Is Fibrinogen and D-dimer Levels Predictors for Cardiovascular Risk in Type 2 Diabetes Mellitus?

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ABSTRACT

Introduction: Diabetes mellitus is a metabolic disorder in which patients are unable to regulate glucose metabolism. It is often associated with cardiovascular diseases due to some coagulation and haemostatic changes in the affected individuals. **Objective**: The aim of the study was to determine any possible relationships between some coagulation parameters and cardiovascular risks in patients with type 2 diabetes mellitus.

Methods and Materials: This cross-sectional prospective study was conducted among 50 diabetic patients and 20 apparently healthy individuals randomly selected. The socio-demographic and other data were obtained from each participant using structured questionnaire. 7mls of blood was collected by venipuncture out of which 2mls was dispensed into EDTA container for determination of platelet count using Mythic 18 haemoautoanalyzer and the remaining 5mls was dispensed into sodium citrate container for prothrombin time test, partial prothrombin time test, fibrinogen assay and D-dimer assay. The prothrombin time and partial prothrombin time tests were done by manual method, fibrinogen assay by Clauss method and D-dimer assay by ichroma method. Basal metabolic index (BMI) of the subjects was also obtained. Data analysis was done using statistical package for social sciences (SPSS) version 21 software, results were expressed as mean ± standard deviation; while the relationship between variables were obtained using independent t-test and Pearson's correlation. The level of significance was set at ($p<0.05$). Ethical approval was sought and obtained from our institution's ethical committee and all participants gave informed consent.

Results: The results showed statistical difference between the diabetic and control groups ($p<0.05$). It was observed that the DM patients showed a shorter APTT-PT, and higher fibrinogen and D-dimer concentration than the control group, possibly contributing to increased prevalence of hypercoagulability in DM patients.

Conclusion: This study suggests that fibrinogen and D-dimer levels could be considered as important predictors for cardiovascular risk in diabetes mellitus. Similarly, D-dimer could serve as a good biochemical marker for predicting thrombus formation in the study subjects.

Keywords: Diabetes mellitus, Body mass index, Platelet counts, Coagulation Testing, Fibrinogen, D-dimer

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Author's contributions:

This work was carried out and approved in collaboration between all authors. INC designed the study, EON sourced for funding, INC wrote the protocol, INC & EON contributed in literature search, INC & ENO did the experiments; INC statistical analysis, INC & ENO wrote the manuscript, and proofread the manuscript.

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1.0 INTRODUCTION

Diabetes mellitus is a metabolic disorder in which patients are unable to regulate glycemia. Wanping and Fukuda (1). It is becoming a worldwide public health issue and a burden to society because of its disabling and common complications. The cause of Type 2 Diabetes is multifactorial and heterogeneous, notably are genetic and environmental factors (2,1) such as excessive visceral obesity (3), inactive lifestyle, lack of exercise and poor dietary habits(4) Semenkovich et al*(5)* posited that the increasing prevalence strongly suggests changes in environmental conditions, such as diet, hygiene, antibiotic use, and other medical practices other than genetics. These factors could likely influence the function of the microbiome in ways that alter the immune and metabolic systems, contributing to the increased risk for these diseases. Obeagu et al (6) reports that over "170 million people worldwide and about 1-7% of Nigerian population are affected with diabetes mellitus". Reports from World Health Organization (WHO) appear to suggest that Nigerians are the worst hit among Africans (7). Cardiovascular disease (CVD) has been attributed as the major cause of morbidity and mortality in persons with diabetes. In fact, Alzahram and Ajjian (8) postulates that about 80% of deaths recorded among diabetes subjects is as a result of CVD and its complications. Studies have shown that progression of atherothrombotic disease in diabetes has been linked with elevated levels of coagulation factors including fibrinogen, plasminogen activator inhibitor-1, D-dimer and von Willebrand factor leading to the risk of hypercoagulability and thrombosis among type 2 diabetic patients (9,10).

This study therefore assessed some coagulation parameters of patients with DM with the aim of determining any possible relationships between some coagulation parameters and cardiovascular risks in patients with diabetes mellitus.

