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Full-Length Research Article

Glycated Haemoglobin, Fasting Plasma Glucose, Plasminogen Activator Inhibitor Type-1, and Soluble Thrombomodulin Levels in Patients with Type 2 Diabetes Mellitus

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Summary: Diabetes mellitus has become increasingly prevalent over the years. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunctions, and failure of different organs suggesting that the most effective tool to prevent complications is the effective control of hyperglycaemia itself. The study is set to determine the effect of glycemic control on plasminogen activator inhibitor type 1 (PAI-1), soluble thrombomodulin (STM) alongside fasting plasma glucose (FPG) and glycated hemoglobin (HbA1c) among type 2 diabetic subjects. One hundred diabetic subjects accessing care at the University of Calabar Teaching Hospital Calabar and 100 non –diabetics that served as controls were enrolled. Blood samples from participants were analyzed for FPG, HbA1c, PAI-1 and STM by standard methods. The result shows 74% of the diabetic to be females. Half of the diabetics were managed on only oral anti-diabetic drugs while the remaining half were either on insulin injection or a combination of oral and insulin injection. Poor glycemic control was observed in 56% of the studied subjects. The mean age of 54.69 ± 9.94 years for the diabetics was comparable to the age-matched controls (p=.097). Diabetics showed significantly higher FPG, HbA1c, PAI-1and STM (P=0.001) compared to control values. Correlations between STM, PAI 1 and glycated hemoglobin (figures 2 p=0.001, p =0.001) and STM, PAI-1 and FPG revealed significantly robust association (p=0.001, p=0.001). The study concludes that there is poor glycemic control among the treated diabetic subjects with PAI-1 and STM showing a very strong positive correlation with HbA1c than FPG.

Keywords*: Diabetes, Hyperglycemia, glycemic control, endothelial function*

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INTRODUCTION

Diabetes mellitus has been reported to be increasingly prevalent over the years (IDF, 2017). while vascular complications have been identified among the important morbidity and mortality factors (WHO, 2019). Persistently increased value of hyperglycaemia in diabetes is associated with long-term damage, dysfunction, and failure of different organs suggesting that the most effective tool to prevent complications from organ impairment in diabetes is the regulation of glycaemia itself. Owing in part to the inconvenience of measuring fasting plasma glucose levels or performing an oral glucose tolerance test, coupled with the day-to-day variability seen in glucose assessment, an alternative glucose measurement for the management of diabetes was introduced known as the glycated haemoglobin (HbA1c). Initially identified as "unusual" haemoglobin in patients with diabetes, glycated haemoglobin is now regarded as an objective measure of glycaemic control. Reports have shown that glycated haemoglobin correlates with coagulation derangements (WHO, 2011; ADA, 2018: Akwiwu *et al.,* 2020). Furthermore, recent endothelial studies done in diabetes suggest endothelial dysfunction, which is the hallmark of vascular diseases (Bretón-Romero *et al.,* 2018; Berra-Romani *et al.,* 2020).

The vascular endothelium, once thought to be simply a passive lining for blood vessels, is now recognized as a key determinant of vascular health. It has also become evident that endothelium is not an inert, single-cell lining covering the internal surface of blood vessels, but plays a crucial role in regulating vascular tone and structure (Iantorno *et al.,* 2014; Brakemier *et al.,* 2016). Other functions include the lining of the internal lumen of all the vasculature serving as an interface between circulating blood and vascular smooth muscle cells thus aiding as a physical barrier between the blood and tissues. Moreover, the endothelial cells facilitate a complex array of purposes in intimate interactions with the vascular smooth muscle cells, as well as cells within the blood compartment (Favero *et al.,*2014; Carrizzo *et al.,*2018). Therefore, injury or activation of the endothelium changes its regulatory functions and results in abnormal endothelial cell function. (Dogné *et al.,* 2018; Schiattarella *et al.,*2018).

Assessment of endothelial functions can be measured by the evaluation of endothelial cell markers. These Cell surface markers are proteins expressed on the surface of cells that often serve as indicators of specific cell types. Because endothelial activation often precedes overt endothelial dysfunction, biomarkers of the activated endothelium in serum or plasma may be detectable before

