Original Research Article

Studies on Haemostatic Parameters of Type 2 Diabetics in Owerri, Imo State

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Abstract

Type 2 diabetes mellitus is a chronic metabolic disorder which has emerged as a health challenge globally due to its insidious on set, late recognition and complications. The present study was aimed at evaluation of haemostatic parameters in Owerri. Cross Sectional Studies was conducted at Federal Medical Center and Imo State specialist Hospital, Owerri. A total of three hundred subjects which include each one hundred and fifty type 2 diabetics and apparently control subjects between the ages forty and sixty nine years were recruited. Ten millimeters of venous blood as aseptically collected from thesubjects. Platelet count and coagulation assay were used for determination of these parameters .The data was analyzed using statistical package for social science 20.0. Test with a probability value of P<0.05 was considered statistically significant. Results from haemostatic parameters showed higher statistically significant value (P = 0.001) in platelet count (226.68 ± 19.40 vs 205 .86 ± 13.33x10^9/l), and fibrinogen (370.19) ± 21.55 v 2.63.56 ± 32.31mg/dl) when type 2 diabetics was compared with control subjects. Inversely, there was statistically significantly lower values; (P = 0.031) in prothrombin time (13.47 ± 0.96 v 14.06 ± 0.96s) and (P = 0.001) in activated partial thrombopalstin time (34,39 ± 2.17 v 37.25 ± 1.82).There was statistically significant progressive increase in activated partial thromboplastin time (33.56 ± 1.56, 34.44 ± 2.32, 36.60 ± 180s) and (P = 0.001) fibrinogen (362.22 ± 21.72, 369.82 ± 22.65, 378.54 ± 17.69mg/dl).Also higher significant value in female compared with male (380.21 ± 16.62 v 360.17 ± 21.35. The increase found in acute proteins, malondialdehyde, platelet count, fibrinogen and decrease in antioxidants, prothrombin time and APTT showed that type 2 diabetics is a state of chronic low grade inflammation, increased oxidative stress and hypercoagulable state which may result in increase morbidity and premature mortality of type 2 diabetes.
Keywords: Haemostatic parameters, type 2 diabetics, Owerri.

INTRODUCTION

Diabetes Mellitus is a chronic metabolic disease which is associated with a high incidence rate of morbidity and mortality globally. Type 2 diabetes mellitus is the most prevalent one and makes up 90% of cases of diagnosed diabetes [1]. Type 2 diabetes mellitus formerly known as non-insulin dependent diabetes mellitus or adult-onset diabetes is a metabolic disorder that is characterized by hyperglycaemia due to insulin resistance and relative reduced insulin secretion by beta pancreatic cells [2]. International Diabetic Federation [3] reported that 425 million (8.8%) audits between the ages of twenty to seventy - nine years had diabetes and predicted to rise up to 625 (9.9%) by the year 2045. According to Adeloye *et al.,* [4], the pooled prevalence of type 2 diabetes in Nigeria in 2017 was 5.7%. Also, the prevalence of 5.7% was reported by

Uloko *et al.,* [5] as pooled geopolitical prevalence in 2018. In Imo State, the prevalence of type 2 diabetes was 8.7% was which reported among geriatric individuals [6].

Haemostasis is the process that maintains the integrity of circulating system after vascular damage [7]. Under normal circumstance, vessel wall injury rapidly initiates series of co-ordinate events designed to seal the breach generated by the injury. This leads to platelets recruitment and activation, blood coagulation; extrinsic and intrinsic way as well as generation of thrombin and fibrin [8]. Circulatory disturbances in diabetes are characterized by alteration in platelet activity, coagulopathy, fibinolytic aberration, haemorreologic factors and changes in endothelial metabolism [9]. Diabetes leads to hypercoaguble state which encompasses abnormalities

such as thrombocytosis or antithrombin iii deficiencies and vascular endothelial cells are exposed to high glucose levels leading to elevation of tissue - type plasminogen activator in the plasma accompanied by impaired fibrinolysis [10].

The study aimed to evaluate the levels of acute phase inflammatory proteins, oxidative stress markers nd haemostatic parameters in Type 2 Diabetics.

