Reference Interval of Glycated Hemoglobin in Adults in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author AFI designed the study, performed the statistical analysis, wrote the protocol and wrote the final draft. Author COA managed the analysis of the study and the literature review. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Measurement of glycated haemoglobin is accepted as the gold standard for the diagnosis and management of diabetes mellitus; but it is rarely used in this environment, a resource-poor setting, largely due to the high cost of the assay. There is need to determine its reference intervals in Port-Harcourt and encourage the use of this assay.
Aim: This study was designed to determine the reference interval of glycated haemoglobin of apparently healthy subjects between the ages of 20-80 years in Port Harcourt, Rivers State, Nigeria.
Methods: A total of 172 subjects who met the inclusion criteria were recruited for the study. A cross sectional sampling method was used to recruit subject. Subjects' past medical history, demographic and anthropometric information were obtained with the help of a questionnaire. Blood was collected from subjects into an EDTA bottle and analysed for glycated haemoglobin using the boronate affinity chromatographic method.
Results: Results were analyzed using Statistical Package for Social Sciences version 20.0 (SPSS 20.0) and the reference interval was determined by the nonparametric method due to the skewness

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of the data. The mean age of subjects was 35.4 years and the minimum and maximum glycated haemoglobin value obtained were 3.5% and 6.0% after eliminating outliers. This gave a mean glycated haemoglobin value of 4.84% and a reference interval of 4.0-5.9%.

Conclusion: The reference interval so determined (4.0-5.9%) is different from that generated by the manufacturer of the diagnostic kit(4.0-6.5%). The introduction of this new local reference interval will enhance patient management in our local hospitals.

1. INTRODUCTION

With the increase in the incidence of obesity and metabolic syndrome worldwide,[1] there is associated increase in type-2 diabetes [2]. Port Harcourt, which is the capital city of Rivers state in Nigeria has one of the highest prevalence of obesity and type-2 diabetes in Nigeria.[2] With diabetes on the increase and with studies showing that a persistently high glycated hemoglobin value positively correlates with the development of diabetes complications,[3] the need for tighter control to prevent diabetic complications has made glycated hemoglobin estimation a reliable means of making diagnosis, monitoring patients and preventing complications. Plasma concentration of glycated haemoglobin is not affected by the day to day variation of plasma glucose or by the quality of patient preparation prior to sample collection[4]. This means that this test can be conducted anytime of the day without prior instruction and this has placed it at an advantage over fasting plasma glucose, but not necessarily replacing fasting plasma glucose for day to day monitoring of patient's blood glucose. To this effect, a local population based reference interval is necessary and important. This study was therefore designed to determine the reference interval of glycated hemoglobin in apparently healthy subjects in Port Harcourt, Rivers state, Nigeria and to evaluate the association of gender, hemoglobin concentration and BMI with glycated haemoglobin values in our population.

2. METHODS

The University of Port Harcourt Teaching Hospital, (UPTH) is a tertiary hospital in Rivers State. It has evolved since its inception in 1980 from a 60 bedded hospital to one with over 800 beds and is presently the apex health institution in Rivers State.

A cross sectional survey method was used to select apparently healthy subjects from the population. Sample size was determined using Reed hypothesis, which suggests a minimum of 120 reference subjects to be used for 90% confidence limits and 150 for 95% confidence limits [5]. The study population consists of apparently healthy individuals between the ages of 20 and 80 years living in Port Harcourt, who were not known diabetic or had history of hemoglobinopathy. Questionnaire was used to exclude hemoglobinopathies, diabetes mellitus and any other chronic illness. Physical examination and packed cell volume was used to exclude subjects with anaemia.

2.1 Measurement of Height

Height of subjects was measured using a height meter. The subject stands with feet together, without shoes or headgear. Back and heels against a vertically ruled bar to which a movable horizontal bar was applied to the vertex of the subject's head and measurement taken in meters (M^2) [6].

2.2 Measurement of Weight

Weight was measured using a weighing scale. Subjects were requested to wear light cloths and then stand erect on the scale. The weight was measured in kilograms (kg). The BMI was then calculated by dividing weight (kg) by the height in meters, squared6 (m^2).

2.3 Blood Collection and Processing

Subjects were made to sit comfortably and a tourniquet was tied at any point of the upper arm to make the veins prominent. A vacutainer was used to collect two milliliters (2 ml) of blood into an ethylenediamine tetra acetic acid [EDTA] bottle. These bottles were gently rolled over to allow the anticoagulant in the bottles mix with the blood. The blood samples were analyzed in batches later same day for glycated haemoglobin and PCV.

2.4 Laboratory Analysis

2.4.1 PCV and haemoglobin estimation

The mixed whole blood in EDTA bottle was aspirated into a capillary tube till the tube was about two third filled. One end of the capillary was sealed with plasticine and placed in the haematocrit centrifuge with the sealed end toward the centrifuge periphery. The packed cell volume (PCV) was determined by centrifuging blood in the capillary tube (also known as a micro haematocrit tube) at 10,000 rpm for five minutes. The haematocrit reader was used to calculate the PCV. The average of the duplicate readings in percentage was taken as the PCV value for each subject. The PCV value was divided by 3 to give a rough estimate of haemoglobin concentration in g/dl. [7].

2.4.2 Glycated hemoglobin

The sample in the EDTA bottle was analyzed same day using the chromatographic technique by Bio system for the glycated haemoglobin analysis [8].

2.4.3 Principle

After preparing the haemolysate, where the labile fraction is eliminated, hemoglobins are retained by a cationic exchange resin. Glycated Hemoglobin (HbA_{1c}) is specifically eluted after washing away the hemoglobin A_{1a+b} fraction. (HbA1c) is quantified by direct photometric reading at 415nm. The estimation of the relative concentration of HbA_{1c} is made by the measure of total hemoglobin concentration by direct photometric reading at 415 nm.

