ORIGINAL ARTICLE

Endothelial cell markers in diabetes and prevalence of uncontrolled type 2 diabetes mellitus in Southern Nigeria

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ABSTRACT

Objectives: Assessment of endothelial cell markers and prevalence of uncontrolled diabetes among diabetic patients on treatment would reveal the extent of glycemic control and management of vascular complications taking place in the given population. This study thus measured plasminogen activator inhibitor-1, soluble thrombomodulin, fasting plasma glucose and glycated haemoglobin (HbA1c) among type 2 diabetic subjects in Southern Nigeria.

Methods: Adult diabetic subjects accessing care at the Diabetic Clinic of University of Calabar Teaching Hospital in Southern Nigeria were enrolled for this study with age and sex-matched non-diabetic control subjects. Blood samples from all participants were analysed for fasting plasma glucose, HbA1c, plasminogen activator inhibitor-1 and soluble thrombomodulin by standard methods.

Results: Significantly (p=0.001) higher values of endothelial markers, plasminogen activator inhibitor 1 and soluble thrombomodulin were observed alongside increased glycemic indices; fasting plasma glucose and HbA1c among diabetic subjects compared to non-diabetic subjects. These parameters were also observed to be higher among the 56.7% subjects with uncontrolled diabetes.

Conclusion: There is a high prevalence (56.7%) of uncontrolled diabetes in the studied population and the expression of endothelial cell markers is related to the degree of glycemic control.

Keywords: Diabetes, glycaemia, haemostasis, endothelium, HbA1c, plasminogen activator inhibitor-1, soluble thrombomodulin.

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INTRODUCTION

Diabetes mellitus is an important non-communicable medical condition currently affecting one out of every ten adults globally (1,2). A uniquely common disorder, diabetes mellitus is a group of metabolic diseases characterised by persistent hyperglycemia. This arises when insulin production is inadequate, when the body's cells do not respond properly to insulin or a combination of both. Diabetes mellitus is a chronic condition that requires medical monitoring for effective management. In Nigeria, type 2 diabetes mellitus is the prevalent subtype affecting mainly the adult population (3,4). Despite associated morbidity and mortality, proper glycemic control ensures a stable health state. However, concerns have been raised over rising global burden of the condition, underdiagnosis, and many patients presenting with complications at the time of diagnosis (5-7). Presently, four out of every five adults living with diabetes reside in low-income and middle-income countries such as Nigeria, while global projection for diabetes in the near future anticipates increase in the number of adults that would be affected (2). It has therefore become necessary to evaluate the prevalence of uncontrolled diabetes in our population and look into morbidity-associated biomarkers for better management of this increasingly common health condition. One such aspect of diabetic complication is the progressive deterioration in cardiovascular health.

A growing interest in cardiovascular health is the role of the endothelium in regulating and maintaining vascular function (8). The endothelial cell lining protects the system from the onset of vascular degeneration as the cells line the internal lumen of all the vasculature serving as an interface between circulating blood and vascular smooth muscle cells. In addition to serving as a physical barrier between the blood and tissues, endothelial cells have been shown to also participate in various functions by interacting with the vascular smooth muscle cells, as well as cells within the blood compartment (8-10). Endothelial response and ultimately dysfunction is associated with the presence of overt inflammatory stimulation as seen in persistent hyperglycemia.

An earlier study on glycemic control among apparently healthy adults in the study area had observed varying degrees of glycemic control with attendant derangements in platelet parameters occurring undetected owing to over-dependence on fasting plasma glucose for routine screening (7). Although glycated haemoglobin is included in the laboratory work-out for management of diabetes in our health facilities, financial constraint remains a challenge for some patients as the national health insurance scheme is limited in coverage. To investigate the prevalence of uncontrolled diabetes and possible relationships between glycemic control and endothelial response, this study was embarked upon.