MATERIAL AND METHODS
Study Area
The cross sectional study was conducted at Federal Medical Centre, Owerri and Imo State Specialist Hospital, Owerri.

Study Population
The sample size for the study was calculated using the formula below, according to Aronye [11]. The prevalence rate of type 2 diabetes mellitus in Imo State is 8.7% [6].

$$n = z^2 p(q)/d^2$$

Where

q = 1-P
n = Sample size
p = prevalence of type 2 of diabetes mellitus in Imo State- 8.7%
z = confidence interval 95% - 1.96
d = Degree of accuracy- 0.05
$n = 1.96^2$ x 0.087(1-0.087)/0.05^2 = 3.8416 x 0.07943/0.0025
= 122.057 = 122

Therefore, the minimum sample size will be 122.

Subjects
Three hundred subjects of both sex between the ages of fifty to sixty-nine years were recruited for the study. One hundred and fifty type 2 diabetics attending clinic of Federal Medical Centre, Owerri and Imo State Specialist Hospital, Owerri for at least six months were eligible for the study. Also, one hundred and fifty apparently healthy individuals were recruited who came for check up for medical fitness and served as the control subjects.

They were further grouped according to their ages 40-49, 50-59 and 60-69 years, and also according to sex; male (n=75) and female (n=75)

Parameters Studied
The parameters evaluated in this study include platelet count, prothrombin time, activated partial thromboplastin time and fibrinogen.

Study Design
This is a cross-sectional study that involved type 2 diabetics and control subjects.

Site of the Study
The analysis was carried out at the Haematolgy laboratory of the Department of Medical laboratory Science of Imo State University, Owerri and Federal Medical Center, Owerri through a letter obtained from the Head of Department through my Supervisor.

Laboratory Procedure
All reagents were commercially purchased and the manufacturer's standard operating procedures were strictly adhered to.

Determination of Glucose
This was carried out by enzymatic oxidase - peroxidase method according to Tietz *et al.,* [12] as modified by Randox Laboratories, United Kingdom. Catalog number; GL 1021, GL 304, GL366.

Procedure
Three dry cleaned plain test tubes were setup in a jack, labeled test, standard and blank. Then, 20µl of sample and standard wore pipetted into the tubes labeled these and standard respectively. In all the tubes, 2000µl of reagents I was added.The tubes were gently mixed, incubated at 37°C for 10 minutes. The absorbance of sample and standard were read spectrophotometrically at 500nm.

Determination of Platelet Count
This was carried out by direct current detecting method, according to Lewis *et al*., [13] using Sysmex Automated Haematology Analyzer KX- 2IN Model.

Procedure
The sequenstrene blood sample was placed in the spiral mixer and allowed to mix well. The whole blood was activated in the LCD screen; the sample number (code) was imput by pressing enter. The sample was mixed well. The cap was removed and the sample inserted into the probe and the stand switch was pressed. The LCD screen displayed the analysis. The sample was removed and re-capped. The unit executed the analysis automatically and the result was displayed on the screen.

Determination of Prothrombin Time
The test was carried out by one stage prothrombin time according to Lewis et al., [13] as modified by Agappe Diagnostic Switzerland. Catalogue Number; 5261003, 52601004

Procedure
A clear dry test tube was set up in a rack. The reagent vial was swirled gently before use. The

prothrombin regent was dispensed from the vial and it was pre-warmed of 370c for 10 minutes. 100 µl of plasma was added to pre - warmed curvette and was incubated at 370C for 3 minutes. 200 µl pre - warmed prothrombin reagent was added to be test curvette containing the pre-warmed sample. The time was started and the clotting time was recorded.

Determination of Activitated Partial Thromboplasmin Time

This was carried out by manual coagulation method according to Lewis *et al.,* [13].

Procedure

The reagent vials were gently mixed to a homogenized suspension and swirled before use. Enough volume of reagent 1 Calcium chloride reagent 2 (APTT reagent) were pre-warmed at 37^oC for ten minutes prior to use. A test curvette was set up at 37^oC in a water bath, $100\mu l$ of plasma was pipette into it, followed by an addition 100μ of pre-warmed APTT reagent 2 well mixed and incubated for three minutes forcibly, 100μ of pre warmed reagent 1 (calcium chloride) was added into it… a timer was started simultaneously and the clothing time was read recorded in formation of clot in seconds.

