

Assessment of Some Coagulation Indices among Type II Diabetic Subjects in a Tertiary Facility in South West Region, Nigeria

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Abstract: Diabetes mellitus (DM) is characterized by fasting hyperglycaemia and a high risk of atherothrombotic disorders affecting the coronary, cerebral and peripheral arterial trees. Thrombo-haemorrhagic complications are well recognized among diabetic populations. The aim of this work is to study the haemostatic profile of diabetic subjects attending Federal Medical Centre, Owo, Ondo State, Nigeria. A total of 50 diabetic subjects aged between 30 – 70 years attending the diabetic clinic of the Federal Medical Centre, Owo were selected for the study. 50 aged and sex matched non – diabetic subjects in the outpatient clinic of the same hospital were recruited as controls. Prothrombin Time (PT) and Partial Thromboplastin Time with kaolin (PTTK) were estimated using standard methods. There was significant prolongation of PT and PTTK of diabetics when compared with the non – diabetic controls ($P < 0.005$). These findings suggest that despite the popular notion of a prothrombotic tendency in diabetes, diabetics may also be prone to developing haemorrhagic complications. It is helpful to bear this in mind, and to incorporate PT and PTTK assay as routine investigations for better management of these patients.

Keywords: Diabetes mellitus, Haemostasis, PT, PTTK, Thrombosis

I. Introduction

Coagulation (also known as clotting) is, the process by which blood changes from a liquid to a gel, the mechanism of coagulation involves activation, adhesion, and aggregation of platelets along with deposition and maturation of fibrin. Disorders of coagulation are disease states which can result in bleeding (hemorrhage or bruising) or obstructive clotting (thrombosis) [1]. Coagulation is highly conserved throughout biology; in all mammals, coagulation involves both a cellular (platelet) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and is the best understood. Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium lining the vessel. Exposure of blood to the space under the endothelium initiates two processes: changes in platelets, and the exposure of subendothelial tissue factor to plasma Factor VII, which ultimately leads to fibrin formation [1].

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycaemia due to disturbances of carbohydrate, fat, and protein metabolism that are associated with absolute or relative deficiencies in insulin secretion, insulin action or both [2, 3]. The current prevalence of diabetes in Africa is 4.3% with about 15 million people living with the disease. Nigeria has a prevalence of 4.04% with about 3 million people living with the disease [4] compared to a previous prevalence of about 2.2% about a decade ago [5]. Urban communities had higher diabetes prevalence (3.3%) than rural communities (2.6%) [5]. The long term effects and complications of diabetes include progressive development of retinopathy, nephropathy, and neuropathy with microvascular and macrovascular diseases. Macrovascular disorders such as atherosclerosis are a recognized major cause of mortality in the diabetic population, and are implicated in the circulatory disturbances that are seen in diabetes. The circulatory disturbances are further compounded by alteration in platelet count and activity, coagulopathy, fibrinolytic aberration, haemorrhological factors, and changes endothelial metabolism [6].

Many studies have shown that diabetes is a hypercoagulable state. Hypercoagulability results from enhanced vascular endothelial cell expression of tissue factor and Von Willebrand factor. Other factors include increased platelet adhesiveness, elevated level of procoagulant factor, and decreased fibrinolytic activity [3, 7]. Numerous studies have shown that coagulation abnormalities occur in the course of diabetes mellitus, resulting in a state of thrombophilia. These observations are supported by epidemiological studies which demonstrate that thromboembolic events are more likely to occur in diabetic patients. The coagulation abnormalities observed in diabetic patients seem to be caused by the hyperglycaemia, which also constitutes the distinguishing feature of this disease [3, 8]. Eighty percent (80%) of patients with diabetes mellitus die a thrombotic death. Seventy-five percent (75%) of these deaths are due to cardiovascular complications, and the remainder is due to cerebrovascular events and peripheral vascular complications. Vascular endothelium, the primary defense

against thrombosis, is abnormal in diabetes. Endothelial abnormalities undoubtedly play a role in the enhanced activation of platelets and clotting factors seen in diabetes. Coagulation activation markers, such as prothrombin activation fragment 1+2 and thrombin-anti-thrombin complexes are elevated in diabetes. The plasma levels of many clotting factors including fibrinogen, factor VII, factor VIII, factor XI, factor XII, kallikrein, and von Willebrand factor are elevated in diabetes [9].

