Some Haemostatic Parameters of Patients with Type II Diabetes Mellitus Attending Metabolic Clinics in Kano Metropolis

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ABSTRACT

Diabetes mellitus (DM) is defined as a metabolic disorder characterized by elevated blood glucose level, resulting from a defect in insulin secretion, insulin action or both. It is accompanied by biochemical alterations in carbohydrate, protein and lipid metabolism. Haemostasis is the body's normal physiological response to thwart haemorrhagic tendencies and consequently, maintain blood fluidity and blood vessel integrity. Abnormalities in haemostasis can result in bleeding (haemorrhage) or blood clots (thrombosis). The study aimed to determine some haemostatic parameters in type II diabetes patients in Kano Metropolis. One hundred (100) participants enrolled between August through November 2019; fifty (50) participants each from diabetic and healthy control subjects were recruited for the study. Routine coagulation studies were carried out using conventional methods. The prothrombin and activated partial thromboplastin times (PT and APTT) were statistically significant ($p<0.05$) compared to controls. A statistically significant positive correlation ($p<0.05$) between PT and APTT in type II diabetic subjects was observed. Decreased PT and APTT values are consistent with abnormal coagulation mechanisms, and this may have clinical implications leading to hypercoagulable state and thrombosis which results in cardiovascular disorders.

Keywords: Blood, Cardiovascular, Diabetes, Glucose, Haemostasis, Thrombosis

INTRODUCTION

Diabetes mellitus (DM) is defined as a metabolic disorder characterized by elevated blood glucose level, resulting from a defect in insulin secretion, insulin action or both.[1] Diabetes mellitus is classified into type I DM, also known as juvenile-onset diabetes or insulin-dependent diabetes mellitus (IDDM) and type II diabetes mellitus or non-insulin dependent diabetes mellitus (non-IDDM), also known as adult-onset diabetes mellitus.[2] Another subset is gestational diabetes mellitus (GDM), described as any degree of glucose intolerance with onset or first recognition during pregnancy. Type I diabetics may be associated with insufficient endogenous insulin secretion by the pancreatic beta cells. While type II diabetes mellitus may be the result of dysfunctionional insulin secretion or resistance to insulin effect.[3]

Over 170 million people worldwide and about 1-7% of Nigerian population are affected with diabetes mellitus.[4] According to diabetes leadership forum, about 12 million persons have diabetes in Sub-saharan Africa in 2010, leading to 330,000 death and other complications.[5] In Nigeria and the world at large diabetes is a major health problem with about 90% of diabetic patients having non-insulin dependent type II diabetes, while about 10% have insulin-dependent.[6] The number of people with type II DM is increasing in every country with 80% of people with DM living in low- and middle-income countries. It is estimated that 439 million people would have type II DM by the year 2030.[7]

Diabetes mellitus is characterized by chronic hyperglycemia with subsequent disturbance of carbohydrates, fats and protein metabolism.[8] Thrombosis is the leading cause of morbidity and mortality in patients with diabetes mellitus.[9] Eighty percent of patients with diabetes mellitus die due to thrombosis and 75% of these deaths are due to cardiovascular complications.[10] Several mechanisms contribute to diabetic pro-thrombotic state. These include endothelial dysfunction, coagulation activation and platelet hyperactivity. In particular diabetic platelet is characterized by deregulation of several signalling pathways tending to enhanced adhesion, activation and aggregation.[9] These alteration result from the interaction between hyperglycemia, insulin

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resistance, inflammation and oxidative stress. Some previous researches suggest that certain haemostatic indices are altered in patients with diabetes mellitus.[11] In patient with diabetes mellitus, persistent hyperglycemia exposes red cells to elevated glucose concentration, thus resulting in the glycation of haemoglobin, prothrombin, fibrinogen and other proteins involved in clotting mechanism.[12] The glycation results in perturbed function of clotting cascade. Glycation of intrinsic and extrinsic clotting proteins which affect the clotting capacity.[13] The pro-thrombotic or hypercoagulable state is not easily detected by routine laboratory tests, unlike the hypercoagulable state.