Determination of Fibrinogen

This was carried out by semi automated coagulation method according to Clauss, 1957. As modified by Giessie Diagonstic, Italy. Catalogue number: 1004, 1005, 1006, 1007.

Procedure

A semi-automated Rayto 220c colgulotmeter was used. In a clean dry tube, $25\mu l$ of plasma was pipette into it and $225\mu l$ of imidazole buffer was added to make 1: 10 dilution. A test curvette was added into the holder and there was a display prompted to add sample displayed on the screen. Then $100\mu l$ of diluted sample was pipette into the test curvette and it was incubated for five minutes and a display showed sample warming. There was another display in the screen prompted to add reagent, $50\mu l$ of thrombin was added with a display showing on the screen testing. The result was displayed on the screen and was recorded.

STATISTICAL ANALYSIS

Data was analyzed using software, statistical package for social science (SPSS), version 20.0. The results were expressed as mean and standard derivation (mean±SD). Comparison of differences in the mean values between the groups were assessed using one way analysis of variance (Anova) and Independent student T – test. Variable between the age groups was assessed using Post Hoc test.

RESULTS

Table-1: Comparison of the Mean Values of Haemostatic Parameters in Type 2 Diabetes Mellitus and Control Subjects of the Study Population

Parameters	Type 2 diabetes mellitus subjects (n = 150) mean ± SD	Control subjects (n = 150) mean± SD	T-test	P-value
Platelet count (10^9/L)	226.68± 19.40*	205.86± 13.33	10.833	0.0001
Prothrombin time (S) Activated partial thromboplastin	13.47 ±1.25*	14.06 ±0.79	7.920	0.031
time (S)	34.39 ±2.17*	37.25 ±2.10	11.402	0.001
Fibrinogen (mg/dl)	370.19 ±21.55*	263.58±32.31	33.616	0.0001

KEY

n: Number of subjects in each group
*: statistically significant when compared with type 2 diabetic group (P<0.05).

Table-1 shows the mean values of haemostatic parameter; platelet count and prothrombin time, activated partial thromboplastin time and fibrinogen in type 2 diabetics and control subjects. Result from this study, showed that the mean value of platelet count (226.68 ± 19.40) was higher in type 2 diabetics and was statistically significant (P = 0.0001) when compared with the mean value (205.86 ± 13.33) of the control subjects.

The mean value of prothrombin time (13.47 ± 1.25) was lower in type 2 diabetics which was statistically significant (P=0.031) when compared with the control subjects (14.06 ± 0.96).

Result showed that the mean value of activated partial thromboplasmin time (34.39±2.17) was lower in type 2 diabetics which was statistically significant (P=0.001) when compared with the mean value (37.25±1.82) of the control subjects.

Inversely, the mean value of fibrinogen (370.19±24.55) was higher in type 2 diabetics which was statistically significant (P=0.001) when compared with the mean value (263.58±32.31) of the control subjects.

Table-2: The Mean Values of Haemostatic Parameters in Type 2 Diabetes Mellitus Subjects in Relation to Age of the Study Population

Parameters	40-49 Years (n=50) mean ± SD	50-59 Years (n=50) Mean ± SD	60-69 (n = 50) mean ± SD	F-value	P-value	P-value a vs b	P-value b vs c
Platelet count (×10⁹ Cells/L)	231.44± 18.44	226.18± 21.58	222.42± 17.23	2.792	0.065	0.358	0.590
Prothrombin time (Seconds)	13.60 ±1.31	13.52 ±1.20	13.28 ±1.23	0.892	0.412	0.945	0.602
APTT (Seconds)	33.56± 1.56	34.44± 2.32	36.60± 1.80**	10.320	0.0001	0.245	0.015
Fibrinigen (mg/dL)	362.22±21.72	369.82±22.65	378.54±17.09*	7.836	0.001	0.160	0.091

KEY

N: Number of subjects in each group

P - value: P- value across all type 2 diabetics age groups

P valuea vs b: P-value of comparison between the age group of 40-49 and 50-59 years in type 2 diabetics

P valueb vs c: P-value of comparison between the age group of 50-59 and 60-69 years

*: statistically significant when compared between the ages of 40-49 years (P<0.05).

