

THE VALUE OF HbA1c THAT GIVES A GOOD YIELD FOR SCREENING

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ABSTRACT

HbA1c is being used as a screening tool for diabetes with its performance varying in different studies depending on the cut-off point of HbA1c used and the reference gold standard. To determine the level of HbA1c that defines the best cut-off point compatible with maximal yield using 2hours post 75gram glucose as reference. A cross sectional study of 207 patients with systemic hypertension attending the renal and cardiology out patient clinics of the Lagos University Teaching Hospital were recruited. One hundred and thirty one (131) of the subjects had OGTT done. A random sampling method was used to select one out of every four who had HbA1c done using DCA 2000® autoanalyzer. Receiver Operative Characteristic Curve was plotted to determine the appropriate HbA1c cut-off point that gave optimal sensitivity and specificity for the diagnosis of diabetes using plasma venous glucose, 2 hours post 75 gram OGTT. A 2x2 tables was utilized to determine the predictive value, and efficiency of the HbA1c cut off point for the diagnosis of diabetes. HbA1c cut-off point of 6.7% was found to give sensitivity of 57.14%, specificity of 75%, positive predictive value of 36.36%, negative predictive value of 85%, and efficiency of 67.74%. the new cut-off point of HbA1c has improved the performance of the method as a screening tool.

Keywords: Diabetes, Screening, Glycated haemoglobin (HbA1c), Oral Glucose Tolerance Test and Receiver Operation Characteristic Curve

INTRODUCTION

The high prevalence of undiagnosed diabetes [1–3] and the large proportion of individuals with complications at diagnosis [4,5] argue for screening for Type 2 diabetes. The World Health Organization (WHO) recommended that the 75-g oral glucose tolerance test (OGTT) should be retained as a diagnostic test [6]. This is based on the fact that, many individuals with diabetes at an early stage have a FPG below the diagnostic criterion, in spite of postprandial hyperglycaemia [7–10]. Therefore, an OGTT may be a more suitable method than an FPG for the early detection of diabetes, especially in high-risk individuals. Glycated haemoglobin (HbA1c), which assesses chronic glycaemia is utilized for assessing glycaemic control in subjects with diabetes. However, HbA1c is not currently recommended for the screening or diagnosis of diabetes [6, 11]. Although some studies have gone ahead to use it as a screening tool with different results base on different gold standard as a reference yardstick and cut-off points (12-14). The purpose of this study is to determine the appropriate cut-off point for HbA1c that is compatible with maximal yield for the diagnosis of diabetes using the 2 hour post 75gramm OGTT venous plasma glucose among people at a risk factor for Type diabetes Mellitus.

METHODOLOGY

Study Design: Cross sectional studies

Study Location: This study was carried out at the Department of Medicine of the Lagos University Teaching Hospital (LUTH) and over the three months period, spanning from January to March 2004. The Lagos University Teaching Hospital is a tertiary public health care centre. **Subjects:** The subject population were people with known history of systemic hypertension on life-style modification and/ or drug (s) for the control of the hypertension and, attending the Cardiology or Renal clinics. Patients with secondary forms of hypertension, chronic renal failure and chronic liver disease were excluded from the study.

SAMPLE SIZE

We recruited 207 persons to give room for attrition. Random sampling method was carried out, to select one out of every four who underwent an OGTT test for HbA1c analysis. Approval was obtained from the Ethical Committee of the Lagos University Teaching Hospital. An informed consent was obtained from the patients/ subjects before commencing the studies. The patients on usual medical follow up were approached and given a brief health talk on the importance of screening for diabetes among people with systemic

The Value of HbA_{1c} that Gives a Good Yield for Screening

hypertension. A questionnaire containing biodata and anthropometry of the subjects was completed. The information captured included: age, gender, height (m) with measurement taken to the closest centimeter, weight (kg) to the closest mg, and BMI calculated as the ratio of weight to the square of height in metres. Waist circumference was taken at umbilical level to the closest centimeter. Hip circumference was measured at the maximum circumference of the buttocks to closest centimeter. Waist to hip ratio was also calculated by finding the ratio of the waist circumference to that of hip circumference. The 2hours venous plasma glucose post standard 75gram OGTT and HbA_{1c} were documented. Performance of OGTT At 7.30am, all had their fasting venous blood sample taken and were given 75gm of dissolved anhydrous glucose in chilled water to drink at once and another sample was taken at 9.30am.

PRESERVATION OF BLOOD SPECIMEN

Immediately, after the venous blood was drawn, all were centrifuged, and aliquots were prepared within 30min of collection and those for HbA_{1c} were run immediately. Plasma aliquots were frozen at -80°C until conduction of the analyses in the same laboratory. Plasma glucose was analyzed according to the method of Trinder (17) using glucose oxidase enzyme buffered in phenoxylate and dissolved in colour reagent. The coefficient of variation for intra-assay was 3.5% and inter-assay was 9%. Diabetes was diagnosed based on a 2hours post OGTT venous plasma glucose of $\geq 11.1 \text{ mmol}^{-1}$.