2.0 MATERIALS AND METHODS

2.1 Study Area

This work was carried out at Nnamdi Azikiwe University Teaching Hospital (NAUTH). NAUTH, a tertiary institution H is located at the heart of the cosmopolitan town Nnewi, in Anambra State. The inhabitants are Igbo by tribe. The hospital, also being a specialist one serves as a referral centre for the State and its neighboring States such as Enugu, Delta, Imo, and Abia. The services obtainable in this institution include diagnosis, preventive and curative services.

2.2 Study Population

The study population consists of 50 DM patients aged 14-86 years that attended the diabetic clinic in the medical out-patient department of hospital and 20 non- diabetic subjects who served as control. These were randomly selected and are age matched. Patients with history of thrombo-embolism or known inherited coagulation disorders, cancer, hyperthyroidism, pregnancy, recent surgery, patients taking standard anticoagulant treatment were excluded from the study based on information obtained from the case note as these could be confounding factors.

2.3 Study Design

Using a structured questionnaire, socio-demographic and other data were obtained from the participants. With the assistance of the physician, information like the most recent fasting blood glucose and blood pressure were obtained from the patients records. Glycemic control means, the regulation and maintenance of blood glucose levels within the normal ranges. The normal range of fasting blood glucose is 60-100mg/dl or 3.9-5.6mmol/l. A poor glycemic control is the persistently elevated blood glucose, thus level above the normal upper limit shows a poor glycemic control, and blood pressure is the pressure of circulating blood on the walls of the blood vessels. It is usually expressed in terms of the systolic pressure over the diastolic pressure and measure in mmHg. The normal range of blood pressure is 120/80 mmHg. Blood pressure readings were taken from each DM participant and those who had ≥ 140 and 90 mmHg for either systolic or diastolic blood pressure respectively were regarded as being hypertensive. Participants who were on blood pressure medication were also regarded as being hypertensive irrespective of the blood pressure readings at the time of sampling.

2.4 Sample Collection

Seven milliliters of venous blood was collected from each consenting study participant aseptically and divided appropriately into EDTA container (2ml) and sodium citrate container (5ml).The EDTA anticoagulated blood was used for platelet count while the sodium citrate anticoagulated blood was centrifuged for 15minutes at 1500g. The plasma was separated and used for prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and D-dimer testing. The platelet count was done immediately using Mythic 18 haemoautoanalyser, the PT, APTT, were also ran immediately by manual methods using PT and APTT assay kits procured from Diagnostic Reagents Ltd., UK. Fibrinogen level was determined using fibrinogen assay kit employing Clauss method obtained from Giesse Diagnostics srl., Italia. The serum for D-dimer was stored at -70^{0}C for batch testing. The assay was by semiautomated method using ichroma^{TM} D-dimer assay kit manufactured by Boditech Med Incorporated, Korea. All tests were carried out according to manufacturer's instructions. Basal metabolic index (BMI) was also obtained.

Ethical approval was obtained from the Institutional Ethical Committee and informed consent approved obtained from the participants.

2.5 Statistical Analysis

The data were statistically analyzed using SPSS version 21. Values were expressed as mean ± standard deviation. The independent t-test and Pearson's correlation were used to determine the statistical difference and relation. It was considered significant if p-value < 0.05.

3.0 Results

A total of 70 subjects aged 14-86 years participated in the study, 30(42.9%) were males and 40(57.1%) females. 50 participants were DM patients and 20 were control subjects. The age of DM participants ranged from 14 - 86 years with the majority of participants (23(46%)) being in the age range of 41-60years. The mean age was 53.2±18.03 year. The age of the control subjects was 21 - 65 years with the majority 10(50%) being in the range of 21-40 years. Their mean age was 42.5 ± 13.61years. The percentages in terms of duration of DM

were≤ 5years, 5-10years ≥ 10 years were 40%, 36% and 24% respectively. The diabetic patients with good glycemic control were 38(76%) and those with poor glycemic control were 12(24%). For diabetic group, the blood pressure in mmHg measured the diastolic between 79-89in 41(82%) patients and ≥90 in 9(18)% while for systolic was between 110-139 in 38(76%) and ≥140 in 12(24%).

Using an independent t-test, the mean platelet count was significantly decreased in study subjects compared with controls, (p=0.000) (Table 1). In contrast no statistical difference was observed with mean PT level (p=0.900) (Table1). However, the mean APTT level was statistically prolonged (p=0.026) (Table1). The mean serum fibrinogen levels concentration was significantly increased (p=0.001) so also the mean D-dimer concentration (p=0.045).and the mean BMI (p=0.000) (Table 1).