**: statistically significant when compared between the ages of 50-59 years (P<0.05).

Table-2 shows the mean values of platelet count, and prothrombin time, activated partial thromboplastin time and fibrinogen in type 2 diabetics in relation to age.

There was progressive decrease in the mean value of platelet count acoss all age groups (231.44± 18.44; 226.18± 21.58; 222.42±17.23) which was not statistically significant (P=0.065). When the value between the ages 50-59 years (226.18± 21.58) was compared with the ages between 40-49 years (231.44± 18.44), there was decrease which was not statistically significant (p = 0.358) and also there was decrease which was statistically significant (p = 0.590) when the value between60 - 69 years (222.42±17.23) was compared with the ages between 50- 59 years (226.18± 18.44).

There was no significant difference (p=0.892) in the mean value of prothrombin time among all the age groups (13.60 ±1.31; 13.52 ±1.20; 13.28± 1.23). There was no significant difference value between 40 – 49 years (13.60 ±1.31) and 50 -59 years (13.52 ±1.20) was not statistically significant (p = 0.412). Also, the mean value of ages between 60 - 69 years (13.28± 1.23) with 50 – 59 years (13.52 ± 1.20) was not statistically significant (p = 0.602).

Result showed that there was progressive increase in the mean value of activated partial thromboplastin time across all ages (33.56 ± 1.56; 34.44 ± 2.32; 36.56 ± 1.80) which was statistically significant (p = 0.001). The mean value between the ages of 50-59 years (34.44 ± 2.32) was increased but was not statistically significant (p=0.245) when compared with the mean value of ages between 40-49 years (33.56±1.56). The mean value between the ages of 60-69 years (34.44±2.32) was increased, but was not statistically significant (p=0.245) when compared with the mean value of ages between 40-49 years (33.56 ± 1.56). Also the mean value of ages between 60-69 years (36.56±1.80) was increased which was increased which was statistically significant (p=0.015) when compared with the mean value of ages between 50-59 years (34.44±2.32).

There was a progressive increase in the mean value of fibrinogen across the ages (362.22 ± 21.72; 369.82 ± 22.65; 378.54 ± 17.09) which was statistically significant (p=0.001). the mean value between the ages of 50-59 years (369.82±22.65) was increased which was no statistically significant (p=0.160+ when compared with the mean value of ages between 40-49 years (362.22±21.72). the mean value of ages between 60-69 years (378.54 ± 17.09) was increased which was not statistically significant (p=0.091+ when compared with the mean value of ages between 50-59 years (369.82±22.65).

DISCUSSION

In the study, higher level of platelet count was observed in type 2 diabetes when compared with the control subjects. Platelet hyperactivity occurs in type 2 diabetes when there was abnormal endothelial vascular dysfunction due to subclinical inflammation or cell wall injury which enhances vasoconstriction, increases release of cytokines and expression adhesion molecules such as intracellular cell adhesion molecules (ICAM) and vascular cell adhesion molecules (VCAM) which mediates platelets activation and adhesion to endothelium. This is in agreement with Soares *et al.,* [14] that reported that hyper platelet activation result in 2 to 3 times higher coronary artery disease risk in diabetics. This is in accordance with studies conducted by Akinsegun *et al.,* [15] that revealed higher mean platelet volume for diabetics. This agrees with the work of Alhadas *et al.,* [16] that reported positive correlation of platelet count with the fasting blood glucose. Kaur *et al.,* [7] observed high level of platelet as a result of endothelial and platelet hypersentivity which may involve in endothelial dyfunction and vascular

