla Vestra *et al* [25] found higher fibrinogen levels in patients with microalbuminuria and overt proteinuria compared to those with no proteinuria. Similar results were obtained in previous 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.

Fibrinogen level in case and control group

In the current study, statistically significant difference was found when mean fibrinogen level was compared among case and control group. This is in accordance with the study conducted by Archana Sachin Bembde *et al* [26] who reported that diabetics had higher fibrinogen level than controls signifying increased cardiovascular risk. The various possible mechanisms for hyperfibrinogenemia in diabetics could be that a procoagulant state often exists in people of diabetes. There is an increase in a number of coagulation factors such as plasminogen activator inhibitor 1, von-Willebrand factor, fibrinogen, factor VII and thrombin antithrombin complexes particularly in association with macrovascular and microvascular disease and glycemic control [27].

Since our study was cross-sectional, it was limited in its ability to find out the relative importance of the above factors in risk stratification. Association between fibrinogen and HbA1c was weak among type 2 diabetic group which was possibly the result of very small sample size. For that, prospective studies with a relatively larger patient population are needed.

Conclusion

In this study, the type 2 diabetic patients were found to have significantly higher levels of fibrinogen in comparison to non-diabetic control subjects. These variables also correlated with the vascular risk markers like age, blood pressure, BMI, and blood glucose. This study shows that these newer risk factors can be measured in diabetic patients for a better risk prediction. Also, the need for better blood pressure and blood sugar control has to be emphasized. The actual impact of these newer risk factors needs to be assessed by doing larger prospective studies.

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