

# Comparative performance of HbA<sub>1c</sub> 6.5% for FPG ≥7.0 vs 2hr PG≥11.1 criteria for diagnosis of Type 2 diabetes

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## Abstract

**Background:** International expert committee on the use of HbA<sub>1c</sub> to diagnose diabetes mellitus in 2009 and World Health Organization (WHO) in 2011 has advocated the use of HbA<sub>1c</sub> to diagnose diabetes mellitus.

**Objective:** To determine and compare the relationship between the new cut off value of HbA<sub>1c</sub> with established criteria.

**Methods:** Thirty-one hypertensive subjects attending Lagos University Teaching Hospital were recruited for HbA<sub>1c</sub> and standard oral glucose tolerance test. Fasting plasma glucose (FPG) and two-hour plasma glucose (2hrpp) value of  $<126\text{mg/dl}$  and  $\geq 200\text{mg/dl}$  were used as standard respectively for diagnosis of diabetes. The HbA<sub>1c</sub> of  $\leq 6.5\%$  was used to diagnose diabetes. The performance and correlation of HbA<sub>1c</sub> with FPG and 2hrpp were calculated and results were compared.

**Results:** Mean age of the subjects was  $53.97 \pm 6.27$  years. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), efficiency and correlation of FPG is 50%, 68%, 27%, 85%, 64% and 0.5 respectively while the sensitivity, specificity, PPV, NPV, efficiency and correlation of 2hrpp is 73.91%, 62.5%, 85%, 41.66%, 70.97% and 0.73% respectively. There was a significant difference between FPG and 2hrpp interms of sensitivity, PPV and NPV.

**Conclusion:** The results of HbA<sub>1c</sub> with 2hrpp has better correlation, sensitivity, and PPV compared to HbA<sub>1c</sub> with FPG.

**Key words:** HbA<sub>1c</sub>, Diabetes diagnosis, Fasting Plasma Glucose, 2-hour post-prandial glucose

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## Introduction

The prevalence of diabetes mellitus (DM) has more than doubled in the last two decades in Nigeria<sup>1,2</sup>. A national survey reported an average prevalence of 2.2% in the late 1990s<sup>1</sup> and it is currently put at 4-5% among 20-79 years old age group<sup>2</sup>. The global projection of DM from now is put at 54% by the year 2030 and poor resource nations, like Africa, it will increase by 98%<sup>2</sup>. Similarly, the projection among those that are 20-79 years old by year 2030 it will be 7-9%<sup>2</sup>. The health implication and financial burden of DM and its consequent complications is estimated to increase dramatically and could consume as much as 40% of some countries budget<sup>3</sup>.

Traditionally, measurement of HbA<sub>1c</sub> levels has been largely restricted to monitoring diabetic patients. However, HbA<sub>1c</sub> was endorsed for diagnosis of DM by the International Expert Committee Report on the role of HbA<sub>1c</sub> assay in the diagnosis (IECRHbA<sub>1c</sub>) of DM and was also

recommended by World Health Organization (WHO)<sup>4,5</sup>. A diagnostic cut-off point of 6.5% was recommended, based on the risk for developing microvascular complications such as retinopathy. The advantages of HbA<sub>1c</sub> assay is that it is quicker to perform, reflect longer term glycaemia (up to three months) and is less affected by current stress levels (physical or emotional). Therefore, it is more likely to give an accurate result. Glucose tests do not have these advantages; however, HbA<sub>1c</sub> is not accurate in individuals with certain conditions like haemoglobinopathies.

Oral glucose tolerance test (OGTT) is considered by the WHO as "gold standard"<sup>6</sup> and recognized by American Diabetes Association (ADA) for the diagnosis of DM. It is more sensitive and specific; however it is less reproducible, more time consuming, expensive and more inconvenient. Because of the problems with OGTT, ADA has adopted use of fasting plasma glucose (FPG) for the diagnosis of DM which is also acknowledged by WHO because it is faster, easier to perform, more convenient and acceptable to patients and less expensive compared to oral glucose tolerance test<sup>7</sup>.