When the body mass index (BMI) and the haemostatic profiles was correlated using Pearson's correlation a significant positive correlation was observed between BMI and APTT (p=0.016) and a weak positive correlation between BMI and fibrinogen (p=0.045) but no significant correlation between BMI and PT and APPT (Table 2).

In a similar vein, A strong correlation was seen between platelet count and APTT (p=0.001) and a weak one between platelet count and PT (p=0.050); while a negative significant correlation was observed between APTT and platelet count (p=0.003) (Table 3).

Table 1: Mean value of Some Haemostatic parameters in Diabetic patients and control subjects

Parameters	Controls (mean±SD)	Diabetics (mean±SD)	t- test	p-value
Platelets (× 10^9/L)	250.35±44.22	180.74±42.22	6.119	0.000**
PT (sec)	11.85±1.42	11.92±2.30	-0.126	0.900
APTT (sec)	31.10±3.71	28.66±4.16	2.284	0.026*
Fibrinogen (g/dl)	2.51±0.84	3.25±0.77	-3.580	0.001**
D-Dimer (ng/ml)	334.59±584.27	1066.15±2349.90	-2.049	0.045*
BMI (kg/m^2)	22.59±1.81	25.29±2.65	-4.173	0.000*
D-Dimer/Fibrinogen Ratio	133.30	328.04		

Key: PT (sec) = Prothrombin time; APTT= Activated partial thromboplastin time; BMI= body mass index; * = correlation is significant at the 0.05 level (2-tailed); **= correlation is significant at the 0.01 level (2-tailed)

Table 2: Correlation between BMI and the haemostatic parameters

Parameters	Control			Diabetic		
	N	R	p-value	N	R	p-value
BMI versus Platelets	20	0.112	0.640	50	-0.059	0.682
BMI versus PT	20	-0.090	0.706	50	-0.058	0.687
BMI versus APTT	20	0.531	0.016	50	0.222	0.121
BMI versus Fibrinogen	20	0.453	0.045*	50	-0.098	0.499
BMI versus D-dimer	20	0.260	0.269	50	-0.216	0.131

Key: PT (sec) = Prothrombin time; APTT(sec)= Activated partial thromboplastin time; BMI(kg/m^2)= body mass index; * = correlation is significant at the 0.05 level (2-tailed); **= correlation is significant at the 0.01 level (2-tailed)

Table 3: Correlation between platelet count and other haemostatic parameters

Parameters	Control			Diabetics		
	N	R	p-value	N	R	p-value
Platelets versusPT	20	0.385	0.094	50	0.279	0.050*
Platelets versus APTT	20	-0.153	0.521	50	0.462	0.001**
Platelets versus Fibrinogen	20	0.312	0.181	50	-0.068	0.639
Platelets versus D-dimer	20	0.447	0.048*	50	-0.178	0.215

Key: PT (sec) = Prothrombin time(sec); APTT= Activated partial thromboplastin time(sec); * = correlation is significant at the 0.05 level (2-tailed); **= correlation is significant at the 0.01 level (2-tailed)

Table 4: Correlation between PT and other haemostatic parameters

Parameters	Control			Diabetics		
	N	R	p-value	n	r	p-value
PT versus Platelets	20	0.385	0.094	50	0.279	0.050*
PT versus APTT	20	-0.246	0.296	50	0.238	0.096
PT versus Fibrinogen	20	0.116	0.626	50	0.086	0.551
PT versus D-Dimer	20	0.226	0.337	50	-0.154	0.284

Key: PT (sec) = Prothrombin time(sec); APTT= Activated partial thromboplastin time(sec); * = correlation is significant at the 0.05 level (2-tailed); **= correlation is significant at the 0.01 level (2-tailed)

Table 5: Correlation between APTT and other haemostatic parameters

Parameters	Control			Diabetics		
	N	R	p-value	N	R	p-value
APTT versus Platelet	20	-0.153	0.521	50	0.462	0.001**
APTT versus PT	20	-0.246	0.296	50	0.238	0.096
APTT versus Fibrinogen	20	0.230	0.330	50	-0.408	0.003**
APTT versus D-dimer	20	0.178	0.453	50	-0.266	0.062