HbA_{1c} SAMPLING AND ASSAY METHODOLOGY

On arrival of the subjects at 7.30am, sample for HbA_{1c} was taken and kept in a specimen bottle for analysis the same day using DCA 2000® autoanalyser machine and reagent kit produced by Bayer Corporation Inc. USA. The assay is based on a latex immunoagglutination inhibition method (DCA 2000®+ Manual). This provides a convenient, quantitative method for measuring the percentage concentration of haemoglobin A_{1c} in blood; both the concentration of haemoglobin A_{1c} specifically and the concentration of total haemoglobin are measured, and the ratio reported as percent haemoglobin A_{1c}. The chemical principle for the measurement of total haemoglobin: potassium ferricyanide is used to oxidize haemoglobin in the sample to thiocyanate to form thiocyan-methemoglobin and the coloured species is measured. The extent of colour development at 531nm wavelength is proportional to the concentration of total haemoglobin in the sample. For the measurement of specific HbA_{1c}, an inhibition of latex agglutination assay is used. An agglutinator (synthetic polymer containing multiple copies of the immunoreactive portion of HbA_{1c}) causes agglutination of latex coated with HbA_{1c} specific mouse monoclonal antibody. This agglutination reaction causes increased scattering of light which is measured as an increased absorbance at 531nm. The HbA_{1c} in whole blood specimens competes for the limited number of antibody-latex binding sites causing an inhibition of agglutination and a decreased scattering of light. The decreased scattering is measured as a decrease in absorbance at 531 nm. The HbA_{1c} concentration is then qualified using a calibration curve of absorbance versus HbA_{1c} concentration. HbA_{1c} of $< 6.1\%$ was considered to have negative screening test and $\geq 6.1\%$ was considered to be positive.

The percentage HbA_{1c} in the sample is then calculated as follows;

$$\% \text{ HbA}_{1c} = \frac{\{\text{HbA}_{1c}\}}{\{\text{Total Hemoglobin}\}} \times 100$$

The coefficient of variation of assay method was 0%, depicting the exceptionally high precision of the machine and method.

DATA PROCESSING AND STATISTICS ANALYSIS

Data were entered into a Microsoft Excel database. We used SPSS version 11 and STATA 8, College Station, Texas 77845, USA, licensed to WHO was used to analyze the data. Continuous variables were expressed as means and standard deviations (SD) and categorical variables as frequencies and proportions. Differences between groups were assessed with the independent t- test for continuous variables. A table (table 1) characterizing those who had OGTT into those with no-diabetes (2hrsPost 75gram glucose $< 11.1 \text{ mmol/l}$) and those with positive screening test for diabetes (2hrsPost 75g glucose $\geq 11.1 \text{ mmol/l}$) was made. Receiver Operative Characteristic Curve (ROC) was used to plot the graph of sensitivity against 1-specificity to determine the best cut-off point of HbA_{1c} that gave maximal yield (Table 2). A two-by-two table (Table 3) was used to compute sensitivity, specificity, predictive values, efficiency of HbA_{1c} as a screening tool and prevalence of Type 2 diabetes among people with systemic hypertension (15). Results – The mean age of those that had OGTT was 53.11 ± 8.69 years. The age differential by gender shows the age of females to be 52.70 ± 8.37 and males to be 53.93 ± 9.34 (p value= 0.06). The mean 2hours post glucose load value was 169.48 ± 93.97 . The mean HbA_{1c} value was $6.62 \pm 1.93\%$. The mean duration of hypertension in

years was 10.04. The mean height, waist and hip measurements were 1.63, 100.79 and 110.27 respectively. The mean weight in kg was 83.42, mean body mass index (BMI) of 31.55 and waist to hip ratio of 0.91. Table 1 shows a comparison of the age, anthropometric and clinical characteristics of those subjects who had 2hour post glucose assay of $<11.1\text{mmol/l}$ and $\geq 11.1\text{mmol/l}$.

Table 1: Features of those who had Oral Glucose Tolerance Test.

	OGTT $<200\text{mg/dl}$	OGTT $\geq 200\text{mg/dl}$	P value
Age	50.78 $\pm$ 8.95	57.14 $\pm$ 7.54	<0.05
Duration of hypertension (years)	9.08 $\pm$ 8.02	13.63 $\pm$ 8.51	<0.05
Height (meters)	1.59 $\pm$ 0.08	1.66 $\pm$ 0.08	<0.05
Weight (kilograms)	78.83 $\pm$ 19.00	88.02 $\pm$ 10.82	<0.05
Body Mass Index(kg/m^2)	29.54 $\pm$ 7.00	33.57 $\pm$ 4.77	<0.05
Waist circumference (cm)	97.18 $\pm$ 12.20	104.40 $\pm$ 8.54	<0.05
Hip circumference (m)	107.09 $\pm$ 12.18	113.45 $\pm$ 7.52	<0.05
Glycated haemoglobin (HbA1c %)	4.97 $\pm$ 1.85	9.17 $\pm$ 2.27	<0.05
Waist/Hip circumference	0.92 $\pm$ 0.08	0.95 $\pm$ 0.07	<0.05

Table 2 shows analysis of various cut -off points of HbA1c for the diagnosis of diabetes based on a 2 hour post OGTT plasma glucose of $\geq 11.1\text{mmol/l}$. It shows that at cut off point of 6.7% gives the best result in terms of sensitivity and specificity, as also depicted in Receiver Operative Characteristic Curve (ROC). As we must strike a balance to get the best sensitivity without compromising specificity, HbA1c of 6.7% gives the balancing point.