However, there is supporting evidence that shortened coagulation parameters values in some cases may reflect a hypercoagulable state, which is potentially associated with increased thrombotic risk and adverse cardiovascular events.[14] Hypercoagulability in diabetes may accelerate atherosclerosis and acts as a risk factor for cardiovascular disease.[15] Measurement of Prothrombin (PT) and Activated partial thromboplastin (APTT) are markers of extrinsic and intrinsic pathway.[16] Therefore reduced PT and PTTK may be basic factors for thromboembolic cardiovascular disease in type II diabetic patients. In this study, we aimed to assess some haemostatic parameters in type II diabetes patients attending urology clinics of some hospitals in Kano metropolis.

MATERIALS AND METHODS

Study area and design

This study was carried out in some selected hospitals in Kano metropolis which includes (Aminu Kano Teaching Hospital, Murtala Muhammad Specialist Hospital and Muhammad Abdullahi Wase Teaching Hospital). The study is a prospective cross-sectional study design.

Study subjects

A total of 100 subjects were recruited in this study, 50 were diabetic patients attending secondary and tertiary hospitals in Kano metropolis together 50 healthy individuals, who served as controls. Both genders were recruited into the study.

Ethical considerations

Ethical clearance to conduct the research was obtained from the ethics committee of Aminu Kano Teaching Hospital (AKTH) and Kano Ministry of Health, before the commencement of the research study. Consent was obtained from all participants before inclusion using an approved protocol.

Inclusion criteria

The inclusion criteria were subjects with type II diabetes mellitus and non-type II diabetes mellitus subjects having the same sociodemographic data with the studied serving as controls.

Exclusion criteria

Patient with thrombotic disorders or on anticoagulant therapy who are type II DM.

Sample collection

Blood Samples were collected by venipuncture aseptically by withdrawing five millilitres (5ml) of blood and placed in a vial containing 0.5ml of 3.2% tri-sodium citrate in the ratio of 1:9 and mixed properly. Platelet-poor plasma was harvested from citrated blood by centrifugation at 3000 rpm for 15 minutes. APTT and PT were assayed using manual method following the manufacturer's guidelines.

Prothrombin time

When tissue thromboplastin and calcium ions are added to plasma at 37°C, coagulation factors involved in extrinsic pathway becomes activated consequently activates factors of the common pathway resulting in the generation of thrombin and the formation of fibrin clot and the time taken for the clot to form is recorded in seconds. The method of Cheesbrough was applied.[17]

1. One hundred Microliter (100µl) of citrated plasma was pre-incubated for 2minutes in a clean glass tube.
2. This was followed with hundred microliters (200µl) of thromboplastin/calcium reagents and stopwatch started immediately
3. The tube was held in the water bath and tilted back and forth for clot formation (and checked at every 5 seconds) till clot formed.

The stopwatch was stopped and the prothrombin time recorded in seconds.

Activated partial thromboplastin time (APTT) test

Kaolin (surface activator) and platelet factor 3 substitute (phospholipid) are incubated with citrated plasma at 37°C for the specified duration. Calcium chloride then added and the time taken for the mixture to clot is recorded in seconds. The method of Cheesbrough was applied.[17]

1. To a test tube, 0.2ml of well-mixed kaolin platelet substitute mixture was added.
2. Then 0.1ml of the citrated plasma was added and the tube incubated for further five minutes.
3. Then after five minutes, 0.1ml of 0.025M calcium chloride solution was added and the timing starts immediately.
4. The tube was held in the water bath and tilted back and forth, for clot formation. After the clot, the time was taken in seconds.

Statistical analysis

The results obtained were analyzed using Statistical Package for Social Science (SPSS) version 20. The mean and standard deviation were calculated and unpaired t-test was used for comparison of values of diabetic subjects and those of apparently healthy individuals. Correlation analysis was also done. All statistics were set at 95% confidence interval i.e. $P<0.05$.