High normal blood pressure and hypertension are associated with the development of type 2 DM<sup>8</sup>. These associations persisted even

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after adjustments for several known or suspected predictors of type 2 DM, including age, body mass index (BMI), smoking habit, leisure-time physical activity, parental history of type 2 DM and daily alcohol consumption<sup>89</sup>. None of the available studies has used the new cut off value of HbA1c advocated by IECRHbA1c/ WHO to compare the diagnostic yield between FPG and 2hour plasma glucose of OGTT (2hrpp) criteria among hypertensive Nigerians. The aim of this study is thus, to compare the performance of IECRHbA1c with FPG and 2hrpp criteria. This comparison shall be useful in giving credence to the test that gives a better performance and help in choice selection of diagnostic standard in subsequent studies.

## Methods

### Study design and location

It is a cross sectional study. It was carried out at the Department of Medicine of the Lagos University Teaching Hospital (LUTH) and the Endocrine Unit Laboratory of the Department of Medicine of the College of Medicine, University of Lagos over a period of three months, from January to March 2004.

### Subjects

The subjects recruited for screening for type 2 diabetes were those with a known history of systemic hypertension on life-style modification and/ or drug(s) for the control of the hypertension. They attended the Cardiology and Renal units' medical out patients' department of the hospital. Those patients with established secondary form of hypertensions, chronic renal failure and chronic liver disease were excluded from the study. They are not previously known to have diabetes.

### Sample Size

Thirty-three subjects were recruited although two HbA1c assay results revealed error. The remaining thirty one results were finally used for the data 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 hypertension. A questionnaire containing biodata and anthropometry of the

subjects were taken. The information taken included: name, age, gender, hospital number, height (m) with measurement taken to the closest centimeter, weight (kg) to the closest mg, and BMI calculated as the ratio of weight in kg to the square of height in m. Waist circumference was taken at umbilical level, to the closest centimeter, hip circumference was measured at the widest dimension of the buttocks, to closest centimeters. Waist to hip ratio was calculated by finding the ratio of the waist to that of hip<sup>10</sup>.

### Procedures for OGTT and HbA1c Assay

At 7.30am, after an eight hour over-night fast, all had their venous blood sample taken for HbA1c and FPG. Subsequently, they were given 75gm of dissolved anhydrous glucose in chilled water to drink at once. Another blood sample was taken at 9.30am, 2hrpp. The samples for FPG and 2hrpp were centrifuged, and aliquots were prepared within 30 minutes of collection. Plasma aliquots for glucose determinations were frozen at -80°C until conduction of the analyses in the same laboratory. Plasma glucose was analyzed according to the method of Trinder<sup>11</sup> 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% for FPG and 2hrpp. FPG of e"126mg/dl and 2hrpp of e"200mg/dl is considered to have DM as "gold standard".

### HbA1c determination

HbA1c assay were run immediately using DCA 2000® autoanalyser machine and reagent kit produced by Bayer Corporation Inc, USA. This assay is based on a latex immunoagglutination inhibition method (DCA 2000®+ Manual). This provides a convenient, quantitative method for measuring the percentage concentration of HbA1c in blood; both the concentration of HbA1c specifically and the concentration of total haemoglobin are measured, and the ratio reported as percent HbA1c.

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 which is measured. The extent of colour development at 531nm is proportional to the concentration of total haemoglobin in the sample.

The procedure for the measurement of specific HbA1c is as outlined by the leaflet that comes with the kit (DCA 2000®+ Manual). Computation

of percent HbA1c is shown in equation 1 below. The HbA1c of <6.5% was considered not to have DM and  $\geq 6.5\%$  was considered to have DM.