classically recognized markers of disease, and therefore, may be clinically useful as biomarkers of disease severity or prognosis in systemic infectious diseases (Page and Conrad, 2013) Predictors of endothelial dysfunction could improve the screening of individuals at increased risk, thus leading to the early diagnosis, appropriate treatment, as well as effective prevention of the complications of type 2 diabetes (Page and Conrad, 2013; Lau *et al.,* 2015). Plasminogen activating inhibitor-1 (PAI-1) is an endothelial damage marker and the primary enzyme inhibitor of plasminogen activation. Plasminogen is an acute-phase protein and precursor to plasmin, which digests fibrin thus playing a key role in the maintenance of the fibrinolytic system. Elevated levels of PAI-1 thus predispose to clot formation by inhibiting fibrinolytic activity and thus may be an early risk marker for disease progression (Pernow *et al.,* 2015; Lau *et al.,* 2015). Soluble thrombomodulin is another marker of endothelial damage. It represents the major substance of the protein C anticoagulant system (Chudy *et al.,* 2011). Thrombomodulin and activated protein C constitute a system that maintains vascular integrity as well as the thrombosis/ haemostasis balance. These roles are facilitated as the system provides anticoagulant, anti-inflammatory, and cytoprotective activities (Ikezoe, 2015). Although soluble thrombomodulin is yet to be extensively studied, its elevation is thought to be linked to widespread vascular damage and could be useful in the assessment of vascular complications in diabetes (Pernow *et al.,* 2015; Lau *et al.,* 2015). To understand more about the extent of activation of the endothelial cells concerning glycemic control, this study was done to determine FPG, HbA1c, PAI-1 and STM of diabetics and their possible relationships among diabetic subjects.

MATERIALS AND METHODS

Participants: A total of 200 participants were recruited for this study. They were made up of 100 persons with type 2 diabetes mellitus who were attending the clinic at the University of Calabar Teaching Hospital. Another 100 age and sex-matched healthy non-diabetic subjects drawn from the general population served as controls.

Ethical consideration: Ethical approval was obtained from the Health Research Ethics Committee (HREC) of the University of Calabar Teaching Hospital. Informed consent was obtained from each participant enrolled in the research and confidentiality was maintained. Bio-data and related information were obtained using a questionnaire.

Data collection: Pre-test counseling was administered to each respondent. Blood specimen was collected from each participant between 8am-9am in the mornings to minimize variability. Fasting plasma glucose was assayed by the glucose oxidase method (Randox, UK). In this method, the glucose oxidase enzyme catalyses the complete oxidation of glucose to produce hydrogen peroxide and gluconic acid. The hydrogen peroxide in the presence of the enzyme peroxidase is broken down and the oxygen released reacts with 4-amino-phenazone and phenol to give a pink coloured derivative whose absorbance is then measured in a colorimeter using a green filter.

Glycated haemoglobin was assayed by ion exchange resin method (Spectrum, Egypt). Glycosylated haemoglobin (GHb) has been defined operationally as the fast fraction haemoglobin HbA1 (HbA1a, A1b, A1c) which elutes first during column chromatography. The non-glycosylated haemoglobin, which consists of the bulk of haemoglobin has been designated HbAo. A haemolysed preparation of whole blood is mixed continuously for 5 minutes with a weakly binding cation-exchange resin. The labile fraction is eliminated during the haemolysate preparation and during the binding. During this mixing, HbAo binds to the ion-exchange resin leaving GHb free in the supernatant. After the mixing period, a filter separator is used to remove the resin from the supernatant. The percent glycosylated haemoglobin is determined by measuring absorbance of the glycosylated haemoglobin (GHb) and total haemoglobin fraction (THb). The ratio of the absorbance of GHb and THb of the control and test is used to calculate the percent GHb of the sample.

Both PAI-1 and soluble thrombomodulin were assayed using enzyme-linked immunosorbent assay kits from Bioassay Technology Laboratory, China. The PAI-1 present in the sample is added and binds to antibodies coated on the wells. Biotinylated Human PAI-1 antibody is added and binds to PAI-1 in the sample. Then streptavidin-HRP is added and binds to the biotinylated PAI-1 antibody. After incubation, unbound streptavidin-HRP is washed away during a washing step. The substrate solution is then added and colour develops in proportion to the amount of human PAI-1. The reaction is terminated by the addition of acidic stop solution and absorbance is measured at 450nm. The STM present in the sample is added and binds to antibodies coated on the wells. Biotinylated human STM antibody is added and binds to STM in the sample. Then streptavidin-HRP is added and binds to the biotinylated STM antibody. After incubation, unbound streptavidin-HRP is washed away during a washing step. Substrate solution is then added and colour develops in proportion to the amount of human STM. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450nm.

Data analysis: Data analysis was done using SPSS version 22.0. Student t-test was used for comparison of means while Pearson's correlation was used to establish a relationship between variables. Statistical significance was drawn at $p \leq 0.05$.

