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Prediabetes and Endothelial Involvement among Apparently Healthy Persons

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ABSTRACT

Prediabetes is a state in between non-diabetic and diabetic conditions. While it is capable of progressing to diabetes, it also holds the potential for reversal of the disease process. Timely detection of this phase is therefore necessary for the control of diabetes. Moreso, persistent hyperglycaemia drives system changes through glycation processes. Notable among these changes are vascular-related complications. This study investigated hyperglycaemic states using glycated haemoglobin among persons previously screened as non-diabetic by fasting plasma glucose. The study also estimated some markers of endothelial involvement among the studied population. This study enrolled 134 apparently healthy middle-aged health workers whose highest fasting plasma glucose level (after 3 consecutive measurements) fell below the lower limit of 6.1 mmol/L used in the classification of prediabetes. Glucose oxidase and ion exchange resin methods were used to respectively assay fasting plasma glucose and glycated haemoglobin. Plasminogen activator inhibitor type1 and soluble thrombomodulin were measured by enzyme-linked immunosorbent assay method. The study observed proportions of 28% prediabetes and 10% diabetes among the participants. Mean values of glycated haemoglobin as well as plasminogen activator type1 and soluble thrombomodulin were significantly higher ($p = 0.001$) in subjects with prediabetes. Significant correlations were observed between glycated haemoglobin and both markers of endothelial involvement ($p = 0.006$ for plasminogen activator inhibitor and $p = 0.013$ for soluble thrombomodulin). This study observed significant vascular involvement in prediabetes.

Keywords: Diabetes mellitus, prediabetes, hyperglycaemia, endothelium

Introduction

Diabetes mellitus (DM) is among the non-communicable diseases of public health concern with risk factors that extend from genetic to socio-economic variables.[1,2] Although DM classification has evolved to recognise emerging sub-groups, the traditionally recognised Types 1 and 2 still contribute in higher proportions to the general prevalence.[3,4,5] Type 2 DM predominates in adult populations, however due to gradually lower age boarder being observed, sustained public enlightenment and timely detection have become necessary. Type 2 DM is the prevalent type of diabetes in Nigeria with Southern Nigeria reporting the highest prevalence of 9.8%.[6,7] Unfortunately, detection and proper management are hampered by inadequate healthcare coverage. The net effect of this situation is the underdiagnosis of DM and consequently inadequate management of the condition.[8,9] Determining the onset of Type 2 DM, though beneficial, remains a challenge under the afore mentioned circumstances. By the time DM is diagnosed many subjects would have been presenting with clinical manifestations of a range of complications. One way of addressing early emergence of complications is to embark on surveys involving the screening of populations particularly vulnerable for developing diabetes. Population screening for diabetes aids detection of undiagnosed cases, and has overtime included investigation of the prediabetic category.[10-13]

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Prediabetes is a state in between non-diabetic and diabetic conditions. It can be diagnosed on the basis of the finding of FGP 6.1-6.9 mmol/L as well glycated haemoglobin level of 5.7- 6.4% among other glycaemic indices. [9,12] Growing interests continue to trail the prediabetic state particularly with regards to prevention and possible reversal of the diabetic process.

Although its concept has been around for a while, the prevalence of prediabetes and its associated morbidity in resource-poor settings such as we have in Nigeria are yet to be fully investigated. More so, of the available diagnostic parameters, for hyperglycaemia, there is an overdependence on the direct measurement of immediate blood glucose level such as the fasting plasma glucose compared to the use of glycated haemoglobin in the study area. This is not unconnected with issues of affordability. Glycated haemoglobin has the advantage of estimating long-term glycaemic state and is a recommended test for the diagnosis of both diabetes and prediabetes. [9,12] This study was carried out among apparently healthy middle-aged health workers with a history for routine health check including that of glycaemic state for possible detection of diabetes. The routine monitoring for blood glucose level among the studied population was by fasting plasma glucose and all of them had levels below 6.0mmol/L as used in the cut-off for normal glycaemic state. The present study in addition to fasting plasma glucose, also measured glycated haemoglobin alongside plasminogen activator inhibitor type1 and soluble thrombomodulin as biomarkers of endothelial response. Hyperglycaemia is a trigger for endothelial involvement and therefore persistent hyperglycaemia is considered a risk factor for vascular complications as the disease state progresses. The risk of underdiagnosis and late diagnosis could be in the progressive development of complications as onset of disease occurs undetected. Thus, this study aimed at investigating possible endothelial involvement in prediabetes in addition to ascertaining its frequency of occurrence in the study area.

