Original Research Article

SIGNIFICANCE OF SOME HAEMOSTASIS PARAMETERS IN TYPE 2 DIABETIC PATIENTS IN THE ENUGU STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY TEACHING HOSPITAL ENUGU STATE NIGERIA

ABSTRACT

Diabetes mellitus has over the years become a public health challenge and a complex disease characterized by chronic hyperglycemia that results in microvascular and macrovascular complications. The present study was designed to determine alteration in haemostatic parameters in type 2 diabetic patients in Enugu State University of Science and Technology Teaching Hospital, Enugu State, Nigeria. A total of 240 subjects comprising 120 Type 2 diabetic mellitus patients (T2DM)(60 males and 60 females) aged 20-55 years and 120 apparently healthy age and gender-matched controls were recruited for the study. Blood samples five milliliters (5.0ml) was collected from subjects by veniipuncture for the determination of the haemostatic parameters. The platelet count was determined using the Mindray 530 BC automated analyzer, Mindray, Japan, the PT, INR and APTT were determined by reagents purchased from Fortress Diagnostics, UK while the VWF antigen was determined with ELISA KIT purchased from Sulong Diagnostics, China. The result revealed an increase in platelet count (216.90 $\pm$ 12.92 vs 208.60 $\pm$ 9.43) and VWF (9.71 $\pm$ 1.16 vs 7.30 $\pm$ 0.42) in the T2DM patients compared to the non diabetic controls but this was not significant. This finding is suggestive of good clinical care and glycemic control for the patients.

Key Words: T2DM, haemostatic parameters, Enugu State.

INTRODUCTION

"Diabetes mellitus is a group of metabolic disorders characterized by abnormal carbohydrate metabolism resulting in chronic hyperglycemia caused by defective insulin production, action or both" (1,2). "Type 2 diabetes mellitus (T2DM) is the most prevalent type of diabetes and accounts for about 90-95% of diabetes cases" (3-5). "It's global prevalence has increased from 4.7% (108 million) in 1980 to 9.3% (463 million) in 2019, postulated to increase to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045" (6,7). It is also estimated that 15.5% (9.8-27.8 million) people have type 2 diabetes mellitus in sub-Saharan Africa with Nigeria having the highest burden of cases (8).Studies have reported that T2DM affects multiple steps of the haemostasis process resulting in a thrombotic state and resultant development of microvascular and macrovascular complications(9),however, there is paucity of data on the haemostatic status of T2DM patients in the Enugu State University of Science and Technology Teaching Hospital (ESUTH), Enugu State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted with blood samples from patients attending the diabetic clinic of the Enugu State University of Science and Technology (ESUT) Teaching Hospital, Enugu State in the South East geopolitical zone of Nigeria. The State derived it's name from her capital and largest city, Enugu. It has an area of 7,161km^2 with a population of 3,267,837 comprising mainly the Igbo tribe of the South Eastern Nigeria. It lies between longitudes 6^o 30 ′E and 6^o 55 ′E and latitudes 5^o 15 ′N and 7^o 15 ′N. It consists of three senatorial divisions namely Enugu East, Enugu North and Enugu West (10). The ESUT Teaching

Hospital is the major tertiary health facility for the State and is located at the centre of the Enugu metropolis (Parklane) for easy accessibility to Enugu residents.

Study Design

This is a cross-sectional case-controlled survey in which patients with Type 2 diabetes mellitus serve as the cases while age-matched healthy non-diabetic served as the controls.

Sample Size

The sample size for the study was calculated using the Leslie Kish formular(11).

$$n = \frac{Z\alpha^2 PQ}{D^2}$$

where n = minimum required sample size

$Z\alpha$ = the α level of the coefficient interval or the standard normal deviate set at 1.96 corresponding to the 95% confidence interval.

P = the proportion in the target population estimated to have diabetic mellitus 8.0% (12).

D = the width of the confidence interval set at 0.05

Q = (1-p); the proportion of non-occurrence.

Substituting into the formula

$$n = \frac{1.96 \times 1.96 \times 0.08(1-0.08)}{(0.05)^2}$$

$$= 120$$

Subjects Recruitment

Subject selection was based on a simple random sampling procedure from a population of diabetic patients who gave their consent to participate in the study.

