Assessment of Human Fibrinogen Antigen and Tissue Plasminogen Activator as Hypercoagulability Markers in Type 2 Diabetic Patients in Obio/ Akpor Local Government Area, Rivers State, Nigeria

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

Original Research Article

ABSTRACT

This is a case controlled study carried out on type 2 Diabetic Patients in Obio/ Akpor Local Government, Rivers State with the aim of determining the haemostatic status. The haemostatic markers were assessed and compared with apparently healthy control subjects without diabetes. A total of 66 Type 2 diabetic patients (36 females and 30 males) aged between 24 and 77 years; 54 subjects (31 females and 23 males) that were apparently healthy of age between 24 and 76years, enrolled as control, with all participants recruited between November 2019 and January 2020. Human fibrinogen antigen and tissue plasminogen activator were analysed with reagents obtained from Elabscience, Wuhan, China and the test carried out with an ELISA machine (STAT FAX-2100).Results showed a significant increase ($p<0.05$) respectively in mean tissue plasminogen activator and mean human fibrinogen antigen when compared with control. Also there were strong significant correlations ($p<0.05$) respectively between human fibrinogen antigen and blood glucose, tissue plasminogen activator and blood glucose.There was no significant differences ($p<0.05$) observed in tissues plasminogen activator and human fibrinogen antigen between Type 2 Diabetic male and female patients. These two haemastatic markers can be used as a predictor indices for management of hypercoagulability in type 2 diabetic patients.

Keywords: Hypercoagulability; type 2 diabetes; haemostatic markers; plasminogen activator.

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1. INTRODUCTION

Diabetes mellitus is a complex disease that affects the metabolism of a variety of components such as carbohydrates, lipids, and proteins, as well as biological processes such as coagulation, which can lead to vascular thrombotic problems [1]. Hyperglycemia is present, as well as abnormalities in carbohydrate, lipid, and protein metabolism [2]. Some haemostatic markers are proteins such as fibrinogen, platelet, mean platelet volume, tissue plasminogen activator concentration. Changes in these proteins favour the development of a hypercoagulable and pro-thrombotic condition, which may increase cardiovascular risk by raising the likelihood of building an occlusive thrombus inside a coronary/cerebral artery, resulting in atherothrombosis [3]. Thrombosis is a hypercoagulable condition with common micro and macrovascular complications in type 2 diabetes. Consistently high blood sugar levels make blood platelets stickier and boost the blood plasma's clotting potential. Diabetics are more susceptible to irregular blood clotting as a result of these side effects.

Fibrinogen is a soluble plasma glycoprotein that is generated by the liver and transformed to fibrin during blood coagulation by thrombin [4]. This is accomplished through coagulation cascade activities that convert the zymogen prothrombin to the serine protease thrombin, which converts fibrinogen to fibrin. Fibrin is then crosslinked by factor XIII, resulting in the formation of a clot. FXIIIa further stabilises fibrin by incorporating the fibrinolysis inhibitors alpha-2-antiplasmin and TAFI(thrombin activatable fibrinolysis inhibitor, procarboxypeptidase B), as well as attaching to a variety of cell adhesive proteins [5]. Fibrin catalyses the activation of Factor XIII by thrombin as well as the activation of tissue plasminogen activator (t-PA). Fibrin binds to and entraps activated coagulation factors Xa and thrombin in a network of fibres, acting as a temporary inhibitor of these enzymes, which remain active and can be released during fibrinolysis. The conversion of plasminogen to plasmin, which leads in clot lysis and the generation of fibrin degradation products, is mediated by tissue plasminogen activator (tPA). One of the most powerful anti-fibrinolytic drugs is plasminogen activator inhibitor-1 (PAI-1), which forms a compound with tPA and inhibits its function [6]. Because vascular endothelium releases tissue plasminogen activator (t-PA), circulating levels of t-PA antigen may be a measure of endothelial dysfunction. A greater plasma t-PA antigen level,

on the other hand, may reflect both endothelial disruption (t-PA and PAI-1 release) and hepatic PAI-1 release, as it reflects mainly inactive circulating t-PA–PAI-1 complexes [7]. Some discrepancies have been observed in some studies on the association between diabetes and haemostasis thus more detailed studies are necessary to elucidate the process and clarify the factors involved. The published study or data on haemostatic markers in type 2 diabetic patient in the south-south part of Nigeria are limited. These informed the reasons to study some haemostatic markers of diabetic patients resident in Obio/Akpor, Rivers State, southern part of Nigeria.