The percentage HbA1c in the sample is then calculated as follows;

$$\% \text{HbA1c} = \frac{\{\text{HbA1c}\}}{\{\text{Total Hemoglobin}\}} \times 100 \quad \text{Equation 1}$$

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

### Statistics

The Analysis of data was done with SPSS version 11. The Means $\pm$ SDs or mean (confidence interval) were assessed for continuous variables, and frequencies and proportions were assessed for categorical variables. Differences among groups with FPG and 2hrpp parameters were assessed using *t*-test and  $p \leq 0.05$  is considered significant. Sensitivity, specificity, predictive values and efficiency were assessed from 2x2 table made between HbA1c and the two diagnostic tests. A plot of correlation was made using a linear regression model where *r*, *r*-square and *y* were estimated.

### Definitions

Sensitivity is the proportion of those with FPG  $\geq 126\text{mg/dl}$  and 2hrpp  $\geq 200\text{mg/dl}$  that were detected as positive by HbA1c. Specificity is the proportion of those with normal FPG  $<126\text{mg/dl}$  and 2hrpp  $<200\text{mg/dl}$  and were detected to have HbA1c  $<6.5\%$ . Positive predictive result is the proportion of HbA1c positive results in a mixed population of sick and healthy people using FPG and 2hrpp criteria. Negative predictive value is the proportion of HbA1c negative results in a mixed population of sick and healthy people using FPG and 2hrpp criteria. Efficiency is the percentage of the sum of the true positives and the true negatives of the grand total population.

### Result

The mean age of the 31 participants in the study was  $53.97 \pm 6.27$  years. Twenty four (77.42%) were females. The mean $\pm$ SD demography of the subjects and the differentiating demography of those who had positive and negative tests by HbA1c and FPG and 2hrpp is shown in table 1 and 2. Twenty five (80.64%) had a negative FPG test. Twenty (64.51%) had HbA1c  $<6.5\%$ . Twenty three (74.2%) had negative 2hrpp.

**Table 1: Demography of two HbA1c categories**

	HbA1c			P value
	All subjects	<6.5%	$\geq 6.5\%$	
Age in years	53.97 $\pm$ 6.27	53.30 $\pm$ 7	56.17 $\pm$ 5.76	0.24
Duration of hypertension in years	12.42 $\pm$ 8.48	13.6 $\pm$ 9.32	11.91 $\pm$ 8.45	0.61
Height in meters	1.63 $\pm$ 0.09	1.64 $\pm$ 0.1	1.62 $\pm$ 0.08	0.70
Weight in Kg	81.56 $\pm$ 13.71	81.24 $\pm$ 14	81.11 $\pm$ 13.67	0.98
Body Mass Index in Kg/m <sup>2</sup>	30.94 $\pm$ 6.01	30.7 $\pm$ 6.9	30.74 $\pm$ 4.62	0.98
Waist circumference in cm	100.19 $\pm$ 10.21	99.55 $\pm$ 11.48	100.75 $\pm$ 7.65	0.75
Hip circumference in cm	109.65 $\pm$ 11.48	108.20 $\pm$ 9.25	111.58 $\pm$ 14.38	0.42
Waist to hip ratio	0.91 $\pm$ 0.06	0.92 $\pm$ 0.06	0.91 $\pm$ 0.1	0.71
Mean HbA1c%	6.61 $\pm$ 1.93	5.65 $\pm$ 0.4	8.36 $\pm$ 2.38	0.00

Among those who had the FPG test; the mean $\pm$ SD FPG was  $126.55 \pm 122.38\text{mg/dl}$  and range was 60-583. The mean FPG among those with negative FPG was  $86.27 \pm 17.41\text{mg/dl}$  (range 60-124), while among those with positive FPG was  $294 \pm 216\text{mg/dl}$  (range 130-583) and had a significant difference ( $p = 0.00$ ). The mean 2-hrpp was  $187.43 \pm 138.59\text{mg/dl}$  (88.24-702). The mean 2-hrpp among those with negative test was  $134.64 \pm 41.68\text{mg/dl}$  (range 88.24-246.60) while among those with a positive test it was

$407.40 \pm 186.84$  (range 165.60-702) which was significant ( $p = 0.00$ ). The mean HbA1c was  $6.61 \pm 1.93\%$  (range 4.60-14). The mean HbA1c among those with negative results was  $5.65 \pm 0.4$ , while among those with positive tests was  $8.36 \pm 2.38\%$  with a significant difference ( $P = 0.04$ ).