Inclusion Criteria

1. All consenting Type 2 diabetic patients on treatment were chosen as cases.
2. All consenting non-diabetic healthy adults were chosen as controls.

Exclusion Criteria

1. Nutritional anaemia can be caused by reactive thrombocytosis, therefore male and female patients having mean hemoglobin (Hb) <12g/dL and <11g/dL respectively were excluded from the study.
2. Non diabetic individuals with any diagnosed malignancy, thrombocytopenia, thrombocytosis or systemic disease were excluded.

Blood Sample Collection and Preparation

Blood sample for the study were collected using venipuncture. Subjects were made comfortable in a sitting position. A tourniquet was gently applied 2-5cm just above the antecubital fossa. The antecubital fossa was cleaned using a cotton wool soaked in 70% alcohol. A hypodermic syringe and 21G needle was

inserted into the lumen of the antecubital vein and seven milliliters (7ml) of blood was drawn quickly by a free flowing ,non-traumatic pull of the piston. The blood was dispensed into sample bottles as follows: (i)5ml into EDTA bottle for the estimation of platelet count within 2hours of collection and (ii) 1.8ml into Trisodium citrate bottle which was centrifuged at 3000 revolutions per minute for 15 minutes within 2 hours of collection using laboratory bench centrifuge (Universal 32, Hettich Zentrifuge, Germany) and the recovered platelet poor plasma stored at -40^{0}C for estimation of VWF,PT,INR and APTT(13).

Determination of the Platelet Count

"The platelet count of subjects were determined using Mindray 530 BC automated analyzer, Japan. The sample was aspirated by letting the machine sample probe into the blood sample and then pressing the probe button. Approximately 20ul of blood was aspirated by the autoanalyser. The result of the platelet count was displayed in the screen after about 30 seconds as part of the full blood count"(14).

Determination of the Von Willebrand Factor

The Von Willebrand factor antigen levels were estimated using ELISA kits obtained from Sunlong diagnostics, China(15). Standard solution and sample (100µl each) were added to each well and the blanks left empty and incubated for 90 minutes at 37^{o}C. The solution was removed, 100µl biotinylated detection antibody was added to it and incubated for 60 minutes at 37^{o}C. The solution was then aspirated, the wells washed thrice and 100µl HRP conjugate was added and let for 30 minutes at 37^{o}C, after which the solution was aspirated and washed five times. Substrate reagent (90µl) was added and incubated for 15 minutes at 37^{o}C,50µl stop solution was added. The optical density (OD) of the blank well was set at zero. The absorbance OD of each well was read at 450nm using a microplata reader. Optical density values were proportional to the concentration of measured parameters.

Determination of the Activated Partial Thomboplastin Time

"The APTT was estimated using a Haemoscan reagent obtained from Fortress Diagnostics, United Kingdom. 0.2ml of the kaolin/platelet mixture (haemoscan reagent) was dispensed into a small tube. 0.1ml of the plasma sample was then added and content mixed and incubated for 2 minutes. With the tube being tilted at intervals, 0.1ml of 0.025m of calcium chloride was then added and the stop watch was started immediately. The tube was tilted back and forth for clot formation and the time for clot to form was recorded" (16).

Determination of the Prothrombin Time

"The PT was determined using a plasmascann reagent obtained from fortress Diagnostics, United Kingdom. 0.2ml of the thromboplastin/calcium reagent (plasmascann reagent) was dispensed into a small tube and placed in a water both at 37^{o}C for about 2 minutes. 0.1ml of plasma sample was added using a calibrated capillary pipette. The contents were mixed and the stop watch started. The tube was held in water bath and the mixture tilted back and forth until a clot was formed. The time at which clot was formed was recorded as the PT in seconds" (16).

RESULTS

There were no significant differences in the Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT), International Normalised Ratio (INR), Platelet Count (PLT) and Von Willebrand factor (VWF) levels between the type 2 diabetic cases and non-diabetic controls (Table 1). There was an increase in the VWF levels and platelet count of type 2 diabetic subjects compared to the non diabetic controls but this was not significant.