The main aim is to determine hypercoagulability state of Type 2 diabetic patients by assessing some haemostatic markers.

2. METHOD

2.1 Research Site

The study was conducted at University of Port Harcourt Teaching hospital, a third level referral hospital for Port Harcourt, Nigeria. It is located in Obio/Akpor local government area, which have a population of 878,800 people as estimated in 2006 census and land mass of 260 km².

2.2 Study Design

This was a cross-sectional study involving type 2 diabetic patients who attended diabetic clinic in University of Port Harcourt Teaching Hospital. Also apparently healthy adult male and female who visited University of Port Harcourt Health Centre for pre-employment medicals and medicals for admission purposes were recruited.

2.3 Eligibility Criteria

People who have being diagnosed to be diabetic and are above 18 years were recruited. People who are healthy and non-diabetic were recruited as control. Women who are pregnant were excluded.

2.4 Sample Size

The Power and Sample Size Software (PASS) was used for calculating the 120 sample size which was a comparison between the proportion of type 2 diabetic patients and normal control participants. The prevalence of type 2 diabetes was based on Akinyimi et al.[11] who reported

that the prevalence in Nigeria varied from 0.65% in rural Mangu (North) to 11% in urban Lagos (south) and data from the World Health Organization (WHO) suggested that Nigeria has the greatest number of people living with diabetes in Africa. Nigeria have the largest number of people with diabetes (3.0 million) compared to some Africa's most populous countries [9].

2.5 Sampling Method

Systematic random sampling was used to group the subjects into male and female, control and test. Some other vital statistics collected were age, glycaemic control and duration of disease.

2.6 Data Collection

The information from the participants was gathered via a questionnaire. The demographic information (age, gender, location of residence, degree of education, and work position), physical measurements (weight and blood pressure), levels of awareness about type 2 diabetic risk factors, and management problems were all included in the questionnaire. The other part of the questionnaire was used to capture information pertaining to the duration of type 2 diabetes disease, diet, glycaemic control for each participants.

2.7 Sample Collection

Four different types of anticoagulant vacutainer were used for the laboratory test. These were EDTA, sodium fluoride, sodium citrate and plain vacutainers. Two (2) ml of venous blood was collected in each vacutainer using the evacuated blood collection system which was very ideal and safe for multiple blood sample collection as blood was delivered directly into the vacutainer. Additional 2ml was added to the plain vacutainer which made it a total of ten (10) ml collected from each research participant.

2.8 Data Analysis

The statistical package for social science (SPSS) and centre for evidence based medicine (CEBM) statistics calculator was used to analyse the results. Statistical analysis of distribution was made using the Kolmogoroff- Smirnoff test. Data were also analysed using Microsoft office excel 2003 version to get the mean and standard deviation of subjects with type 2 diabetes mellitus and control subjects that were apparently

healthy. The significance of the difference between patients and controls for normally distributed parameters were determined using the independent samples T-test for continuous variable and chi-square test for categorical variables. The association between glycaemic control and hypercoagulable state were determined by ANOVA. A level of correlation between blood glucose level and tissue plasminogen activator-fibrinogen (tPA-FG) was determined by spearman method. The p values of less than 0.05 were taken as significant.

2.9 Prevalence of Hypercoagulabiltity in Type 2 Diabetic Patients

Tissue plasminogen activator and fibrinogen are normal (tPA <10.0 ng/ml and fibrinogen (200-500 ng/ml) and hypercoaguable when (tPA >10.0ng/ml and fibrinogen >500 ng/ml). The proportion of participants who were at risk of hypercoagulability was compared between type 2 diabetic patients and control subjects. The values were based on the proposed 1st WHO International Standard for the measurement of tissue Plasminogen Activator (tPA) antigen in plasma 94/730 in 2007.

3. RESULTS

3.1 Sample Population Distribution by Demographic Factors

A total of 120 subjects aged 24 -77 years participated in the study out of which 53(44.2%) were males and 67(55.8%) were females. Sixty-six (66) participants were type 2 diabetes mellitus and 54 were control participants. The age of type 2 diabetes mellitus ranged from 24 years to 76 years with the majority of participants [20 (30.3%)] being in the range of 44 - 53 years. The mean age was 49.3 ±12.5. The age of control subjects also ranged from 24 -77 years with majority [17 (31.5%)] being in the range of 44 - 53. The mean age for control subjects was 47.2 ±13.0. Fig. 4.1 depicts the sample population distribution.