Calculations

$$\frac{\text{Absorbance of } HbA_{1C}}{\text{Absorbance of } Hb_{TOTAL}} \times 100 = \%HbA1c$$

2.5 Quality Assurance

All guidelines for specimen collection, processing, storage and handling was strictly adhered to. Laboratory analysis of samples was carried out with inclusion of quality control (QC) samples in which within run and between run quality control samples were assayed. Instrument efficiency was checked through regular calibration and recalibration.

3. RESULTS

The data generated was analyzed using Statistical Package for Social Sciences versions 20.0 (SPSS 20.0) Data was ranked and mean, median and mode were calculated and outliers were removed using the Dixon outliers' statistic [9]. The non-parametric method was used to determine the reference interval due to the skewed nature of the data where the 2.5^{th} and the 97.5^{th} percentile of the ranked data represented lower and upper reference limits [10]. Multiple regression analysis was used to determine the association of PCV, age and BMI with glycated haemoglobin values of the reference population and the level of significance was set at p-value < 0.05

A total of 172 subjects recruited for this study were made up of 99(51.7%) female and 83(48.3%) male subjects. A total of 99 subjects had a BMI above 25 kg/m^2 while 73 subjects subjects had BMI below BMI ≤ 25 kg/m^2.

The mean age for the study population was 35.4 years and the mean glycated hemoglobin was 4.84%. The data, when divided into male and female groups had a mean of 4.63% for the female and 4.61% for the male group. The reference subjects had minimum and maximum glycated heamoglobin values of 3.5% and 6.0% respectively (Table 1) and a reference interval of 4.0%- 5.9% (Table 1).

Table 1. Results of HbA1c

	Total subjects(n=172)
Mean age	35.35
Male	83
Female	89
HbA1C (%)	3.5
Min value	6.0
Max value	4.84 (0.04)
Total population (SEM)	4.83 (0.05)
Males (SEM)	4.85 (0.05)
Females (SEM)	**4.0-5.9**
Ref interval HbA1c	

4. DISCUSSION

The advantages of glycated haemoglobin over fasting plasma glucose have made it an important index in the screening, diagnosis and management of diabetes.

Table 2. Test of association between HbA1c and other factors using multiple linear regression analysis in the total population

Model	Unstandardized coefficients		Standardized coefficients	Students' t-test	Sig.
	Beta	Std. error	Beta		
(Constant)	2.740	.225		12.18	0.001
Ages	.012	.002	.324	5.69	0.001
PCV	.014	.005	.120	2.57	0.01
BMI	.012	.005	.128	2.38	0.02
Alcohol	.042	.033	.063	1.27	0.20

a. Dependent variable: hba1c

More so, studies have shown that diabetics with glycated haemoglobin value around the upper reference limit are more likely to develop diabetic complications than those with lower values [11]. To this effect, a population based reference value of glycated haemoglobin is very necessary as the upper limit of normal varies from one population to another. The reference interval of glycated haemoglobin of a local population will greatly influence the management of diabetic subjects, bearing in mind the association of glycated haemoglobin value and diabetic complications.

The changes in lifestyle and eating habits, apart from genetic, age and race factors has been shown to affect blood glucose value and so by extension affect the level of glycation of red blood cells.[12] Though the reference interval of fasting plasma glucose in this region was determined in a study done in 2008, [13] no literature has been cited on the reference interval of glycated heamoglobin in this area.

From this study the mean glycated heamoglobin value was 4.84% and the reference interval of glycated heamoglobin was found to be 4.0 – 5.9%. This is lower than the reference interval given by the diagnostic kit manufacturers, which was given as 4.0 – 6.5% [14] and also different from those obtained in other sub Saharan African studies[15,16,17]. The glycated heamoglobin value determined in a study in Kumasi Ghana using the chromatographic method was 3.7 – 7.0% [15]. This interval was found to be statistically and clinically significant when compared to the reference interval of this study even though both cities are located in the sub-Saharan Africa region. This difference may be due to the type of diet and the level of diabetes awareness which may have led to lifestyle modifications [18] A study done in Pakistan showed a reference interval of 4.6 – 6.6% [19]. This was also found to be both statistically different and clinically significant from the reference interval determined in this study and in

other studies. The variation of the reference intervals shown above is quite remarkable and only re-emphasizes the fact that even when using the same method, every laboratory should determine the reference interval of its population [15,20] for better management of patients. The use of reference interval given by diagnostic kits manufacturers for our population would therefore make for poor screening, diagnosis, monitoring and treatment of diabetic patients.

In this study, it was also noted that the mean glycated heamoglobin value for females was slightly higher than that of males but the difference was not statistically significant. This finding is in agreement with other studies, [21,22] though some had contrary findings [23]. It also may explain why the prevalence of diabetes among African Americans is higher in female than in males [24]. The positive association of glycated heamoglobin with PCV and age in this study, as in other studies, affirms that glycated hemoglobin value may not be too reliable in anemic subjects and in those with heamoglobinopathies. Anemia has been shown to be an independent determinant of glycated haemoglobin value [21]. The positive association of glycated haemoglobin with age may call for a possible age related partitioning of the reference interval of glycated haemoglobin.

5. CONCLUSION

The reference interval for this study is significantly different from that provided by the kit of manufacturer and those from other regions of the world. The introduction of this local reference interval will improve the management of diabetic patient in our region.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Ethical committee of the University of Port Harcourt Teaching hospital and all subjects gave informed

written consent. A total of 172 apparently healthy reference subjects who met the inclusion criteria and signed the consent form were recruited for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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