Many of the previous studies on haemostatic changes in diabetic Nigerians were conducted in the western and south eastern parts of Nigeria with dearth of reported data from local region areas in Nigeria. For these reasons, it was important to study the haemostatic profile of diabetic patients resident in Owo, Ondo State, Nigeria because the study will help the scientist, physician and/or patients to know whether diabetic patients will be prone to hemorrhage especially during surgery or thrombotic crisis.

II. Materials And Methods

Study population

Ondo State at 7 10'N and 5 05'E and occupies a land area of 15,500km² while Owo lies at latitude 710'59.998''N and longitude 534'59.988''E with an altitude of 306m. The study area is located in the south-western part of Nigeria. It has an estimated population of 276, 593 [10]. It is located within the low rain forest zone of Nigeria and has two seasons, dry and wet. The dry season last from mid- October to March while the rainy season lasts from April to September.

Study design

This is a case-control study and it was conducted at Federal Medical Centre, (FMC) Owo, which serve as a tertiary health institution in Ondo State. The research was conducted between February to August, 2015. A total of fifty (50) diabetic subjects (both males and females) aged between 30 – 70 years attending the diabetic clinic of Federal Medical Centre, Owo were randomly recruited for the study. Diabetes in this study was defined based on laboratory findings as a fasting plasma glucose levels greater than 7.0mmo/L on two or more occasions [11]. Their medical history and personal data were obtained via a comprehensive questionnaire after due approval from the ethnical committee of the hospital. Fifty (50) age and sex–matched non-diabetic subjects attending the family medicine outpatient clinic of the hospital were used as controls in this study. Informed consent was obtained from all the participants.

Socio- Demographic Data

A pre-designed structural questionnaire was utilized to collect bio-data, and socio-demographic characteristics of the patients. Some of the questionnaires on these issues include sex, age, oral anticoagulant therapy, clinical manifestations, bleeding tendencies etc. prior to specimen collection. Approval for this study was obtained from the Federal Medical Centre, Owo and Ethical Clearance (FMC/OW/380/VOL.XXIX/190) was issued by Ethical Committee Federal Medical Centre, Owo.

Sample Collection and Storage

Blood samples were obtained from each subject by applying a tourniquet around the arm above elbow. The ante-cubital forsa was disinfected with a 70% alcohol soaked swab. Eight milliliters (8mls) of venous blood was collected from each subject using aseptic procedure after 12 hours fast. Five (5mls) of venous blood was dispensed into 5 ml sterile vacutainer bottle containing 0.5ml of 3.2% tri- sodium citrate solution in a ratio of blood- citrate, 9:1 (v/v) as an anticoagulant and gently mixed by inverting the container several times for the determination of PT and PTTK. Plasma was separated from the blood after centrifuging at 2000g/m for 10minutes in standard bench centrifuge to obtain platelet poor plasma required for these coagulation assays. Tests were performed within 3 hours of sample collection and in duplicates [12]. Similarly, three (3mls) of venous blood left over was dispensed into fluoride oxalate bottle for glucose estimation.

Analytical Methods

Height (m) was taken using a Stadiometer while body weight (kg) was taken using a body weight weighing scale with the subject wearing light clothing and without shoes. Body mass Index (BMI) was calculated as the ratio of weight (kg) to the square of height (m²). Blood levels of fasting blood sugar were determined using standard spectrophotometric method [13] and standard methods of Dacie and Lewis, (12) were employed for the determination of PT and PTTK.

Statistical analysis of data

A statistical package for social scientist (SPSS) 17.0 was used for the analysis of the data appropriately. Continuous variables were displayed as means and standard deviation (SD) and categorical variables were

displayed as percentage. The level of significance was taken at 95% confidence interval and P value less than 0.05 was considered significant.

III. Results

A total number of 100 subjects comprising 50 diabetic subjects with mean age (51.84±9.04) years and 50 non-diabetic subjects (control) with mean age (46.92±9.84) years were studied. Fifteen (15) out of the diabetic subjects were naïve (i.e. not yet placed on diabetic drugs) while the remaining 35 were already undergoing treatment.

Table 1 show the age and sex distribution of all participants. Participants were aged between 30 and 70 years. There were 28 females and 22 males, and 30 females and 20 males in diabetic and non-diabetic groups respectively. Thus, females constituted 58% while males constituted 42% in overall. Table 2 shows anthropometric indices in both diabetic and non-diabetic subject population. The mean age was significantly higher in diabetic subjects compared with controls, while there were no statistical significant in mean height, weight and body mass index (BMI).