Key: PT (sec) = Prothrombin time(sec); APTT= Activated partial thromboplastin time(sec); * = correlation is significant at the 0.05 level (2-tailed); **= correlation is significant at the 0.01 level (2-tailed)

Table 6: Correlation between fibrinogen and other haemostatic parameters

Parameters	CONTROL			DIABETIC PATIENTS		
	N	R	p-value	N	R	p-value
Fibrinogen versus Platelet	20	0.312	0.181	50	-0.068	0.639
Fibrinogen versus PT	20	0.116	0.626	50	0.086	0.551
Fibrinogen versus APTT	20	0.230	0.330	50	-0.408	0.003**
Fibrinogen versus D-dimer	20	0.505	0.023*	50	0.131	0.364

Key: PT (sec) = Prothrombin time(sec); APTT= Activated partial thromboplastin time(sec); * = correlation is significant at the 0.05 level (2-tailed); **= correlation is significant at the 0.01 level (2-tailed)

4.0 DISCUSSION

Evidence suggest that certain haematological indices are altered in patients with diabetes mellitus (11). The circulatory disturbances in diabetes are characterized by alteration in platelet count and activity, coagulopathy, fibrinolytic aberration, haemorrheologic factors and changes in endothelial metabolism (12). In these patients, persistent hyperglycaemia exposes red blood cells (RBC) to elevated glucose concentration, thus resulting in glycation of haemoglobin, prothrombin, fibrinogen and other protein involved in clotting mechanisms (13). This study found platelet counts to be decreased in study subjects, but not statistically significant, this observation is in line with previous studies that reported counts within normal range (14) or decreased in others (15).

Activated partial thromboplastin time (APTT) in the study subjects was significantly shorter than that of control subjects while that of prothrombin time (PT) was insignificantly shorter than in controls group. These finding is in line that reported by Zhao et al. (16) and Lippi et al (17). The earlier report of *Ng* (18) had suggested that shortened APTT values in some cases may reflect a hypercoagulable state; this potentially is associated with increased thrombotic risk and adverse cardiovascular events in subjects. Solter and Dayer (19) are of the view that shortened APTTs may result from an accumulation of circulating activated coagulation factors in plasma caused by enhanced coagulation activation *in vivo*. The observation of insignificant shortened PT levels in the present study support the hypothesis proposed in the work of Merill (20) that "there is less involvement of the extrinsic pathway in hypercoagulability state in diabetic conditions due to the fact that injury occurring to the vascular system in diabetic patients does not involve tissue factor from outside the vascular system".

Various researchers have reported increased serum fibrinogen levels in diabetes. The mean fibrinogen concentration of DM patients in this study was significantly higher than the levels in the controls ($P < 0.001$). This higher fibrinogen concentration found in the diabetic group agrees with that of Mark (21) and Zhao et al. (16) who also posited that elevated fibrinogen concentration is one of the risk factors for atherosclerosis among diabetics. The work of Koening et al (22) suggests that Fibrinogen induces thrombus formation by causing platelets and erythrocytes to form aggregate and consequently promoting increased blood viscosity. Kannel et al. (23) in his study brought in another dimension on the effect of raised fibrinogen level in T2DM patients, as been associated with poor glycaemic control. The raised fibrinogen levels in study subjects may be due to chronic fibrinogen hypersecretion associated low-grade inflammation.

The results of this study support several previously published studies that reported increased D-dimer in DM (24; 25). Elevation has been associated with increased risk of future myocardial infarction, stroke and peripheral vascular disease. The clinical utility of the plasma D-dimer test could be limited to seeking 'negative for exclusion' of deep vein thrombosis and pulmonary embolism (26). It can therefore be considered as a good biochemical marker of thrombosis as reported by Zhao (16). It is also interesting to note that D-dimer/fibrinogen ratio in the study subjects is well above 328 as against 132 for the control subjects and when compared with documented range of 100. This suggests that our study population is at risk of developing

deep venous thrombosis if preventive measures are not put in place.

5.0 CONCLUSION

This study observed significant increase in fibrinogen and D-dimer levels in the diabetics compared with the non diabetic control, and therefore concludes that fibrinogen and D-dimer levels and D-dimer/fibrinogen ratio could be considered as important predictors for cardiovascular risk in diabetes mellitus. Similarly, D-dimer could serve as a good biochemical marker for predicting thrombus formation in the study subjects.

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