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Original Article

The use of glycated haemoglobin ($HbA1_C$) in determining glycemic control (and relevance of BMI) in diabetic patients in Ahmadu Bello University Teaching Hospital Zaria, Nigeria

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ABSTRACT

This study was carried out to specifically investigate the local $HbA1_C$ level and determine extent of (if any) variation from the WHO (World Health Organization) recommended threshold for the diagnosis of diabetes and prediabetes using blood glucose as a benchmark. In addition, we also looked to see what role BMI (Body Mass Index) plays among subjects used for the study.

152 subjects were used for the study: 101 diabetic subjects and 51 non-diabetic control subjects. 5 mL of blood sample was collected from each of the subjects after about 8–10 h of overnight fasting. 3–4 mL of the sample was centrifuged and the serum analysed for glucose. The remaining 1–2 ml was transferred into EDTA bottles and analysed immediately for glycated haemoglobin ($HbA1_C$). The BMI (kg/m^2) was calculated by dividing the weight in kilograms (kg) by the square of the height in metres (m^2).

For the BMI, no significant difference was observed between the diabetic subjects (mean = 25.75 kg/m^2) and the non-diabetic control subjects (mean = 25.09 kg/m^2). Thirty-seven (37) of the diabetic subjects and twenty-three (23) of the non-diabetic subjects had $HbA1_C$ levels (mean = 6.96% and 6.29% respectively) that would imply either prediabetes or diabetes but were actually normal going by their fasting blood glucose (FBG) levels. A new chart for the interconversions between FBG and HbA1c and for predicting their expected values from each other was realized, drawn up and recommended for consideration in the management of diabetic patients along with the WHO recommended chart.

There are a lot of normal individuals with HbA1c level that does not conform to (or that are simply higher than) what is regarded as the threshold for the onset of diabetes or prediabetes. Generally, the local (Nigerian) glycated haemoglobin (HbA1c) level can therefore be said to be distinctly higher for a given blood glucose range and should be taken as such in the management of diabetes in this environment. Being overweight or obese is not prerequisite to the development of diabetes or abnormal glycated haemoglobin level.

1. Introduction

Diabetes mellitus is a metabolic disease or condition characterized by hyperglycaemia (abnormally high blood glucose). The hyperglycaemia could be caused by defects in insulin secretion, defects in insulin action or usually a combination of both. Already a global menace, it is predicted to even have about 110% increase in prevalence in Africa between 2013 and 2035 (especially Type 2 diabetes), with Nigeria having the highest number of cases among African countries [1]. The diagnosis of diabetes can be challenging, as it is usually not made on a single blood test; albeit an elevated fasting blood glucose will raise suspicion that a patient may have diabetes, and this often times leads to additional workup and testing. As our understanding of diabetes evolves, the options for diagnostic criteria have been changing too. The era of tasting urine to know if it is sweet have evolved into portable devices and laboratory machines that can multiple tests within minutes to diagnose and monitor diabetes.

Glycated haemoglobin (haemoglobin A1C or HbA1C) is a form of haemoglobin (a blood pigment that carries oxygen) that is bound to