RESULTS

A total number of one hundred (100) participants were enrolled in this study for a period of four months, August through November 2019. Fifty (50) were diabetic subjects while fifty (50) were apparently healthy controls. Among the test group, twenty-seven (27) were males while twenty-three (23) were females. While in the control group, twenty-nine (29) were males and twenty-one (21) were females.

Table 1: Shows age distribution of type II diabetic subjects and controls. The mean age of T2DM was 43.98±7.81 and 42.78±8.07 for controls, respectively. There was no significant difference ($p>0.05$) between subjects and controls. A chi-square test of independence was used to determine the differences in the proportion of Type II DM subjects and controls.

Table 1: Distribution of type II diabetic subjects and controls by age

Variables	Subjects (n, %)	Control (n, %)	x^2	p-value
Age				
15-24	0(0%)	34(68%)	0.344	0.440
25-34	1(2%)	12(24%)		
35-44	9(18%)	2(4%)		
45-54	13(26%)	2(4%)		
55-64	19(38%)	0(0%)		
65-74	8(16%)	0(0%)		

X^2=Chi square test, n = number, %=Percentage, p=probability value

Table 2: Shows distribution of type II diabetic subjects and controls by gender. Of the total 100 participants studied, equal gender distribution was taken (50) and there was no significant difference ($p>0.05$) between type 2 diabetic subjects and controls based on gender.

Table 2: Distribution of type II diabetic subjects and controls by gender

Variables	Subjects (n, %)	Controls (n, %)	x^2	p-value
Gender				
Male	27(54%)	29(58%)	0.14	0.70
Female	23(46%)	21(42%)		

Key:X^2=Chi square test, n= number, %=Percentage, p value=probability value

The mean ± standard deviation of prothrombin time (PT) in type II diabetic subjects was 11.60±1.67 seconds while in non-diabetic individuals was 13.42±1.68 seconds. The mean ± standard deviation of activated partial thromboplastin time (APTT) in type II diabetic subjects was 27.20±4.63 seconds while in non-diabetic individuals was 36.38±3.71 seconds, respectively. Statistical analysis for PT indicates statistically significant differences among type II diabetic subjects and non-diabetic individuals with a p-value of <0.001. The APTT also shows significant difference among type II diabetic subjects and non-diabetic individuals with a p-value of <0.001, (Table 3).

Table 3: Comparison PT and APTT tests of type II diabetes subjects and controls

Parameters	T2DM Subjects n=50	Healthy Control n=50	p-value
PT (sec)	11.60 ± 1.67	13.42 ± 1.68	<0.001
APTT (sec)	27.20 ± 4.63	36.38 ± 3.70	<0.001

Key: t-test= Independent t test, p-value = Probability value, Prothrombin time (PT), APTT = Activated partial thromboplastin time, T2DM = Type II diabetes mellitus

This study has shown that, a statistically significant difference ($p< 0.05$) was observed in both Prothrombin (PT) time and activated partial thromboplastin (APTT) time between type II diabetic subjects and non-diabetic controls.

Table 4: Indicates the relationship between PT and APTT in type II diabetic subjects and controls. There was a positive correlation between prothrombin time (PT) and activated partial thromboplastin time (APTT) of type II diabetic subjects with (r = 0.48). Also, a significant positive correlation exists between prothrombin and activated partial thromboplastin time of non-diabetic individuals with r-value of 0.26.

Table 4: Relationship between PT and APTT in type II diabetes subjects and controls

Groups	PT (sec)	APTT (sec)	*r
Subjects	11.60 ±1.67	27.20 ± 4.63	0.48
Controls	13.42 ± 1.68	36.38 ± 3.71	0.26

Key: *r=Correlation Coefficient, Prothrombin time (PT), Activated partial thromboplastin time (APTT)

The distributions of PT and APTT by gender in type II diabetic mellitus subjects are depicted in Table 5.