**Table 2: Comparative demography of positive diagnostic tests**

Features	FPG $\geq$ 126mg/dl	2hrPP $\geq$ 200mg/dl
	(6 subjects)Mean (95% CI)	(8 subjects)Mean (95%CI)
Age in years	55 (48, 60)	55.50(51, 60)
Duration of hypertension in yrs	11.6 (1, 24)	13.25(1, 26)
Height in meters	1.63(1.52, 1.80)	1.62(1.52-1.72)
Weight in kg	90.41(80.20, 109.20)	81.56(48.8, 109.2)
Body mass index	34.48(24.75, 47.26)	34.48(30.42, 47.26)
Waist measurement in centi meters	105.16(90, 119)	106.87 (102, 119)
Hip measurement in centimeters	116.67(102, 125)	117 (107, 125)
Waist to hip measuremnt	0.90(0.82, 1.02)	0.91(0.82, 1.02)

The prevalence of new diagnosed DM by HbA1c in both groups was 11/31 (35%) and by FPG was 6/31 (19%). Cross tabulation of 2x2 table revealed true positive were three, true negative were 17, false positive and false negatives were eight and three

respectively. Evaluation of performance in terms of sensitivity, specificity, correlation of HbA1c and FPG, positive predictive value, negative predictive value and efficiency is shown in table 3.

**Table 3: Comparative performance of the two diagnostic tests; ADA and WHO Criteria**

Features	ADA	WHO	P value
y equation	$y=5.16+790E^{-09}x$	$y= -158+52x$	0.00
Regression coefficient	0.50	0.73	0.06
R-square	0.25	0.53	0.02
sensitivity	50%	73.91%	0.05
specificity	68%	62.5%	0.06
Positive predictive value	27%	85%	0.00
Negative predictive value	85%	41.66%	0.00
efficiency	64%	70.97%	0.56
HbA1c	11/31(35%)	11/31(35%)	1
Blood sugar	6/31(19%)	8/31(25.8%)	0.51

Among those who had 2hrpp, the mean 2hrpp was  $187.43\pm 138.59$  (range 88.24-702). The mean 2hrpp among those with a negative test was  $124\pm 25.28$  (range 88.24-176.40) while among those with a positive test it was  $369.67\pm 170.72$  (205.20-702) which was statistically significant ( $P=0.00$ ). The mean FPG among those with a negative test was  $85.86\pm 19$  (range 60-130), while among those with a positive test it was  $243.56\pm 204.84$ mg/dl (range 99-583) which was found to be significant ( $p=0.00$ ). The mean HbA1c among those with a negative test was  $6.15\pm 1$  (range 5-9.70) while among those with positive test it was  $8\pm 3.22$  (range 4.60-14) which was also statistically significant ( $p=0.02$ ).

The prevalence of new DM by 2hrpp was 8/31(25.8%). Evaluation of performance of HbA1c and 2hrpp in a 2x2 table showed true positive result of five, false positive of six, true negative of 17 and

false negative of three. Results of sensitivity, specificity, efficiency, correlation regression between HbA1c and 2-hrpp, positive predictive value and negative predictive value is shown in table 3.

### Discussion

The interest in the use of HbA1c in the diagnosis of DM had recently crescendoed with the recommendation of IECRHbA1c/WHO report. This will serve as a possible replacement for FPG and / or OGTT in diagnosing DM. The proposed advantage of using HbA1c in the diagnosis of DM is the fact that it would obviate the need for the patient to undergo fasting and the associated inconvenience with OGTT.