3.2 Haemostatic and Biochemical Markers of Type 2 Diabetes Mellitus Patients and Control Subjects

An independent sample t-test was conducted to compare the haemostatic markers in type 2 diabetes mellitus and control participants as shown in Table 1 that the mean tissue plasminogen activator (t-PA) for control

participant (5.8 + 2.4ng/ml) was significantly lower than type 2 diabetic patient (12.7+ 3.9ng/ml) t-value= 11.8; P-value 0.0001.

The mean human fibrinogen antigen (HFA) for type 2 diabetic patients (745.6 + 323.9 ng/ml) was significantly higher than control participants (354.0 + 101.8ng/ml) t-value 9.27; P-value =0.0001.

The mean glycated haemoglobin (HbIAc) for type 2 diabetic patients (7.5 ± 1.6%) was significantly higher than control participants (5.0 ± 0.7%) t-value = 11.5; P-value = 0.0001.

Table 2 reveals results of an independent sample t-test that was conducted to compare the haemostatic markers in type 2 diabetic male and female patients. Table 2 shows that the mean tissue plasminogen activator for type 2 diabetic female patients (12.3+ 4.1 ng/ml) was lower than type 2 diabetic male patients (13.1 + 3.6ng/ml). The difference in the two means was not significant t-value = 0.902; p-value = 0.370.

The mean human fibrinogen antigen for type 2 diabetic female patients (735 + 317.6ng/ml) was lower than type 2 diabetic male patients (757.3 + 336.5ng/ml). The difference in the two means was slightly significant t-value= 0.264; p-value= 0.396.

3.3 Prevalence of Hypercoagulability

Tissue plasminogen activator and human fibrinogen antigens which were continuous variable in Statistical Package of Social Science, were recorded so as to divide the results into two categories; those who had tissue plasminogen activator less than >10.0ng/ml and human fibrinogen antigen between the range of 200-500ng/ml were regarded as normal. A chi-square test was done to determine the proportion of hypercoagulability in type 2 diabetic patients and control participants. Table 3 shows that type 2 diabetic patients had higher prevalence of hypercoagulability [41(62.1%)] than control participants [16 (29.6%)]. The difference was significance P-value = 0.000.
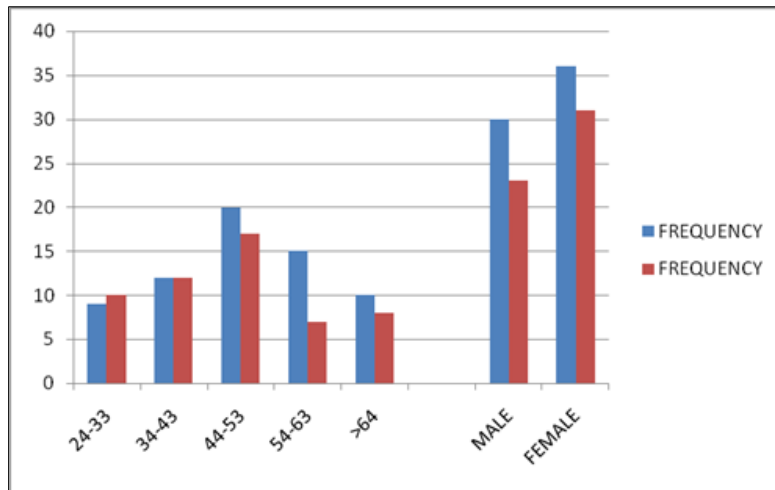

Fig. 1. Graphic representation of sample population distribution

Table 1. Haemostatic and biochemical markers of type 2 diabetic patients and control subjects

Parameters	Status	N	Mean ± SD	t-value	p-value
t-PA (ng/ml)	Control	54	5.8 ± 2.4	-11.8	0.0001
	Diabetic	66	12.7 ± 3.9		
HFA (ng/ml)	Control	54	354.0 ± 101.8	-9.27	0.0001
	Diabetic	66	745.6 ± 323.9		