disorders. Ferrein *et al.,* [17] observed that high level of platelet count result from increase platelet reactivity which was induced by non-glycation of membrane protein that decreased membrane fluidity and platelet adhesion. This is also in agreement with Alhadas *et al.,* [16], Pulani *et al.,* [18] who observed higher platelet count and suggested that long term complication of diabetes with marked platelet morphorlogy could be associated with risk of developing vascular complication in diabetes contrary to present findings Buch *et al.,* [19], reported that platelet count was significantly decreased in diabetics due to increased platelet activity. In contrast in a cross-sectional studies conducted by Chen *et al.,* [20], there was no significant difference between platelet count and among diabetics when compared with non-diabetics. The study observed increase in various ages in type 2 diabetes which is not significant. This may be due to age related changes in platelet function in translocation platelets and unstable platelet interaction with von willie brand factor which result in increase adhesion to surface. This is in agreement by observational study conducted by Jones (2016). Also, Cowman reported that platelet function decreases in age due to decrease number of platelet tracking and interaction in von Williebrand factors. There was a slight increase in female type 2 diabetics which was not significant when compare with male diabetics. This may be due to reduction in oestrogen and deletion of oestrogen receptor in aged female which is expresses by platelet. This observation is supported by studies conducted Cowman *et al.,* [21]; Zhao *et al.,* [22].

The present study observed variation in blood coagulation. Lower level of prothrombin time was observed in type 2 diabetics when compared to control subjects. Prothrombin time is a screening test used to detect disorder involving contact with tissue factor which involve activation factor, ii, v, vii, ix, of extrinsic and common pathway. The shortened prothrombin time result from increased activation of hyperglycaemia induced glycation of coagulable protein ; prothrombin activation of coagulation factor which result in clot formation thrombosis increased pre-maline atherosclerotic plague formation rupture occlusive thrombus and hypercoagulable state include endothelial dysfunction, coagulative activation, platelet hypersensitivity as a result of hyperglylaemia insulin resistance inflammation of oxidative stress. The coagulation detect contributes lot micro vascular and macro vascular complication such as diabetic nephropathy, retinopathy, neuropathy cardiovascular, cerebrovascular peripheral vascular disease. The finding of this study is consistent with previous studies. Karim *et al.,* [23] reported lower prothrombin time and concluded that type 2 diabetes were prone to develop coagulation defects. This is in concordance with the work of Ephram *et al.,* [24] that found the association shortened PT. with or without diabetes complication as

a coagulation risk factor in contrary to this study. Abdeen and Hamza *et al.,* [25] observed prolongation of PT due to deficiency of coagulation factors in relation to diabetic duration. Thukral *et al.,* [26] observed higher level of PTin type diabetics which indicated abnormal coagulation mechanism involving tendency of bleeding due to thromboembolic haemorragic disorder. A cross sectional study conducted by Obeagu *et al.,* [27] showed no significant difference in the level of prothrombin time between diabetics and control subjects. Abdulla *et al.,* [28] reported no significance in type 2 diabetics and control subjects. This study showed no significant difference in all age groups in type 2 diabetics. The findings are similar to the work of Ephraim *et al.,* [24]; Thukral *et al.,* [26] that reported no difference in age in PT level among diabetics. The findings of the study observed elevated level in female T2D which was not statistically significant. This may be due to increase in visceral obesity and hormonal changes such decrease in estrogen which may factor development of thrombosis. This agrees with the work of Ephraim *et al.,* [24] that found insignificant increase in female diabetics. In contrary, there was no significant difference in PT level between male and female type 2 diabetics which was reported by Elendo *et al.,* [29]; Abdulla *et al.,* [28].

The current study observed low level of activated partial thromboplastin time (APTT).This is a coagulation maker used in assessing abnormalities in intrinsic and common pathway, contact with prekallikren high molecular, Kinnogen ,fibrinogen , coagulation inhibitors and natural anticoagulant. The low level of APTT in diabetics is a risk of hypercoagulable state which enhances thromboemobolic or occlusive thrombus within coronary or cerebral artery contributing to development of atherosclerotic lesion. This occurs due to hyperglycaemia glycation of intracellular and extracellular proteins which affect intrinsic clotting factors and triggers accumulation of these activated coagulation factor, delay action of coagulation inhibitors and fibinolytic system. The formation of uncontrolled thrombin is indicated by shortened APTT. The findings of this study are consistent with previous studies. Zhao *et al.,* [30] reported low level of APTT in type 2 diabetes which reflects pro coagulation imbalance with increase level of coagulation of factor. Mwambungu *et al.,* [31] reported low significant APTT in type 2 diabetes because T2DM patients are more prone to hyper coagulable state with increased thrombotic risk of cardiovascular events. This is in agreement with the work of Ankalayya *et al.,* [32] who reported shortened APTT in type 2 diabetes with thrombotic risks. Ambelu *et al.,* [33] reported low APTT level of 85% in untreated and 2.5% in treated type 2 diabetes. This agrees with the findings of Pan *et al.,* [34] that reported shortened APTT in T2D was associated with diabetic complication such as