Comparing biochemical parameters in both diabetic and non-diabetic subject population using independent student t test, the mean fasting blood sugar (FBS), PT and PTTK were also significantly higher in diabetic subjects compared with controls (Table 3). Comparing the anthropometric indices and biochemical parameters in diabetic subjects (naïve and under treatment) and controls using One way analysis of variance (ANOVA), the mean FBS, PT and PTTK were significantly different among the three groups (Table 4).

Similarly, in Table 5 which shows comparison of anthropometric indices and biochemical parameters in diabetic subjects (Naïve and DM subjects under treatment), the mean FBS, PT and PTTK were significantly higher in naïve diabetic subjects compared with those under treatment, while there were no statistical significant in mean age, height, weight and BMI. Finally, Table 6 shows correlation of plasma levels of FBS in diabetic subjects with BMI and haemostatic parameters. FBS has positive correlation with PT and PTTK while it shows negative correlation with BMI in diabetic subjects.

Table 1: Age and Sex distribution of the Subject population in percentage

Age group (Years)	Diabetic Subjects		Non-diabetic subjects		Total
	Male	Female	Male	Female	
30-39	1	5	2	9	17
40-49	9	7	11	10	37
50-59	5	11	2	8	26
60-69	7	5	5	3	20
Total	22	28	20	30	100

Table 2: anthropometric indices in both diabetic and non-diabetic subject population

	Diabetic subjects (n=50)	Non-diabetic subjects (n=50)	P-Value	Rmk
Age (Years)	51.84±9.04	46.92±9.84	0.011*	S
Height (m)	1.63±0.07	1.62±0.09	0.949	NS
Weight (Kg)	62.96±9.90	62.84±8.65	0.748	NS
BMI (Kg/m ²)	23.83±3.31	23.84±2.88	0.983	NS

* significant at p≤0.05

Key: n=sample size, Rmk= remark, S=significant, NS= non-significant

Table 3: Biochemical parameters in both diabetic and non-diabetic subject population

	Diabetic subjects (n=50)	Non-diabetic subjects (n=50)	P-Value	Rmk
FBS (mmol/l)	9.13±3.85	4.15±0.47	0.000*	S
PT (Secs)	15.26±0.46	14.50±0.30	0.000*	S
PTTK (Secs)	49.59±3.06	45.35±2.78	0.000*	S

* significant at p≤0.05

Key: n=sample size, Rmk= remark, S=significant, Secs= seconds

Table 4: Anthropometric indices and biochemical parameters in diabetic subjects (naïve and under treatment) and controls using One way analysis of variance (ANOVA)

	Naïve DM (n=15)	DMUT (n=35)	Control (n=50)	F-value	Rmk
Height (m)	1.64±0.08	1.62±0.07	1.62±0.09	0.765	NS
Weight (Kg)	62.13±9.09	63.31±10.33	62.84±8.65	0.918	NS
BMI (Kg/m ²)	23.13±3.20	24.13±3.35	23.84±2.88	0.580	NS
FBS (mmol/l)	12.97±5.18	7.48±1.03	4.15±0.47	0.000*	S
PT (Secs)	15.78±0.32	15.04±0.31	14.50±0.30	0.000*	S
PTTK (Secs)	52.37±2.04	48.40±2.63	45.35±2.78	0.000*	S

* significant at p≤0.05

Key: n=sample size, Rmk= remark, S=significant, NS= non-significant

Table 5: Comparison of anthropometric indices and biochemical parameters in diabetic subjects (Naïve and DM subjects under treatment)

	Naïve DM subjects (n=15)	DM subjects under Treatment (DMUT) (n=35)	P-Value	Rmk
Age (Years)	54.00±8.10	50.91±9.37	0.273	NS
Height (m)	1.64±0.08	1.62±0.07	0.703	NS
Weight (Kg)	62.13±9.09	63.31±10.33	0.471	NS
BMI (Kg/m ²)	23.13±3.20	24.13±3.35	0.332	NS
FBS (mmol/l)	12.97±5.18	7.48±1.03	0.000*	S
PT (Secs)	15.78±0.32	15.04±0.31	0.000*	S
PTTK (Secs)	52.37±2.04	48.40±2.63	0.000*	S

* significant at p≤0.05

Key: n=sample size, Rmk= remark, S=significant, NS-non-significant, Secs= seconds

Table 6: Correlation of plasma levels of FBS in diabetic subjects with BMI and haemostatic parameters

	FBS		Rmk
	r-value	p-value	
BMI (Kg/m ²)	-0.027	0.851	NS
PT (Secs)	0.467**	0.001	S
PTTK (Secs)	0.335*	0.017	S