METHODS

Ninety adults with type 2 diabetes mellitus attending clinics at the University of Calabar Teaching Hospital were enrolled with ninety age and sex-matched non-diabetic subjects who served as control subjects. Apart from prescribed antidiabetic drugs taken by the diabetic subjects, the study participants were not on any other medication at the time of the study. Ethical approval was obtained from the Health Research and Ethics Committee of the University of Calabar Teaching Hospital. Informed consent was obtained from each participant enrolled in the research and confidentiality was maintained. Biodata and related information were obtained using a questionnaire. Pre-test counselling was administered to each respondent. Blood specimens were collected from each participant between 8am-9am in the mornings for analyses of fasting plasma glucose by glucose oxidase method (Randox, UK) and glycated HbA1c by ion exchange resin method (Spectrum, Egypt). Both plasminogen activator inhibitor-1 and soluble thrombomodulin were assayed using enzyme-linked immunosorbent assay kits from Bioassay Technology Laboratory, China. Data analysis was done using SPSS version 22.0. Student t-test was used to compare means between test and control subjects as well as controlled and uncontrolled diabetic subjects after testing for normal distribution of data. Pearson's correlation was used to analyse relationships between the measured parameters and only the significant correlations have been reported. Statistical significance was drawn at $p \leq 0.05$.

RESULTS

Apart from comparable body mass index between diabetic and non-diabetic subjects, the results of fasting plasma glucose, HbA1c, plasminogen activator inhibitor-1 and soluble thrombomodulin from the study participants indicate significantly higher values among diabetic subjects (Table 1). The diabetic subjects were grouped into two on the basis of glycemic control as measured by the HbA1c using a cut-off value of 59.57mmol/mol. This cut-off value is the upper limit for 'fair glycemic control', while values beyond 59.57mmol/mol fall under the 'poor glycemic control' category (normal control: <42.08 mmol/mol, good control: 42.08-50.82 mmol/mol, fair control: 50.82-59.57 mmol/mol and poor control: >59.57 mmol/mol). Therefore, this study categorised subjects with values greater than 59.57mmol/l under uncontrolled diabetes. They were 51 in number constituting 56.67% of the enrolled diabetic subjects. Mean values of fasting plasma glucose, HbA1c, plasminogen activator inhibitor-1 and soluble thrombomodulin were all significantly higher among the group with uncontrolled diabetes compared to those with controlled diabetes (Table 2). The various Pearson's correlation values for all the measured parameters are shown in Table 3.

DISCUSSION

This study on endothelial cell markers and prevalence of uncontrolled diabetes among subjects with type 2 diabetes in Southern Nigeria measured fasting plasma glucose, HbA1c, soluble thrombomodulin and plasminogen activator inhibitor-1. In this study we found that fasting plasma glucose and HbA1c of diabetics were significantly raised when compared with the control subjects and this is expected as the test subjects were already diagnosed and were being managed for diabetes, a condition characterised by hyperglycemia. The concentrations of plasminogen activator inhibitor-1 and soluble thrombomodulin were observed to be also raised significantly in diabetics. The former is secreted in vascular and metabolic tissues and is considered an acute phase reactant with the potential of increased expression under sustained inflammation as seen in persistent hyperglycemia. The up-regulation of plasminogen activator inhibitor-1 has thus been linked to endothelial dysfunction as well as obesity by virtue of its secretion sites (11-14). Soluble thrombomodulin on the other hand represents major substance of the protein C anticoagulant system and its elevation has been found in chronic diseases related to inflammation and endothelial dysfunction (15-18). Up regulation of thrombomodulin is thought to reflect widespread vascular damage (19-22).

Table 1. Selected morbidity indicators of diabetic and control subjects.

Parameter	Diabetics (N=90)	Non-diabetics (N=90)	P-value
BMI (Kg/m^2)	28.90 ± 5.33	28.13 ± 6.52	0.382
Fasting plasma glucose (mmol/L)	10.39 ± 4.87	4.35 ± 0.77	0.001
HbA1c (mmol/mol)	62.00 ± 18.20	36.68 ± 9.41	0.001
Plasminogen activator inhibitor-1 (µg/L)	13.14 ± 4.12	4.86 ± 1.54	0.001
Soluble thrombomodulin (µg/L)	8.59 ± 0.65	3.21 ± 0.27	0.001

Table 2. Selected morbidity indicators of controlled and uncontrolled diabetic subjects.