RESULTS

The assessed variables of both diabetic and non-diabetic subjects have been captured in Table 1. Approximately three-quarters (74%) of the diabetic subjects were females while the remaining one-third (26%) were males. Fifty percent (50%) of the diabetic subjects were on only oral anti-diabetic drugs, 31% on oral and insulin combined while 19% were on insulin alone. More than half (56%) of the studied subjects had poor glycemic control. Fifteen persons (15%) out of the 100 diabetic subjects had normal glyceamic control while 8% and 21% respectively represent good and fair glyceamic controls.

Table 1.
Measured Parameters of Diabetic and Control subjects

Parameters	Diabetic Subjects n = 100	Control n = 100	p-Value
Gender			
Males	26	26	
Females	74	74	
Treatment			
Oral drugs	50	-	
Insulin	19	-	
Oral & insulin	31	-	
Glycemic Control			
Normal	15	66	
Good	8	20	
Fair	21	14	
Poor	56	0	
Age (years)	54.69±9.94	52.40±9.50	0.097
FPG (mmol/l)	10.45±4.82	4.36±0.76	0.001
HbA1c (%)	7.97±1.80	5.56±0.86	0.001
PAI-1 (ng/ml)	13.26±4.13	4.86±1.54	0.001
STM (ng/ml)	8.77±0.65	3.18±0.28	0.001

The mean age of 54.69 ± 9.94 years for the diabetics was comparable to that of the age-matched controls (52.40±9.50 years). The FPG (10.45±4.82mmol/l), HbA1c (7.97±1.80%), PAI-1 (13.26±4.13ng/ml) and STM (8.77±0.65ng/ml) were significantly higher in diabetics compared to control values (4.36±0.76mmol/l, 5.56±0.86%,4.86±1.54ng/ml and 3.18±0.28ng/ml respectively). The chart in figure 1 represents the percentage impact of treatment types on glycemic control. Oral agents alone achieved more of good glycaemic control followed by oral/insulin combination and the least being insulin alone.
The correlation between STM and glycated hemoglobin in Fig. 2 (p=0.001, r=0.845) was stronger than that between STM and FPG (p=0.001, r=0.691). Same pattern was observed between PAI-1 and glycated hemoglobin (p =0.001, r=0.812) and PAI-1 and FPG (p=0.001, r=0.652) Fig 3.