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glucose and primarily used to identify the average plasma glucose concentration over extended periods of time (at least 6–8 weeks) [2]. Effective monitoring of diabetic control (and diagnosis) requires a good marker for estimation of the average blood glucose over a longer period and glycated haemoglobin (HbA1c) fulfils this requirement for such a reliable compass to guide therapy. HbA1c is the chromatographic fraction of haemoglobin that has glucose attached to the N-terminal valine (or lysine) of the β-chain. The glycation reaction depends on how long red blood cells have been in circulation and the ambient (blood) glucose levels. As red cells have a lifespan of 3–4 months, HbA1c then logically should reflect the average blood glucose levels in the preceding three months [3]. Thus, in patients with diabetes mellitus, HbA1c is used to assess and monitor effectiveness of treatment (glycaemic control) given over a past period, usually the past two to three months. The results from HbA1c testing can then be used to determine the course of future treatment for the patient in order to guard against hyperglycaemic-induced complications [4]. HbA1c has the advantage of being accurate, simple and, now, reproducible with worldwide standardization and harmonization of the assays. One advantage HbA1c has over glucose measurement is the lack of a fasting requirement and the difficulties with OGTT (Oral Glucose Tolerant Test). The precise cut-off to diagnose diabetes remains controversial. A level of 6.5% (IFCC (International Federation of Clinical Chemistry) 48 mmol/mol) is specific for the diagnosis of diabetes in most studies, but lacks sensitivity and may miss or misdiagnose many cases. The accuracy of the test is further complicated by many factors, which modify levels of HbA1c due to biological variability, genetic factors (such as red cell life span, ethnicity and haemoglobinopathies), environmental factors (e.g., iron deficiency) and interferences (e.g., vitamin C) [3]. Clinicians caring for people with diabetes encounter individuals in whom HbA1c and blood glucose simply do not match [5]. This discordant information leaves clinicians with the question of how to care for these patients especially when it occurs in people with reliable blood glucose records and ostensibly normal peripheral blood and reticulocyte counts, without evidence of haemoglobinopathy, hemolytic disorder, blood loss/transfusion, or nutritional deficiency such as iron, folate, or vitamin B12 [5]. There is the need therefore, to investigate the local peculiarities (if any) in HbA1C levels (in Nigeria) and statistically determine extent of these peculiarities.

2. Methods

2.1. Study design

2.1.1. Study subjects

Participants consisted of 152 subjects of both genders comprising of 101 diabetic subjects and 51 non-diabetic control subjects. The diabetic volunteer-subjects were those receiving treatment in the teaching hospital, and for at least duration of one year. They were recruited over a period of six (6) months from July through December 2018. The control non diabetic volunteer-subjects were chosen from Zaria residents that meet the criteria for selection (see inclusion and exclusion criteria). They were recruited over a period of six (6) weeks from November through December 2018. The age range of both diabetic and non-diabetic subjects was similar to eliminate bias.

2.1.2. Study site

Zaria is a major city in Kaduna State in North-western Nigeria, and has a population of about 700,000 people [6]. The inhabitants of Zaria are of diverse ethnicity and livelihood. Zaria houses Nigeria's largest University, Ahmadu Bello University. Ahmadu Bello University Teaching Hospital (ABUTH) is a modern hospital and serves patients with myriad forms of ailments including diabetes mellitus.

2.1.3. Informed consent and Ethics Committee approval

The study was approved by the Ethical Committee on Human Research of Ahmadu Bello University, Zaria with the **Approval No**: ABUCUHSR/2017/002. Informed consent was also obtained from individual participant.

2.1.4. Inclusion and exclusion criteria

Diabetic patients with diabetes for at least a year were selected for the study.

The diabetes was Type II diabetes with age range of patients being 35 years and above. Also the control (non-diabetic) subjects were of a similar age range (35 years and above). Subjects with conditions that affect erythrocyte production; or with evidence of chronic medical conditions like hypertension, renal failure, liver disease and urinary tract infection were excluded from the study. Also, patients with diabetes for <1 year were excluded. For the control group, subjects with fasting blood glucose level >120 mg/dL were excluded from the study because fasting blood glucose level greater than 120 mg/dL was considered hyperglycaemic. Inclusion and exclusion was made using information about health history filled in a questionnaire and by inspecting patients' medical records.

2.1.5. Sample size [7]

$$n = \frac{Z^2 P(1-P)}{d^2}$$

Where:

n = Sample size, Z = Z statistic for a level of confidence (for the level of confidence of 95%, Z's value is 1.96), P = Expected prevalence or proportion (expressed in proportion of 1 instead of percentage), d = Precision (expressed in proportion of 1 instead of percentage).

Choosing a prevalence of 10% [8] at 95% confidence interval, the expected prevalence P = 10% (or 0.1) and Precision = 5% (or 0.05), [9].

Substituting the values in the equation:

$$n = \frac{1.96^2 0.1(0.9)}{0.05^2} = 138$$

This indicates that the sample size should at least be 138 (for both experimental and control subjects), but 152 subjects were used for the study, with the attrition rate at 10%.