The result of our study showed that 2hrpp criteria had performed better than FPG criteria in terms of establishing new cases of DM and

efficiency. This also applied to the correlation between HbA1c and the plasma glucose test; the correlation between HbA1c and 2hrpp criteria is better than between HbA1c and FPG criteria. The reported  $r^2$  in the 2hrpp group is more than twice  $r^2$  in FPG group. The result of 2hrpp criteria is also better than FPG criteria in sensitivity and positive predictive factor. the FPG criteria were marginally better than 2hrpp criteria in specificity and FPG is more than twice better than 2hrpp in negative predictive factor.

A similar result of a better correlation between total glycated haemoglobin (GHb) with 2hrpp compared to correlation between GHb with FPG was reported by Agboola-Abu *et al*<sup>3</sup>. They reported a correlation between GHb with FPG of 0.59 while the correlation between GHb with 2hrpp was 0.73. The similarity of results may be related to race or geography, as GHb was measured and not HbA1c and the sampling method is also different compared to our study.

Similar studies carried out in other parts of the world that correlated FPG, 2hrpp criteria with GHb were at aberrant to ours. Those other studies reported a better correlation between GHb with FPG compared to GHb with 2hrpp<sup>14,15</sup>. Gary *et al*<sup>4</sup> reported a correlation between HbA1c with FPG of 0.640 and correlation between HbA1c with 2hrpp of 0.511 in a study of 2,877 Hong Kong Chinese subjects. The HbA1c was specifically measured in this study using automated ion-exchange chromatographic method and the sampling method is also similar to ours, since consecutive at risk subjects were recruited in this study. Despite similarity in the HbA1c measured and the sampling method, the result still differs giving credence to the earlier idea of race and geography giving rise to similarity in our result with that of Agboola-Abu *et al*<sup>13</sup>.

Likewise, Randie *et al*<sup>5</sup> reported a correlation between HbA1c with FPG of 0.91 and correlation between HbA1c with 2-hrpp of 0.88 in a study of 381 Pima Indians of U.S. The HbA1c was measured by highly precise high-performance liquid chromatography. The study design, HbA1c and sampling method was similar to ours but gave similar result to that of Gary *et al*<sup>4</sup> supporting race and geography as a factor responsible for this. A dissimilar correlation between HbA1c with FPG of 0.852 and HbA1c with 2-hrpp of 0.806 was reported by Yoshihiko *et al*<sup>6</sup> in a study of 2,621 Japanese subjects. HbA1c was specifically measured in this study using high-performance liquid

chromatography and a similar sampling method was also used.

It is important to note that HbA1c is significantly correlated with 2hrpp but not FPG in subjects without DM<sup>17</sup>. Postprandial glucose contributes more than FPG to HbA1c at the lowest levels of HbA1c, particularly among those without DM<sup>18</sup>. This statement goes in tandem with the result of our study; however, the subjects used in our study were a mixed population of DM and non-DM. The same thing applied to the randomized controlled study of Agboola-Abu *et al*<sup>13</sup>.

Unlike the well established fact that HbA1c is specific for diagnosing DM, but not sensitive to diagnose DM<sup>19</sup>, our result of comparison of HbA1c with 2hrpp showed a higher sensitivity result compared to specificity. However, comparison of HbA1c with FPG showed a lower sensitivity result. There is no available study that had compared FPG and 2hrpp as “gold standard” using the IECRHbA1c or any other form of GHb measurement in terms of sensitivity, specificity, predictive values and efficiency. Thus, we find it difficult to compare our study with others in this regard.

The limitation we encountered is the small sample size of this study. It would have been my desire to recruit more subjects for the study.

## Conclusion

The HbA1c correlated better with 2-hrpp compared to FPG. The comparison of HbA1c with 2-hrpp is much better than comparison of HbA1c with FPG in all parameters of statistical measurement except in negative predictive value and specificity.

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