Parameter	Controlled diabetics (N=39)	Uncontrolled diabetics (N=51)	P-value
BMI (Kg/m^2)	28.79 ± 6.48	28.99 ± 4.32	0.866
Fasting plasma glucose (mmol/L)	8.86 ± 4.14	11.56 ± 5.09	0.009
HbA1c (mmol/mol)	46.23 ± 11.40	74.06 ± 12.18	0.001
Plasminogen activator inhibitor-1 (µg/L)	10.61 ± 4.05	15.08 ± 2.98	0.001
Soluble thrombomodulin (µg/L)	5.59 ± 2.59	10.89 ± 0.90	0.001

Table 3. Pearson's correlation values of measured parameters among diabetics.

Parameters	Pearson's correlation (r) N = 90	p-value
HbA1c vs PAI-1	0.677	0.001
HbA1c vs STM	0.845	0.001
PAI-1 vs STM	0.642	0.001
FPG vs PAI-1	0.235	0.026
FPG vs STM	0.297	0.004
FPG vs HbA1c	0.383	0.001

PAI-1: plasminogen activator inhibitor-1. FPG: fasting plasma glucose. STM: soluble thrombomodulin.

More than half (56.67%) of the enrolled study participants had uncontrolled diabetes as at the time of the study and the measured endothelial cell markers were observed to be significantly higher in this group compared to the controlled diabetic group. Proper management of diabetes aims at achieving stable glycemic control. It is thus unfortunate to find more than half of the studied population with the challenge of poor glycemic control which has in turn increased the risk of vascular complications. Soluble thrombomodulin and plasminogen activator inhibitor-1 both had stronger positive correlations with glycated HbA1c than the fasting plasma glucose. This finding confirms the importance of effective glycemic control in relation to vascular health. A healthy endothelium is said to inhibit platelet and leukocyte adhesion to the vascular surface and maintains a balance of pro-fibrinolytic and pro-thrombotic activity (8,17). Vascular complications have been noted to be among the leading causes of morbidity and mortality in diabetes and endothelial dysfunction is thought to be at the onset of such deterioration (22,23). The mechanisms by which hyperglycemia triggers endothelial injury and dysfunction include enhanced generation of reactive oxygen species and reduced reserves of antioxidants as well as increased production of advanced glycation end products (24-27). Interestingly, our earlier study among apparently healthy adults in the study area had observed platelet activation in response to progressive loss of effective glycemic control (7). Haemostatic disturbance thus, remains an important aspect of diabetic morbidity in Nigeria.