Table 1: Haemostasis Parameters of Type 2 diabetic mellitus cases and non-diabetic controls

Parameter	Reference Range	Type 2 Diabetes (n = 120)	Non-diabetic (n = 120)	T-Test (P-value)
PT (secs)	14 – 16	20.63 ± 0.11	20.82 ± 0.11	0.2510
INR	0.8 – 1.0	1.03 ± 0.01	1.03 ± 0.01	0.7186
APTT (secs)	20 – 25	24.89 ± 0.08	24.90 ± 0.12	0.3940
VWF (ng/ml)	2 – 100	9.71 ± 1.16	7.30 ± 0.42	0.0656
PLT (10^9/L)	150 – 400	216.90 ± 12.92	208.60 ± 9.43	0.6170
FBS (mmol/L	3.6 – 5.6	9.6 ± 1.21	3.6 ± 0.35	0.021*
HbAIC (%)	< 7	9.54 ± 2.02	3.86 ± 1.12	0.007*

Key: Data are expressed as mean ± standard deviation, * significant difference between compared to non-diabetic control, PT = prothrombin time, INR = International normalized Ratio, APTT = Activate partial thromboplastin time, VWF = Von willebran factor, PLT = platelet count, FBS = fasting blood sugar, HbAIC = glycated hemoglobin.

Determination of the International Normalised Ratio

The INR was calculated using the formula(17).

$$\text{INR} = \frac{\text{Prothrombin time of subject}}{\text{Prothrombin time for laboratory reference plasma)}}^{\text{ISI}}$$

Where 'ISI stands for international sensitivity index for the thromboplastin reagent

DataAnalysis

Data was analysed using SPSS version 23 (SPSS Inc. Chicago). Statistical significance was defined as $p < 0.05$. Differences in the PT, APTT, INR and VWF between the cases and controls was tested using T-test.

DISCUSSION

The Prothrombin time (PT), International Normalised Ratio (INR), Activated Partial Thromboplastin Time (APTT), Platelet Count (PLT) and the Von Willebrand Factor (VWF) are hematological parameters that provide an understanding of an individual haemostatic condition in health and disease(18).

There are currently some controversies in reports on these hematological parameters in type 2 diabetic patients. Some studies reported significant lower levels of these parameters,some reported significant higher levels while others failed to establish a significant differences the type 2 diabetic patients and non-diabetic controls. Our present study did not record significant differences in the PT, INR and APTT of type 2 diabetes mellitus patients compared to controls. However, the studies of Abdelrhman and Ahmed(18), Ephraim et al(17), Karim et al(19), Ankalaya et al(20) and Ukamaka et al(21) reported a decrease in PT, INR and APTT in type 2 diabetes mellitus subjects compared to non diabetic controls. These hematological parameters when decreased in patients are markers for the activation of coagulation

pathways both the intrinsic and extrinsic pathways leading to a hypercoagulable state and in the case of type 2 diabetic mellitus patients with poor glycemic control may aggravate to occlusive thrombus which is the major factor underlying the development of microvascular and macrovascular complications in patients. In contrast, Alao et al(22) reported a prolonged PT and APTT in type 2 diabetic patients while in a similar study Ismail et al(23), Abdelrahaman and Dallatu(24) and Madans et al(25) reported no significant differences in these parameters. An increased PLT and VWF levels recorded for the type 2 diabetic mellitus patients though not significant in the present study could be attributed to glycaemic control in patients since most of the patients that enrolled for the our study are already on treatment regimens. This is not in agreement with the findings of Adane et al(1),Ukamaka et al(21) and Umeji et al(26) who recorded significant increase in PLT and Umadevi et al(27) who recorded significant increase in VWF in the type 2 diabetic patients compared to controls. The basic function of platelets is to uphold blood clotting so increased platelet count or activation will result in a hypercoagullable state which may present a poor prognosis for type 2 diabetic patients. The principal physiological function of VWF is to uphold haemostatic balance within the vascular endothelium but because the endothelium is the primary some of VWF, their increased levels in the type 2 diabetic patients in the present study suggests stimulation or injury to the endothelium which may result to a hypercoagulable state with poop prognosis such as cardiovascular disorders.

CONCLUSION

The findings of non significant differences in the haemostasis parameters between the type 2 diabetic subjects and the control suggests a good glycemic control and clinical care for the patients since all the patients recruited were already being managed for the condition. This presents a good prognosis for the patients.

Ethical Approval and Consent:

Ethical clearance was obtained from the Ethical Review Committee of the ESUT Teaching Hospital (ESUT NP/C-MAC/RA/034/Vol. 1/290) as well as informed consent from the patients.

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