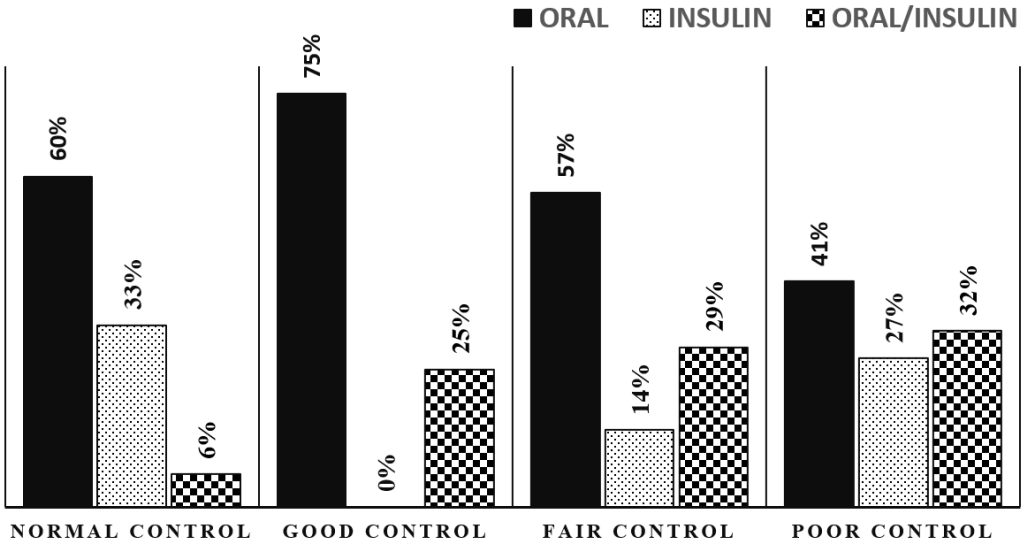

Figure 1
Categorisation of glycaemic control drug regimens

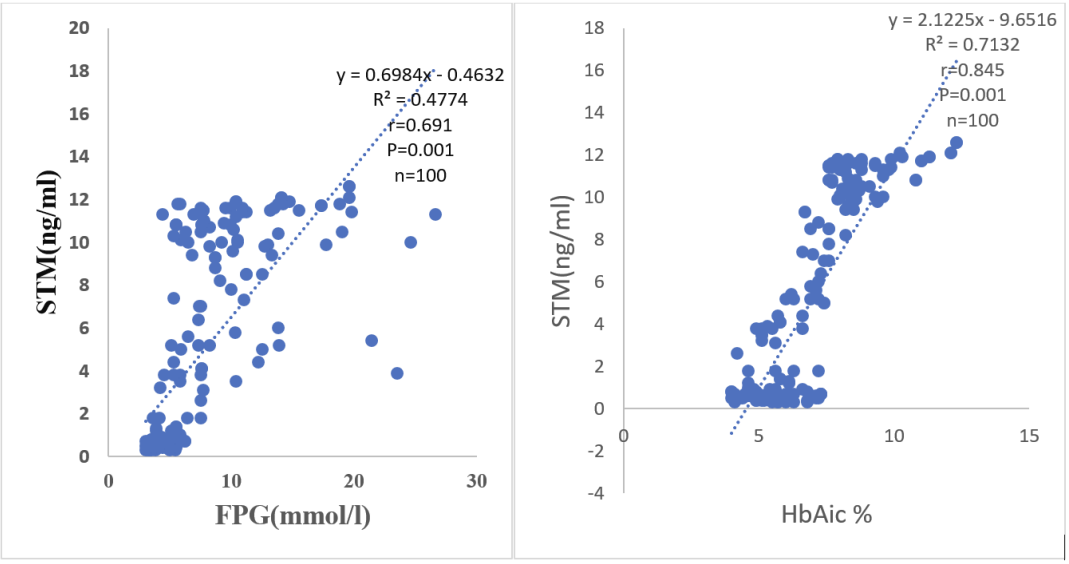

Figure 2:
Correlations of soluble thrombomodulin with fasting plasma glucose and glycated haemoglobin levels of diabetic subjects

DISCUSSION

Diabetes mellitus is a frequent and increasing public health problem mainly due to changes in dietary habits and general lifestyle during the last few years as well as rapid epidemiological transition (CDC, 2014; Sabir *et al.*, 2017; WHO, 2019). This study observed a mean age of 54.69±9.94 years and female preponderance of 74% within the studied population. Age and gender distribution differences in diabetes vary across the world. Depending on the region, distribution of these factors is influenced by prevailing risk factors as determined by socio-cultural and economic situations. These include poor dieting and physical inactivity particularly in low income countries with inadequate access to health care services (Kavanagh, *et al.*, 2010; Agardh *et al.*, 2011). Central obesity as well as high levels of estrogen and progesterone (both of which can reduce whole body insulin sensitivity) in women may also be implicated in the diabetic gender skew (Christensen *et al.*, 2008; Hilawe *et al*, 2013).