** correlation is significant at the 0.01 level (2-tailed)

* correlation is significant at the 0.05 level (2-tailed)

IV. Discussion

Diabetes mellitus (DM) is characterized by fasting hyperglycaemia and a high risk of atherothrombotic disorders affecting the coronary, cerebral and peripheral arterial trees [14]. The PTTK is a performance indicator measuring the efficacy of both the intrinsic and the common coagulation pathways. Apart from detecting abnormalities in blood clotting, it can be used to monitor the treatment effects in patient at risk of thrombosis on heparin therapy. It is used in conjunction with PT which measures the extrinsic pathway. Shortening of the PT and PTTK predisposes patients to an increased risk of thrombosis [15, 16]. Numerous studies have shown that coagulation abnormalities occur in the course of diabetes mellitus, resulting in a state of thrombophilia. The coagulation abnormalities observed in diabetic patients seem to be caused by the hyperglycaemia, which also constitutes the distinguishing feature of this disease [3, 8].

It was observed in this study that the Prothrombin time (PT) of diabetic subjects (15.26±0.46) was significantly prolonged compared to that of non-diabetic controls (14.50±0.30). Partial thromboplastin time with kaolin (PTTK) in the diabetic subjects was also significantly prolonged than that of controls, although the values were within normal limits. This finding is in agreement with previous report by Alao et al. [3] who also reported significant decrease in the mean value of the PT between diabetic subjects and controls. These changes might be as a result of circulatory disturbances in diabetic subjects which are characterized by alternation in platelet count and activity, coagulopathy, fibrinolytic aberration, haemorrhologic factors and changes in endothelial metabolism as also reported by McFarlane, [6].

Similarly, this study also showed statistical significant in some haemostatic indices (PT & PTTK) among naïve diabetic subjects compared with those under treatment; so also with non-diabetic controls. These might be attributed to hyperinsulinaemia. Insulin resistance is accompanied by the development of a chronic inflammatory, atherothrombotic phenotype which appears to underpin the strong association with both Type 2 diabetes and coronary vascular disease (CVD). Haemostatic mechanisms are altered both in response to insulin resistance itself, with further changes secondary to abnormal glucose metabolism as impaired glucose tolerance and frank Type 2 diabetes develop [14].

This research work showed positive correlation between PT, PTTK and blood glucose levels, and negative correlation between BMI in diabetic subjects and blood glucose levels. Hyperglycaemia per se has been implicated in the development of prothrombotic changes in clamp studies [17]. Apart from the accelerated development of atherosclerosis in patients with diabetes, these patients were also found to have an increased risk of thrombotic events, explained by an increased procoagulant activity combined with a decreased fibrinolytic capacity [9, 18]. A prominent feature of type 2 diabetes is the concurrent existence of hyperglycemia and hyperinsulinemia. Recent work had demonstrated that hyperglycemia and hyperinsulinemia have differential and selective effects on the hemostatic balance in healthy humans [17]. In a strictly controlled setting, it had been showed that acute hyperglycemia activates coagulation independent of insulin levels, whereas hyperinsulinemia inhibits fibrinolysis irrespective of plasma glucose levels [17, 18].

It is obvious that this study found the PT and PTTK to be significantly prolonged among diabetics when compared with the controls even though they were still within normal limits. The significant prolongations of these parameters might be as a result of hypercoagulable tendency resulting from a shift of thrombo-haemorrhagic balance in favor of thrombosis in diabetes.

The significantly prolonged PT and PTTK found in the diabetic group in this study which did not go as expected (relative shortening of these parameters) may appear to be at variance with this submission. Even though, this study could not ascertain the mechanism for these observations because we could not investigate separate effects of hyperglycemia and/or hyperinsulinemia on coagulation. After all, much of the evidence surrounding thrombotic risk in diabetes has been amassed in relation to the role of underlying insulin resistance [14]. The outcome of this study may also be due to in-vitro interference of fibrin clot formation by inhibitors such as fibrinogen fragments 1 & 2 and D-Dimers as reported in several studies [3, 19]. Whatever the reasons may be, haemorrhagic tendencies and complications should not be entirely ruled out in management of diabetic subjects.

V. Conclusion And Recommendations

In conclusion, this study shows that PT and PTTK are both significantly prolonged among diabetic subjects when compared with the non-diabetic controls. These findings thus suggest that haemorrhagic tendencies and complications should not be entirely ruled out in management of diabetic patients. In respect to this fact, coagulation screening is advised to be incorporated as part of routine tests for proper management of these subjects.

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