Table 2. Gender comparison of Haemostatic markers of type 2 diabetic patients

Haemostatic parameters	status	N	status	t-value	p-value
Tissue Plasminogen activator(ng/ml)	Male	30	13.1 ± 3.6	0.902	0.370
	Female	36	12.3 ± 4.1		
Human Fibrinogen antigen(ng/ml)	Male	30	757.3 ±336.5	0.264	0.396
	Female	36	735.8 ±317.6		

Table 3. Comparison of hypercoagulability prevalence between type 2 diabetic patients and control participants

Status	Total	No. of participant	%	χ^2	p-value
Control participant	54	16	29.6		0.000
Type 2 diabetic patients	66	41	62.1		

3.4 Correlations between the Haemostatic Markers and Blood Glucose

Fig. 2 shows that there was a strong significant positive correlation between tissue plasminogen activator and human fibrinogen antigen. It indicates that as tissue plasminogen activator is increasing, human fibrinogen antigen is also increasing. [R=0.538; R2= 0.041, P=0.0001]

Fig. 3 shows that there was a very weak positive correlation between tissue plasminogen activator and fasting blood sugar. The correlation was not significant. [R=0.054 R2=0.003, P=0.664]

Fig. 4 shows that there was a weak correlation between human fibrinogen antigen and fasting blood sugar. The correlation is not significant. [R=0.133, R2 = 0.018, P= 0.286].

3.5 Association between Glycaemic Control and Haemostatic Markers

Table 4 reveals results of ANOVA single factor that was conducted to associate glycaemic control with haemostatic markers in type 2 diabetic patients and control. Table 4 shows the association between tissue plasminogen

activator of poor glycaemic control group and good control group were significantly different F-value = 63. 4; P-value = 0.001.

The association between human fibrinogen antigen of poor glycaemic control group and good control group were significantly different F-value =36.9; P-value = 0.001.

4. DISCUSSION

The mean human fibrinogen antigen of persons with type 2 diabetes in this study was significantly higher than in the controls (p < 0.05). This higher fibrinogen antigen found in the diabetic group agrees to the findings of Zhao et al. [8] who reported increased fibrinogen concentrations among Chinese type 2 diabetic patients. Increased fibrinogen may induce thrombus formation by affecting platelets and erythrocytes to aggregate which also promotes increased blood viscosity. Kannel et al. [5] reported that fibrinogen is often elevated in type 2 diabetic patients and this elevation is associated with poor glycaemic control. The increase in human fibrinogen antigen levels in type 2 diabetic patients may be due to chronic fibrinogen hyper secretion. Increased fibrinogen levels in diabetes may be related to the associated low-grade inflammation [12].
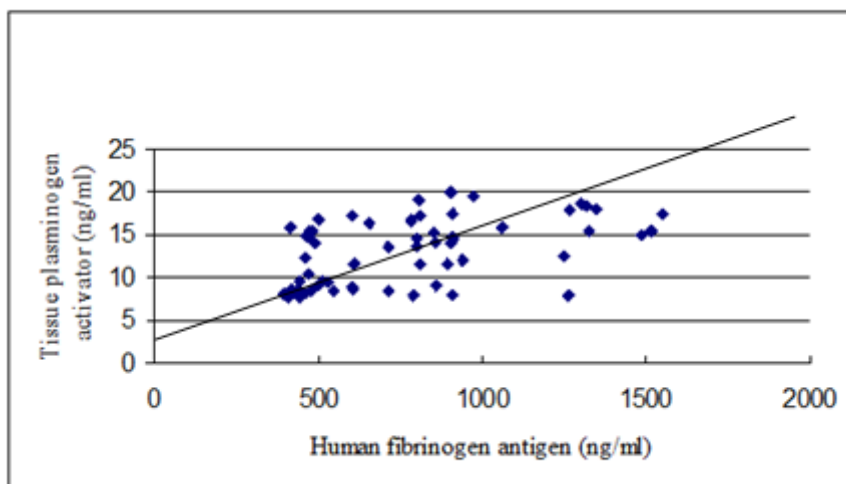