nephropathy. This is consistent with the study of Agarwal *et al.,* [32] that reported that low APTT occurred with or without diabetic complications. In contrary to this study, Alao *et al.,* [35] reported significant prolonged due to thrombo haemorrhage shift in haemostasis balance. This is supported by the work of Obeagu *et al.,* [27]; Abdeen and Hamza [25] found significant prolonged APTT level in type 2 diabetics as a result of inhibitors of intrinsic system and abnormal coagulation. Thurkral *et al.,* [26] reported elevated plasma level of APTT which occurs due to defect in intrinsic coagulation, no significance difference was observed in the level of APTT between T$_2$D and control subjects which was reported by Abdulla *et al.,* [28]. The finding on the study observed significant decrease in APTT level in diabetes as the ages progress, this indicates that coagulation defect increase the thrombotic risk as the ages increase .The findings is not in accordance with the study of Elendo *et al.,* [29]; Ephriam *et al.,* [24] reported no significant difference between various age groups in type 2 diabetics. This study observed no significant difference between male and female type 2 diabetics. This is consistent with the work of Abdulla *et al.,* [28] that reported no significant difference in type 2 diabetics in relation to sex .The findings to this study is inconsistent with work done by Elendo *et al.,* [29] that reported significant increase in female when compared to male type 2 diabetics.

Higher level of fibrinogen was observed in type 2 diabetes when compared with the control subjects. This is due to non enzymatic glycation of fibrinogen that promotes adhension of endothelial receptors to platelet membrane, increases aggregation due to increase blood viscosity, reduction in tissue fibrinolysis from plasminogen to fibrinogen. Higher level of fibrinogen plays a role in formation of occlusive thrombosis over a rupture atherosclerotic plug in development of atherosclerosis in type diabetes. This implies that fibrinogen stimulates smooth cells proliferation and migration, increases coronary arterial diseases. The findings of this study agrees with the study of Bembale *et al.,*[36] that reported higher level of fibrinogen due to increased thrombotic tendency in diabetics. Grupta *et al.,* [37] also reported significant higher level of fibrinogen in diabetic with poor glycaemic control and its role in plague formation in form of occlusive thrombus over ruptured atherogenesity .This is similar to the findings of Yang *et al.,* [38] that reported higher level of fibrinogen which is a marker of thromboembolism and inflammation that promotes ischemic stroke risk in diabetics. This is also consistent with the study by El-sayeed *et al.,* (2018) reported higher fibrinogen level in type 2 diabetes due to changes in haemostatic mechanism that contributes to development of diabetic vascular diseases. There was progressive increase in fibrinogen level as the ages progress due to hepatic stimulation of fibrinogen which as associated with

inflammation, indicated a link between inflammation and hypercoagulation in advanced ages. This is in agreement with the work of Gupta *et al.,* [37]; Yang *et al.,* [38] that reported that plasma level of fibrinogen increases with ages. There was higher level of fibrinogen in female when compared with male type 2 diabetics. This is because fat content and hormonal difference in woman. This is not in agreement with the study conducted by Bembale *et al.,* [36] when reported insignificant higher level in female type 2 diabetics.

CONCLUSION
The findings of this study have shown that type 2 diabetics mellitus is associated with alteration in haemostatic system which is signified by platelet hyperactivity and hypercoagulable state. These undesirable state which play roles in the pathogenesis of type 2 diabetes may result in unexpected complications increased morbidity and premature death in type 2 diabetics.

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