2.1.6. Sample collection and analysis

Information (personal, lifestyle and medical history) about each subject was collected via questionnaire. Their blood pressure was also measured at the point of blood collection to ascertain absence of hypertension. Subjects' weights in kilogram was obtained using a weighing scale manufactured by Vins Medical England and height in meters by a stadiometer manufactured by Vins Medical England. The BMI (Body Mass Index) was calculated and expressed as weight(kg)/height(m^2). Blood samples were collected from subjects after 8–10 h of fasting. 5 mL of blood sample (each) was collected via venepuncture. About 3–4 ml of the blood was transferred from syringe into plain bottles, and centrifuged at 4000 rpm for 10 min. The serum was kept in the freezer (at −18 °C). The remaining 1–2 ml was transferred into EDTA bottles and analysed immediately for glycated haemoglobin (HbA1C).

The glycated haemoglobin (HbA1c) level in the blood samples was measured using the Finecare™ fluorescence immunoassay (FIA) meter and cartridges. 10 μL of whole blood was transferred by a micropipette into the buffer tube containing the buffer. 75 μL of the mixture (blood specimen plus buffer) was then pipetted out and loaded onto the sample well of the test cartridge. The test cartridge was then inserted into the test cartridge holder in the meter and "Test" was clicked by a touch pen on the display touch screen present on the meter. After 5 min the result of the HbA1c test was shown on the display screen and also printed out on a pre-installed paper roll after "Print" was clicked.

Automated Erba® Mannheim XL-200 Full-Auto Chemistry analyser by Erba Diagnostics Mannheim, Germany was used in the analysis of the serum samples which were prior brought out of the freezer and defrosted for about 30 min. The samples were then poured into the sample cups – 30 cups at a time – that fit into the sample tray of the analyser. After analysing each batch, the (results) level of glucose present in each sample was then displayed on the computer screen and carefully copied out. The Analyser employs the colorimetric analysis method where the amount of resultant colour change produced by mixing the serum sample (containing the measured analyte) with the measured analyte's specific reagent is proportional to the amount of the analyte present in a quantified amount of the serum sample.

2.2. Statistical analyses

Results were presented as mean ± SD and data was analysed using independent student's T-test and one-way analysis of variance (ANOVA) where it is normally distributed, while relationships between variables was determined using the Pearson's correlation test. A linear regression analysis was also conducted to generate a regression equation. A P-value of ≤0.05 was considered statistically significant. All analysis was done using SPSS version 24.

3. Results

The mean values of the measured parameters of diabetic patients were compared with those of the healthy (non-diabetic) control subjects (Table 1). The diabetic patients had significantly ($P < 0.001$) higher levels of HbA1c and FBG. However, no significant BMI difference observed between the two categories.

A Pearson's correlation analysis was also conducted to test the interrelationship between all parameters and each variable (parameter) with itself. Parameters for the diabetic subjects and the parameters for the non-diabetic control were analysed separately (Tables 2 and 3 respectively). With itself, the variables expectedly gave a perfect correlation of 1. For the diabetic subjects, FBG and HbA1c showed a strong positive correlation with an R value of 0.84. The relationship was also significant at a P level of 0.01. For the control, the HbA1c and FBG did not show a significant correlation with each other (Table *4.3*)

Fig. 1 below is a graph showing the relationship between HbA1c and FBG for the diabetic subjects.

Table 1
Comparing mean values of HbA1c, FBG and BMI of diabetic subjects with those of non-diabetic control subjects.

	Diabetic Mean ± SD (n = 101)	Control Mean ± SD (n = 51)	P-value	t-value
HbA1c (%)	9.31* ± 3.24	5.67* ± 0.71	0.001	10.78
FBG (mg/dL)	160.62* ± 87.61	76.31* ± 8.89	0.001	9.57
BMI (kg/m2)	25.75 ± 5.21	25.09 ± 4.93	0.449	0.76

The asterisk (*) indicates significant difference at the level of P ≤ 0.001.

Table 2
Exploring the relationship (correlation) between HbA1c and FBG for the diabetic subjects.

	HbA1c (%)	FBG (mg/dL)
HbA1c (%)	1	0.84*
FBG (mg/dL)	0.84*	1

Asterisked (*) R-values indicates significant correlation at the level of P = 0.01.