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REFERENCES

1. Ma D, Sakai H, Wakabayashi C, et al. The prevalence and risk factor control associated with non-communicable diseases in China, Japan and Korea. *J Epidemiol* 2017; 27(12) :568-573.
2. International Diabetes Foundation. IDF diabetes atlas. 10th edition 2021. http://www.diabetes.atlas.org.
3. Sabir AA, Balarabe S, Sani AA, et al. Prevalence of diabetes mellitus and its risk factors among the suburban population of northwest Nigeria. *Sahel Med J* 2017; 20(4): 168-172.
4. Uloko AE, Musa BM., Ramalan MA. et al. Prevalence and Risk Factors for Diabetes mellitus in Nigeria: A Systematic Review and Meta-Analysis. *Diabetes Ther* 2018; 9(3): 1307–1316.
5. Amos AF, McCarty DJ, Zimmet P. The rising global burden of diabetes and its Complications; Estimates and projections to the year 2010. *Diabet Med* 1997; 14 Suppl 5: S1-S85.
6. Unwin N, Sobwugi E, Alberti G. Type 2 diabetes: the challenge of preventing a global epidemic. *Diabet Int* 2001; 11: 4-8.
7. Akwiwu EC, Edem MS, Akpotuzor JO, et al. Glycemic control and associated platelet indices among apparently healthy caregivers in Southern Nigeria. *N Z J Med Lab Sci* 2020; 74: 87-90.
8. Favero G, Paganelli C, Buffoli B, et al. Endothelium and its alterations in cardiovascular diseases: life style intervention. *Biomed Res Int* 2014; 2014: 801896.
9. Knapp M, Tu X, Wu R. Vascular endothelial dysfunction, a major mediator in diabetic cardiomyopathy. *Acta Pharmacol Sin* 2019; 40(1): 1–8.
10. Berra-Romani R, Guzmán-Silva A, Vargaz-Guadarrama A, et al. Type 2 Diabetes Alters Intracellular Ca^{2+} Handling in Native Endothelium of Excised Rat Aorta. *Int J Mol Sci* 2020; 21(1): 250.
11. Boos CJ, Lane DA, Karpha M, et al. Circulating endothelial cells, arterial stiffness and cardiovascular risk stratification in hypertension. *Chest* 2007; 132(5): 1540-1547.
12. Pirro M, Bagaglia F, Paoletti I, et al. Hypercholesterolemia -associated endothelial progenitor cell dysfunction. *Ther Adv in Cardiovasc Dis* 2008; 2(5): 329-39.
13. Stäsko J, Chudy P, Kotuhëová D, et al. Type 2 diabetes and fibrinolysis In: M Zimering (Ed). Recent Advances in the Pathogenesis and Management of Type 2 diabetes and its Complications. In Tech Rijeka: In Tech, 2011; 67-90.
14. Chudy P, Kotulićová D, Stäsko J, Kubisz P. The relationship among TAFI, t-PA, PAI-1 a.nd F1+2 in Type 2 diabetic patients with normoalbuminuria and microalbuminuria. *Blood Coagul Fibrinolysis* 2011; 22(6): 493-498.
15. Aso Y, Inukai T, Takemura Y. Mechanisms of elevation of serum and urinary concentration of soluble thrombomodulin in diabetic patients; possible application as a marker for vascular endothelial injury. *Metabolism* 1998; 47(3): 362-365.
16. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001. 414(6865); 813-820.
17. Libby P. Inflammation in atherosclerosis. *Nature* 2002; 420 (6917) 868-874.
18. Kubisz P, Chudý P, Stäsko J, et al. Circulating vascular endothelial growth factor in the normo- and / or microalbuminuric patients with type 2 diabetes mellitus. *Acta Diabetol* 2010; 47(2): 119-124.
19. Tohda D, Oida K, Okada Y, et al. Expression of thrombomodulin in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 1998; 18(12): 1861-1869.
20. Califano F, Giovaniello T, Pantone P, et al. Clinical importance of thrombomodulin serum levels. *Eur Rev Med Pharmacol Sci* 2000; 4(3): 59-66.
21. Raitakari OT, Celermajer DS. Testing for endothelial dysfunction. *Ann Med* 2000; 32(5): 293-304.
22. Carizzo A, Izzo C, Oliveti M, et al. The main determinants of diabetes mellitus vascular complications: Endothelial dysfunction and platelet hyperaggregation. *Int J Mol Sci* 2018; 19 (10): 2968.
23. Elsalakawy WA, Farweez BAT, Sallam MTH, Hamza MA. High levels of soluble thrombomodulin maybe a marker of arterial disease and peripheral ischemia in Egyptian patients with diabetes mellitus. *Egypt J Haematol*. 2014; 39(2): 52-57.
24. King GL, Loeken MR. Hyperglycemia-induced oxidative stress in diabetic complications. *Histochem Cell Biol* 2004; 122(4): 333-338.
25. Mabley JG, Soriano FG. Role of nitrosative stress and poly (ADP-ribose) polymerase activation in diabetic vascular dysfunction. *Curr Vasc Pharmacol* 2005; 3(3): 247-252.
26. Brakemeier S, Eichler I, Knorr A, et al. Modulation of Ca^{2+} activated K^+ channel in renal artery endothelium in situ by nitric oxide and reactive oxygen species. *Kidney Int* 2016; 64(1): 199-207.
27. Bretón Romero R. Weisbrod RM, Feng B, et al. Liraglutide treatment reduces endothelial endoplasmic reticulum stress and insulin resistance in patients with diabetes mellitus. *J Am Heart Assoc* 2018; 7 (18): e009379.