Table 5: Comparison of PT and APTT of type II diabetes subjects and controls by gender

Parameters	Male (n=27)	Female (n=23)	p-value
PT (sec)	12.04 ± 1.69	11.22 ± 1.58	0.08
APTT (sec)	27.35 ± 5.19	27.07 ± 4.18	0.84

Key: Prothrombin time (PT), Activated partial thromboplastin time (APTT)

The mean ± standard deviation of Prothrombin time of males and females were 12.04 ± 1.69 seconds and 11.22 ± 1.58 seconds, respectively. There was no statistically significant difference ($p>0.05$). Also, the mean ± standard deviation of the activated partial thromboplastin was 27.35 ± 5.19 seconds and 27.07 ± 4.18 seconds, respectively and also not statistically significant ($p>0.05$).

DISCUSSION

In our study, activated Partial thromboplastin time (APTT) in type II diabetic subjects was significantly reduced ($p<0.05$) compared to controls. The result is inconsistent with that of Lippi *et al.*[18] who found a lower APTT in type II diabetic subjects than in non-diabetic control subjects. Acang *et al*.[19] also found that there was a significantly reduced APTT value in type II diabetic subjects, especially in patients with diabetic complications. Benjamin *et al.*[1] found similar results in the control group. Notwithstanding, our results contrasts with that of Abdulrahman *et al*.[8] who reported significant elevated APTT between treatment naïve diabetic patients and control subjects, but no significant elevation between diabetics subjects on treatment and control subjects. Similarly, our result was also not in agreement with studies by Hassan[20] who reported significant elevation of APTT in type 2 diabetic patients compared to control individuals and with the result of Shaffy *et al.*[21] who also reported a significant increase in APTT in type II diabetic subjects compared to controls.

Despite the differences between our result and that of other researchers, the implication of reduced APTT in type II diabetes mellitus could be a risk factor for hypercoagulable state, as has been demonstrated in various studies-shortened APTT associated with an increased risk of thrombosis and hence hypercoagulability.[8,18,22]

Furthermore, the in our study prothrombin time (PT) in type II diabetic subjects was significantly lower ($p<0.05$) than that of the control. This was in agreement with the study conducted by Lippi *et al*.[18] who reported significantly low PT in type II diabetic subjects compared to controls. However, it was also in accord with the study carried out by Benjamin *et al.*[1] who documented significantly shortened PT in type II diabetic subjects compared to the control group. Our result also followed that of Fayeza *et al.*[15] who reports shortened PT in type II diabetic subjects than normal individuals. Acang *et al.* observed that there were significantly shortened PT and APTT values, in diabetic patients, especially in patients with diabetes complications, which are consistent with the results of this study.[19] Karim *et al.* further asserted that diabetic conditions lead to hypercoagulable conditions among diabetic patients which enhance thromboembolic risk for cardiovascular disease.[23] Kakouros *et al.* reported that patients with diabetes mellitus have enhanced chances of vascular disease such as thrombosis due to rupture of an atherosclerotic plaque.[24] Merlo *et al*. reported an increase in tissue factor (TF) and subsequent conversion of inactive factor VII to activated factor VIIa which triggers the extrinsic pathway.[25] Lower activated Prothrombin time often indicates hypercoagulable conditions leading to increased risk of adverse cardiovascular and thrombotic events.

The result of this study was contrasted with the study of Abdulrahman *et al*. who reported significant elevation of PT between type II subjects and non-diabetic controls.[8] The result of this study was also discordant with the studies by some workers, who reported significant increase in PT in type II diabetic subjects compared to controls.[20-21]

Here, we reported no statistically significant difference between PT and APTT of males and females with type II diabetes mellitus ($p>0.05$), which was not in agreement with the finding by Abdullah *et al*., who reported a higher elevation of PT and APTT in female than male diabetic patients.[26]

CONCLUSION

In our study, we documented that Type II DM subjects had shortened APTT and PT than healthy non-diabetic controls and this is more evident in female than male subjects. This may contribute to increased predisposition to hypercoagulable state, consequently leading to cardiovascular disorder in type II diabetic subjects.

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