Table 3
Exploring the relationship (correlation) between the parameters (HbA1c and FBG) for the non-diabetic control subjects.

	HbA1c (%)	FBG (mg/dL)
HbA1c (%)	1	0.10
FBG (mg/dL)	0.10	1

After establishing a strong correlation between HbA1c and FBG among the diabetic patients a linear regression analysis was conducted and a regression equation (y = mx + c) generated: $\boldsymbol{y = 0.031x + 4.29}$. Where y is the dependent variable HbA1c, and x is the independent variable FBG. "m" is the slope of the graph and it equals 0.031 while c is a constant and it equals 4.29. This was then used to generate a chart of varied hypothetical FBG values in order to see their respective equivalent HbA1c values (Fig. 2) (see Fig. 3).

Using the 5.7% and above as a benchmark for pre-diabetes and diabetes, subjects were classified according to their equivalent blood glucose (Tables 4 and 5). Those with fasting blood glucose at the expected pre-diabetes and diabetes level (120 mg/dL and above) were classified as "Conforming HbA1c" while those with normal blood glucose range (110 mg/dL and below) were classified as "Deviating HbA1c". The number of subjects in each category was counted and expressed as percentage of the total number of subjects. Also, the mean levels of HbA1c and fasting blood glucose in each category were compared with each other; Table 4 for the diabetic subjects and Table 5 for the non-diabetic control subjects. For both groups of subjects (diabetic and non-diabetic control), the difference between the mean HbA1c of the "Conforming" group and the mean HbA1c of the "Deviating" group is seen to be significant (P = 0.0001). Also, the difference between the mean fasting blood glucose of the "Conforming" group and the mean fasting blood glucose of the "Deviating" group is seen to be significant (P = 0.0001)

4. Discussion

The results from HbA1c testing is used to monitor blood glucose level thereby determining the course of future treatment for the patient in order to guard against hyperglycemic-induced complications [4]. However, the accuracy of these results is being reported to be doubtful and highly varied amongst individuals especially along racial lines; with reports of significantly higher levels in non-Caucasian diabetic patients for a given blood glucose range [10,11]. The rate of glycation of haemoglobin – the level of HbA1c – is generally proportional to the level of blood glucose and this study alludes to that. Though still proportional to the level of fasting blood glucose, the results obtained from this study indicate that the HbA1c range that should be generally considered normal in the locality of the patients/subjects used is significantly higher than what was presupposed. It is also varied from individual to individual. In the end, we are able to identify a trend in the HbA1c level of diabetic patients that attend Ahmadu Bello University Teaching Hospital Zaria and indeed Zaria's (the Nigerian city where the research was carried out) very diverse residents in general even