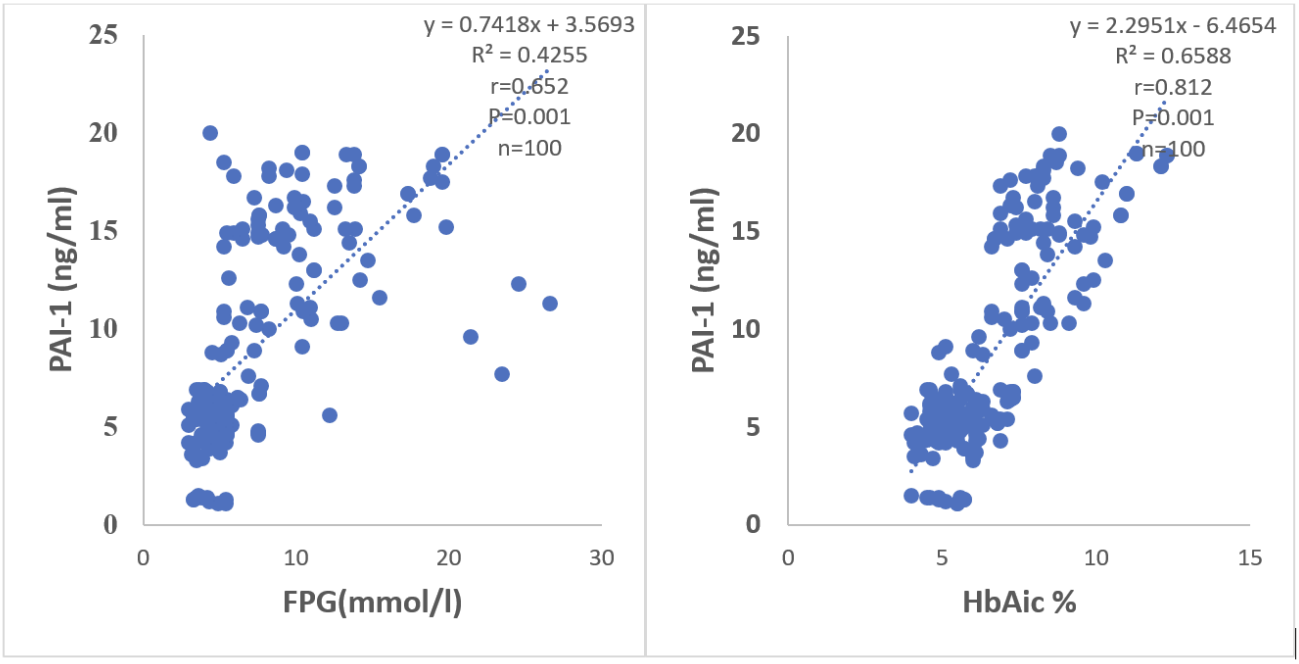

Figure 3:
Correlations of plasminogen activator inhibitor type-1 with fasting plasma glucose and glycated haemoglobin levels of diabetic subjects

This study measured fasting plasma glucose, glycated haemoglobin, plasminogen activator inhibitor type-1 and soluble thrombomodulin of subjects with type 2 diabetes on treatment. In proper management of diabetics, the primary goal is achieving glycaemic control. In this study, the glycated haemoglobin which is a measure of glycaemic control presents the results as, normal when patients value is <6.0%, good when the value is between 6.0-6.8%, fair when it is 6.8 -7.65% and poor when value is >7.65%). Findings of this study has shown that despite accessing diabetic care at the study center, attempts at maintaining near normal blood glucose levels among the diabetic subjects have not been very successful as only 15% of them had normal results while 56% had poor glycaemic control. Type 2 diabetes being a syndrome characterized by insulin resistance and increased hepatic glucose output (Kahn *et al.*, 2014; WHO, 2019), require medications that can correct one or more of the metabolic abnormalities. Prescriptions for the subjects are in the form of oral agents, insulin injection or combination of oral agents and insulin. The main goal is to achieve such therapeutic effects that will give good glycaemic control. It was observed as at the time of this study that half (50%) of the diabetics were on oral antidiabetic therapy and this achieved better glycaemic control than the combination therapy and insulin alone.

The current study also observed that glycaemic indices (FPG and HbA1c) of diabetics were significantly raised when compared with the control subjects. This format of result is expected as the test subjects were already known and managed diabetic subjects.

Furthermore, plasminogen activator inhibitor type-1 concentrations were observed to be raised significantly in diabetics. This may be due to its role as an acute phase reactant whose concentrations are raised in inflammatory conditions. Patients with diabetes also have insulin resistance, which contributes to inflammation that leads to the potential of causing endothelial dysfunction. Previous studies had observed correlation between plasma insulin and PAI-1 in different groups including obese subjects and non-insulin dependent diabetic patients (Rosenson *et al.*, 2011).

Soluble thrombomodulin is a marker of endothelial damage, representing major substance of the protein C anticoagulant system. Elevated levels of soluble thrombomodulin have been found in association with chronic diseases related to inflammation and endothelial dysfunction. More specifically, hyperglycaemia has been noted to induce increased expression of soluble thrombomodulin in the system (Kubisz, *et al*, 2010; Dietrich *et al.*, 2013; Elsalakawy *et al.*,2014; Hayden, 2019). Apparently, the elevated soluble thrombomodulin in diabetes as seen in this study is indicative of ongoing

endothelial injury induced by hyperglycaemia. In fact, elevated plasma concentrations of soluble thrombomodulin in patients with type 2 diabetes could be having deeper implications, such as widespread vascular damage (Chudy *et al.,* 2011; Brakemier *et al.,* 2016).

Apart from the observation that both fasting plasma glucose and glycated haemoglobin were significantly raised among the diabetic subjects when compared with the control subjects, there was also positive correlation between the endothelial cell markers with both fasting plasma glucose and glycated haemoglobin, (among the diabetics). However, glycated haemoglobin provided more sensitive relationships with the endothelial cell markers investigated than fasting plasma glucose. Thus, challenge in achieving glycaemic control is indicatively the more underlying mechanism for diabetic endothelial dysfunction rather than just the presence of hyperglycaemia. This obviously has implications in the areas of routine laboratory monitoring and effective management of type 2 diabetes. This study concludes that for better management of type 2 diabetes, at least one of either PAI-1 or STM alongside HbA1c should be used in the laboratory tests for the routine evaluation of affected persons.

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