Materials and Methods

Ethical approval
Ethical approval was sought and duly obtained from Health Research Ethics Committee at University of Calabar Teaching Hospital (UCTH/HREC/33/655). Informed consent was obtained from each participant.

Sampling technique
This cross-sectional study was carried out among 134 apparently healthy care givers engaged at University of Calabar Teaching Hospital, Southern Nigeria. Considering the 9.8% prevalence of DM in Southern Nigeria,[6] a minimum sample size of approximately 136 was derived. A total of 134 subjects who were consistent with the study appointments and requirements were recruited as participants.
The subjects were aged 30 to 55 years and equal number of males and females were enrolled for this study. Criteria for inclusion were; granting of consent, not being on medication for at least 3 months prior to the study and having fasting plasma glucose level within the reference range of 3.5-5.5mmol/l. The fasting plasma glucose (FPG) was measured for 3 consecutive days to obtain an average. It was also ensured that for each participant, all three measurements were within the reference range. Volunteers to the study who could not meet the inclusion criteria were excluded.
In the sequence of testing, participants with fasting plasma glucose level within the reference range of 3.5-5.5 mmol/l had their glycated haemoglobin measured. Those found to be prediabetic with values between the range of 5.7-6.4% were selected alongside age and sex-matched controls from the non-diabetic group. Plasminogen activator inhibitor type1 and soluble thrombomodulin assays were carried out on the selected subjects.

Methods
Blood sample was collected and dispensed appropriately into corresponding sample bottles from each subject for the analyses of glycaemic indices and markers of endothelial involvement. Glucose oxidase method using kit from Randox UK was used to measure fasting plasma glucose, while ion exchange resin method (kit from Spectrum, Egypt) was used for glycated haemoglobin assay. Plasminogen activator inhibitor type1 and soluble thrombomodulin were measured by enzyme-linked immunosorbent assay method using kits from Bioassay Technology Laboratory, China.

Statistical analysis
Generated data were keyed into a spreadsheet (Microsoft Excel) for analysis on the SPSS software (statistical package for social sciences) version 22.0. Frequencies, student T-test and Pearson's correlation were used for analysis of data. Statistical significance was considered at ≤0.05.

Results and Discussion

The study enrolled a hundred and thirty- four apparently healthy adults aged 30 - 55 years whose highest fasting plasma glucose level after 3 consecutive measurements fell below 5.5 mmol/L each. Using glycated haemoglobin, observed proportions of 28% prediabetes and 10% diabetes were recorded in the present study (Table 1). Mean values of glycated haemoglobin as well as plasminogen activator inhibitor type1 and soluble thrombomodulin were significantly higher (p = 0.001) in prediabetes (Table 2).
Pearson's correlation of measured parameters among the subjects with prediabetic condition revealed some significant relationships including moderate positive correlations between glycated haemoglobin and plasminogen activator inhibitor type 1 (p = 0.006) as well as between glycated haemoglobin and soluble thrombomodulin (p = 0.013) (Table 3).
Reliance on fasting plasma glucose alone for the screening of diabetes is common in resource-poor settings but may not be sufficient for early detection of the disease. The use of glycated haemoglobin in the study area is predominantly considered in the monitoring of treatment among known diabetics.[14,15] Glycated haemoglobin reflects a more long-term