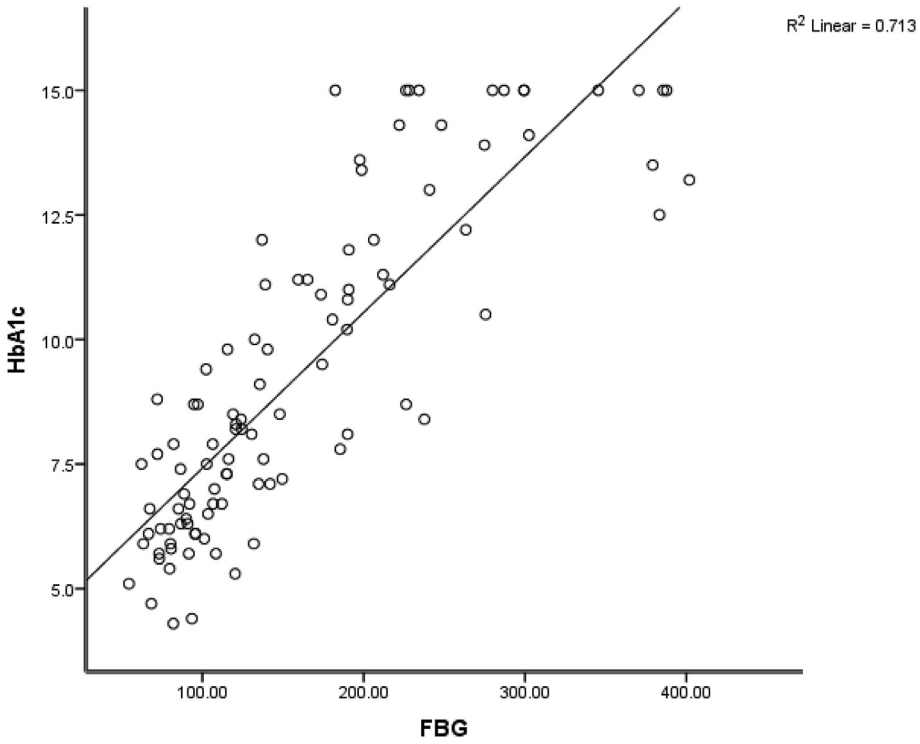

Fig. 1. Relationship (with line of best fit) between HbA1c and FBG in the diabetic subjects.

This Study	
FBG (mg/dL)	HbA1c (%)
68	6.40
97	7.30
126	8.20
152	9.00
183	9.96
212	10.86
240	11.73
269	12.63
298	13.53
326	14.40
355	15.30

WHO (World Health Organization)	
FBG (mg/dL)	HbA1c (%)
68	4
97	5
126	6
152	7
183	8
212	9
240	10
269	11
298	12
326	13
355	14

Fig. 2. Glucose values and equivalent glycated haemoglobin levels based on the regression equation: $y = 0.031x + 4.29$ gotten from this study (your left). The supposed standard equivalents from WHO (your right).

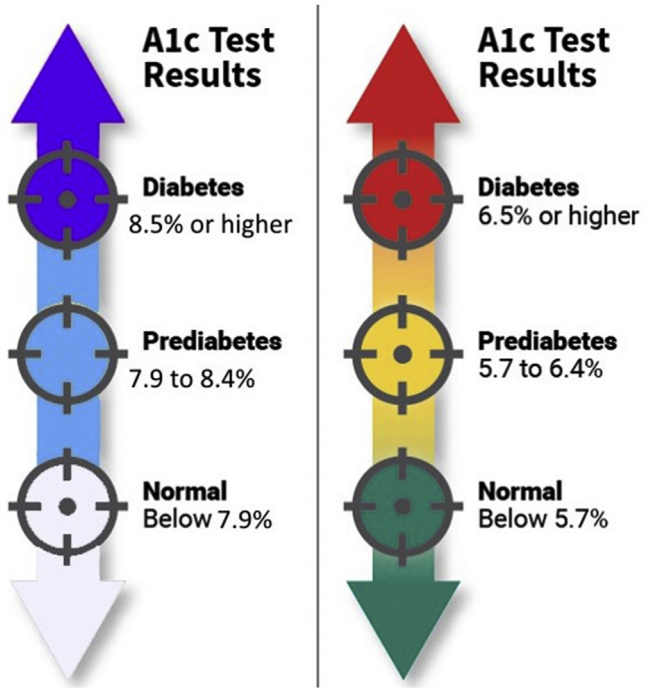

Fig. 3. Comparing the glycaemic chart recommended by WebMD (supported by WHO) with the chart gotten from this study.

though only instant samples were collected and not continual monitoring. It is still alright though, since the within individual day-to-day variability of HbA1c level is even said to be less than 0.02 [12]. A review by Oghagbon (2014) [1] concluded that caution should be taken in the application of HbA1c in our environment – despite its usefulness in monitoring diabetic patients in Nigeria –

Table 4
Proportion of diabetic subjects with deviating HbA1c levels (5.7% and above) not conforming to their corresponding FBG levels.

	N (No. of individuals)	% of total N	Mean HbA1c (%) ± SD	Mean FBG (mg/dL) ± SD
Conforming HbA1c	64	63.37	10.67 ± 3.31	200.65 ± 87.15
Deviating HbA1c	37	36.63	6.96* ± 1.08	91.37* ± 15.89
P-value			0.0001	0.0001

Table 5
Proportion of non-diabetic control subjects with deviating HbA1c levels (5.7% and above) not conforming to their corresponding (normal ranged) FBG levels.