Fig. 2. Correlation between tissue plasminogen activator and human fibrinogen antigen
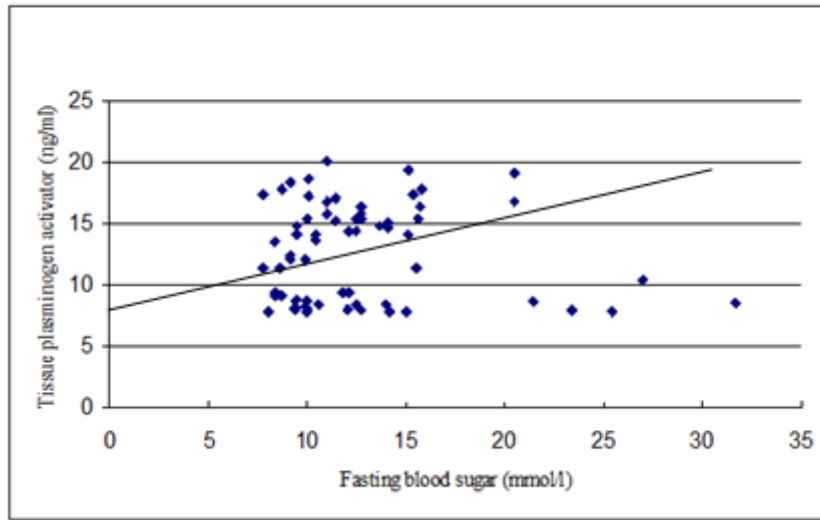
Fig. 3. Correlation between tissue plasminogen activator and fasting blood sugar
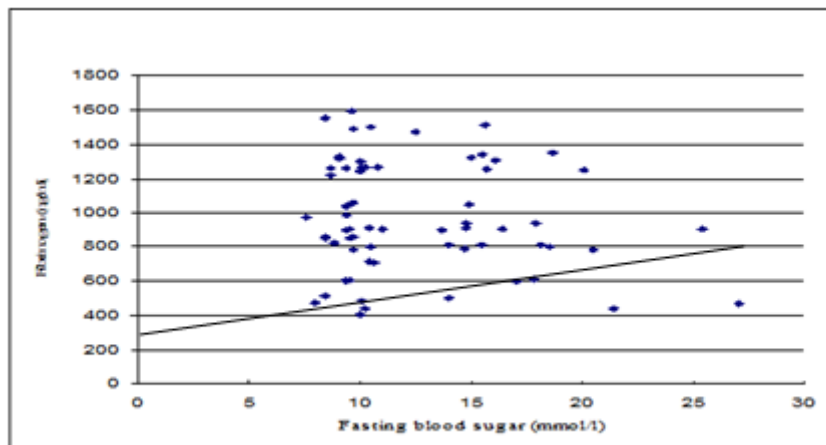

Fig. 4. Correlation between human fibrinogen antigen and fasting blood sugar

Table 4. Association between glycaemic control and haemostatic markers

Haemostatic parameters	Glycaemic control	N	Variance	F-values	P-values
t-PA (ng/ml)	Control	54	5.92	63.4	0.001
	Good control	40	16.53		
	Poor control	26	13.77		
HFA (ng/ml)	Control	54	10380.4	36.9	0.001
	Good control	40	102547.4		
	Poor control	26	110267.0		

The mean tissue plasminogen activator of persons with type 2 diabetes was also significantly higher than controls ($p < 0.05$). This agrees to the findings of Lowe et al. [9] who reported that an elevated t-PA antigen level is considered to be an integral feature of the insulin resistance syndrome and is also related to the inflammatory response.

The correlation between tissue plasminogen activator and fasting blood sugar was significant. This agrees to the finding of Goya et al., (2008) who reported the increased risk of diabetes associated with t-PA. It also does not agree with Nsakashalo-senkwe [10] findings which shows no significant interaction between glucose levels and t-PA. The reason for this correlation may be due to the effects of hyperglycemia on the endothelium over a prolonged period of time.

5. CONCLUSION

The main aim of this study was to assess the haemostatic markers of type 2 diabetic patients. It was observed that type 2 diabetic patients had increased levels of tissue plasminogen activator and human fibrinogen antigen than healthy non-diabetic control participants. Type 2 diabetic patients had higher human fibrinogen antigen, tissue plasminogen activator than the healthy non-diabetic control participants contributing to increased prevalence of hypercoagulability in diabetic patients than control participants. Therefore type 2 diabetic patients especially those with poor glycaemic control, are more prone to hypercoagulable state than non-diabetic healthy individuals.

CONSENT AND ETHICAL APPROVAL

The study was approved by the ethical and medical committee of University of Port Harcourt Teaching Hospital. Each of the participants in this study gave informed consent to participate.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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