glycaemic state uninfluenced by the daily variability in blood glucose hence its utility in the management of diabetes. In the light of the foregoing and considering the increasing prevalence of diabetes, glycated haemoglobin could be much needed at the screening level too. This study considered the possibility of observing hyperglycaemic states using glycated haemoglobin among apparently healthy adults whose highest fasting plasma glucose level of 5.5 mmol/L (after 3 consecutive measurements) fell below the lower limit of 6.1 mmol/L employed in the classification of prediabetes.[9,12] Using glycated haemoglobin, observed proportions of 28% prediabetes and 10% diabetes were recorded in the present study. However, for the purpose of statistical inference, the study focused on the prediabetic group, and further investigated the group by comparing mean values of some markers of endothelial response (PAI-1 and STM) with values obtained from an age and gender-matched group.
There exists indication of endothelial response in prediabetes compared to age and gender-matched controls as observed in the elevated mean value of plasminogen activator inhibitor type 1. This study also observed that among the prediabetic group, age correlated positively with BMI (r = 0.367, p = 0.023) and soluble thrombomodulin (r = 0.476, p = 003). More importantly, there were significant moderate positive correlations between glycated haemoglobin and both plasminogen activator inhibitor (r = 0.438, p = 0.006) and soluble thrombomodulin (r = 0.401, p = 0.013). Endothelial dysfunction is usually preceded by its activation, thus implying that indicators of endothelial activation could be detectable in circulation ahead of classical disease manifestation. This could be of potential clinical importance in determining disease progression. Plasminogen is an indicator of inflammation and precursor to plasmin. The latter is responsible for digesting blood clot. Plasminogen activating inhibitor-1 (PAI-1), mainly secreted by the endothelium, is the primary inhibitor of plasminogen activation. Thus, down-regulation of the fibrinolytic pathway is a likely consequence of PAI-1 elevation. This marker has been associated with the development of type 2 diabetes independent of body fat and insulin resistance.[16,17] Another interesting endothelial biomarker is soluble thrombomodulin which constitutes a major part of the protein C anticoagulant system. Its association with type 2 diabetes has been in the area of widespread vascular damage and as such could serve as a of microvascular complications. [18,19]
Previously reported in the studied population is the observation of deranged platelet parameters alongside loss of normal glycaemic control.[7] Hyperglycaemia at whatever degree, whether intermediate or at a full diabetic scale, apparently holds the risk of compromised physiological function via glycation processes including those of platelets and the endothelium.[20-24] Platelet activation sequel to its glycation and also arising from endothelial dysfunction potentiates the haemostatic derangement of diabetes.[25,26] Studies of endothelial physiology and functions have increasingly led to the understanding of its mediation in both health and disease states even though it was once thought to be inert.[27-31] Vascular function as an integral aspect of haemostasis interplays in the pathophysiology of different diseases including diabetes where it significantly mediates organ involvement and general morbidity.

Table 1: Frequency distribution of prediabetes within the studied population

Diabetic status	Frequency	Percent
Participants without diabetes (HbA1c <5.7%)	83	62
Participants with prediabetes (HbA1c 5.7-6.4%)	38	28
Participants with diabetes (HbA1c >5.7%)	13	10
Total number of participants	134	100

HbA1c = Glycated haemoglobin

Table 2: Effect of prediabetes on other measured parameters

Parameter	Normal control n = 38	Prediabetes n = 38	p-Value
Age (years)	41.45 ± 5.04	41.71 ± 3.74	0.797
BMI (Kg/m^2)	27.79 ± 7.68	27.25 ± 3.54	0.698
FPG (mmol/l)	4.50 ± 0.68	4.39 ± 0.82	0.524
HbA1c (%)	4.98 ± 0.48	6.04 ± 0.17	0.001
PAI-1 (µg/l)	9.15 ± 3.58	16.36 ± 2.52	0.001
STM (µg/l)	6.40 ± 1.41	10.16 ± 1.88	0.001

BMI = Body mass index, FPG = Fasting plasma glucose, HbA1c = Glycated haemoglobin, PAI-1 = Plasminogen activator inhibitor type1, STM = Soluble thrombomodulin

Table 3: Pearson's correlation values for subjects with prediabetes

Parameters	Pearson's correlation (r) n = 38	p-value
HbA1c vs PAI-1	0.438	0.006
HbA1c vs STM	0.401	0.013
PAI-1 vs STM	0.473	0.003
Age vs BMI	0.367	0.023
Age vs STM	0.476	0.003
BMI vs STM	0.360	0.026

BMI = Body mass index, FPG = Fasting plasma glucose, HbA1c = Glycated haemoglobin, PAI-1 = Plasminogen activator inhibitor type1, STM = Soluble thrombomodulin

Cardiovascular risks alone, rank high among the many complications of diabetes and contributes to the associated mortality, while endothelial involvement reflects onset of vascular complication in type 2 diabetes.[23,24,32] The findings of this study therefore allude to the importance of the prediabetic state as one in need of urgent public health attention as a wider range of haemostatic involvement in association with intermediate hyperglycaemia has been established. Timely detection of the prediabetic state therefore holds the possibility of reversal and consequently the arrest of further complications.

Conclusion

Prediabetes was observed in this study to the tune of 28%. Significant vascular involvement is associated with the prediabetic state even when the individuals appear to be healthy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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