	N (of individuals)	% of total N	Mean HbA1c (%) ± SD	Mean FBG (mg/dL) ± SD
Conforming HbA1c	28	54.90	5.16 ± 0.35	73.97 ± 8.94
Deviating HbA1c	23	45.10	6.29* ± 0.51	79.16* ± 8.13
P-value			0.0001	0.0001

due to its variability with race. This is evident from the results obtained from this study. About 37% of diabetic subjects used and 45% of non-diabetic control subjects had HbA1c level that does not match their glucose level – they had higher HbA1c (at the supposed diabetic or prediabetic level) for their (normal) blood glucose range. Earlier, a multi-center study involving seven teaching hospitals in Nigeria revealed that 68% of diabetic patients had their mean HbA1c level greater than 7% but only about half of these showed sign of complications development [13]. In contrast to a study by Sakpa and Idemudia, (2014) [14] carried out in University of Benin Teaching Hospital, Benin City, Edo state, Nigeria, the HbA1c and fasting blood glucose of the non-diabetic control subjects in this study though showed some correlation with each other, the correlation is weak and insignificant. This is simply due to haphazardness of values of HbA1c within the narrow range of normal FBG levels among the individuals. There appears to be a loss of proportionality of HbA1c to blood glucose level due to the narrow range of normality for FBG within which HbA1c levels hover haphazardly.

These findings that point to a local peculiarity in what should be regarded as normal HbA1c is buttressed by earlier studies that indicate a high hereditability of native HbA1c estimated to be approximately 50% [16,17]. And the contributory genetic factors are similar to those indicated by Florez, 2010 [15] which are involved in physiologic factors such as glycaemic control (e.g., b-cell function, insulin sensitivity and incretin physiology), and non-glycaemic factors (e.g., RBC glucose transport, deglycation pathways and altered RBC turnover) [16,17]. Others include iron handling, glucose distribution across, and rates of uptake into the erythrocyte membrane, rates of glucose attachment to or release from haemoglobin and rates of intra-erythrocytic glucose metabolism [15]. This implies that the genetic factors involved in the manifestation of these physiologic factors having been inherited by generations will then determine the HbA1c normal-level (uniqueness) of such locality.

This study further iterates that caution in translating and using HbA1c results is vital. From the results obtained, the study recommends that confirmatory investigations (mainly blood glucose tests) should be carried out on individuals or patients with high (apparently diabetic or prediabetic) HbA1c level especially when the HbA1c level is below 7.9%. This is because values below that level might just be normal in this locality and indeed Nigeria as Zaria city domiciliates individuals from all over the country. BMI (Body Mass Index) which is used to determine whether an individual is of normal weight, overweight or obese and positively correlated with diabetes is observed to be normal and similar among the diabetic and non-diabetic subjects used for this study. This definitely implies that being overweight though a risk factor is not prerequisite for the development of diabetes. It is not likely that the normality seen in the diabetic subjects' BMI is due to weight loss due to diabetes because of the relatively short duration of their condition (average number of years since diagnosis = 5 years) and the treatment they are getting. The study also went ahead to recommend a local HbA1c chart that could be used in the diagnosis and management of diabetes. This could be considered together with the HbA1c chart recommended by World Health Organization which is also useful but could result in misdiagnosis or over-treatment of patients if used alone. As stated earlier, instant samples were collected from subjects and analysed. There could be need for a repeat of the study using samples collected at intervals from study subjects over a period, and results compared with those obtained from this study. Also the underlying physiologic factors that is causing such a relatively wide range in normal HbA1c level will be investigated in the nearest future.

5. Conclusion

The possibility of a patient having a glycated haemoglobin (HbA1c) level that indicates diabetes without the patient having the condition should be taken into consideration while caring for diabetic patients. Similar caution should be taken while testing for the presence or absence of diabetes. Generally, the "bar of normality" in HbA1c testing should be raised a little higher in this environment. Further study is needed to investigate the physiological mechanisms responsible for the normally higher glycated haemoglobin (HbA1c) level observed among some individuals.

Other information

Data are available on request.

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Conflicts of interest

No conflict of interest declared.

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