Table 2: Performance of the Screening Test Using Different Cut-Off Points
Detailed report of Sensitivity and Specificity

Cut point	Sensitivity	Correctly Specificity	Classified	LR+	LR-
(≥ 4.6)	100.00%	0.00%	22.58%	1.0000	
(≥ 5)	85.71%	0.00%	19.35%	0.8571	
(≥ 5.2)	85.71%	4.17%	22.58%	0.8944	3.4286
(≥ 5.4)	71.43%	4.17%	19.35%	0.7453	6.8571
(≥ 5.5)	71.43%	8.33%	22.58%	0.7792	3.4286
(≥ 5.6)	71.43%	16.67%	29.03%	0.8571	1.7143
(≥ 5.7)	71.43%	29.17%	38.71%	1.0084	0.9796
(≥ 5.8)	71.43%	33.33%	41.94%	1.0714	0.8571
(≥ 5.9)	57.14%	45.83%	48.39%	1.0549	0.9351
(≥ 6)	57.14%	58.33%	58.06%	1.3714	0.7347
(≥ 6.2)	57.14%	62.50%	61.29%	1.5238	0.6857
(≥ 6.3)	57.14%	66.67%	64.52%	1.7143	0.6429
(≥ 6.6)	57.14%	70.83%	67.74%	1.9592	0.6050
(≥ 6.7)	57.14%	75.00%	70.97%	2.2857	0.5714
(≥ 6.8)	42.86%	75.00%	67.74%	1.7143	0.7619
(≥ 7)	28.57%	83.33%	70.97%	1.7143	0.8571
(≥ 7.1)	28.57%	87.50%	74.19%	2.2857	0.8163
(≥ 9.7)	28.57%	91.67%	77.42%	3.4286	0.7792
(≥ 10.2)	28.57%	95.83%	80.65%	6.8571	0.7453
(≥ 14)	0.00%	95.83%	74.19%	0.0000	1.0435
(> 14)	0.00%	100.00%	77.42%		1.0000

An assessment of the performance of HbA1c using a new cut-off point of 6.7% as shown in table 2, reveals a positive predictive value of 36.36%, negative predictive value of 85%, efficiency of 67.74 and a prevalence of 35.48%. The correlation and Receiver Operative Characteristic Curve (ROC) of the screening are as

The Value of HbA1c that Gives a Good Yield for Screening

reported in the previous study, showing usefulness of HbA1c as a screening tool (11).

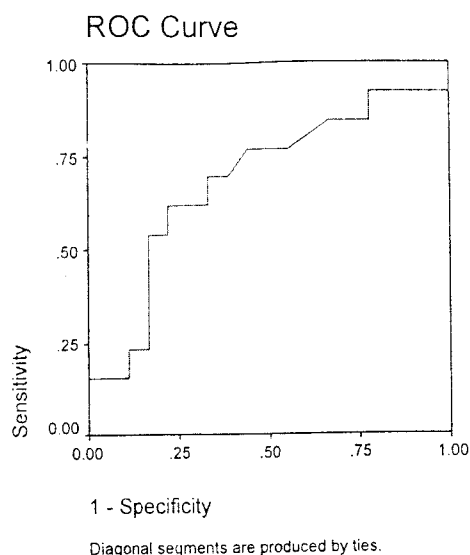


Fig 1 showing ROC derived from appendix 1.

Table 2- Showing 2x2 features at a Cut-Off Point of HbA1c of 6.7%

OGTT 2HrPP x HbA1c

Count		GHbA1c%		Total
		<6.7	=>6.7	
OGTT2hrpp(mg/dl)	<200	18	6	24
	=>200	3	4	7
	0			
Total		21	10	31

DISCUSSION

It has been known for several years that in any diabetic population there is proportion of people who appear to glycate haemoglobin at a faster or slower rate than most others (16). Even within a non-diabetic reference range of 4-6%, subjects tend not to vary between 4% and 6%, but instead stay very close to their own "set points" (17,18). By inference, this means that two subjects with the same degree of glucose tolerance might well have HbA1c values that vary by up to 2%. The reason for these differences between high and low glycaters was originally thought to be the result of interindividual differences in tissue glycation, but recent data suggest that much of these differences can be explained by the fact that high glycaters seem to have red blood cells that survive longer than low glycaters (19). Going by the fact that the tropical environment of the Sahara desert is laden with a population of people with haemoglobinopathy, red blood cells with a short survival rate should give rise to lower HbA1c values for screening for diabetes.

CONCLUSION

This study has shown that the established cut-off point of HbA1c of 6.7%, has improved the performance remarkably as seen in the result.

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