Original Article

Reference interval of fasting plasma glucose in apparently healthy adults in Port Harcourt, Nigeria

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Abstract

Background: Reference values of most assays in use in many chemical pathology laboratories in Nigeria are usually those of the manufacturer of diagnostic kit using non-indigenous reference populations. This is professionally unacceptable, so this study was aimed at determining a reference value for fasting plasma glucose (FPG) in adults living in Port Harcourt, Nigeria, using our indigenous population.

Methods: A total of 332 non-diabetic subjects who met the inclusion criteria for this study were recruited after they had signed the consent forms. After an overnight fast, about 2 ml of blood was collected into the fluoride oxalate bottles and was assayed the same day using the glucose oxidase method. Each sample was measured in duplicate and an average was calculated. The data were statistically analysed using the SPSS version 20 and the significance level was set at $P \leq 0.05$.

Results: The reference interval of FPG was calculated by the non-parametric method after data had been screened for outliers and ranked. This was done using the 2.5th and 97.5th percentile due to the skewed nature of the data, and the reference interval of FPG obtained was 3.1–5.8 mmol/l.

Conclusion: The reference interval obtained from this study was different but close to that obtained in 2008 in Port Harcourt and more importantly different from those provided by manufacturers of the diagnostic kit (4.2–6.4 mmol/l). The use of this new reference interval is recommended to be necessary for better management of patients in Port Harcourt, Nigeria. The need to use locally determined reference intervals is emphasised.

Keywords: Diabetes mellitus, glucose oxidase method, reference interval

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INTRODUCTION

In most cities in Nigeria, the lifestyle of individuals is fast becoming sedentary and it is accompanied by an increase in the intake of 'fast food'.[1] This, therefore, has led to an increase in the incidence of obesity and metabolic syndrome and the conditions are associated with an increased incidence of type-2 diabetes mellitus, which has a prevalence rate of 4.6% in Nigeria[2] and 6.8% in Port Harcourt.[3] Since the incidence of diabetes in Port Harcourt is one of the highest in the country, it is important that reference intervals given by the manufacturers of diagnostic kits need to be verified for better patient management. It is important to have a population-based reference interval and those in existence are expected to be reviewed periodically[4]

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since population demographics are dynamic.[5] A local population-based reference interval will therefore improve the result interpretation and the overall management of diabetes patients. Most times, the upper cut-off limits of reference intervals pose a challenge as it determines the cut-off values for making diagnosis, treatment and monitoring of patients.

The aim of this study, therefore, was to determine the reference interval of fasting plasma glucose (FPG) among apparently healthy individuals living in Port Harcourt, the capital of Rivers State, Nigeria, and to compare the reference interval so generated with existing ones, both locally and internationally. The association between FPG and age and between gender and body mass index (BMI) was also evaluated.

METHODS

The study population consists of a cosmopolitan population of people living in Port Harcourt.

Approval of the Ethical Committee of the University of Port Harcourt Teaching Hospital was obtained before the commencement of this study. Subjects were selected by cross-sectional study method. A total of 332 apparently healthy subjects between the ages of 18 and 70 years who were willing to participate and signed the consent form were selected based on Reed's hypothesis.[6]

For those who could not read, the information was read to them and those who were willing to participate gave their consent by thumb printing the consent forms. Only those who gave their consent were recruited for the study. Information was obtained from the subjects with the help of a questionnaire. Subjects were told to eat their last meal of the day before 10 pm the previous night. Venous blood sample (2 ml) was taken between 7 and 10 am the next day into a fluoride oxalate bottle. This was gently rocked to allow the anticoagulant in the bottle mix with the blood sample. This sample was kept in a rack at room temperature and later centrifuged at 3000 rpm for 5 min to separate the plasma from the blood cells. This plasma was transferred into a plain sample bottle and batch analysed for FPG using the glucose oxidase method kit manufactured by Randox laboratories.[7]

Procedure

One millilitre of glucose reagent was taken into the test tubes and 10 ml of blank, standard and blood samples are added to each of the tubes. This was gently mixed and left to stand for 10 min at room temperature and colour read at 520 nm using an Apel PD303S spectrophotometer, after zeroing the spectrophotometer with the blank. The concentration of plasma glucose was calculated as absorption of test over absorption of standard multiplied by the concentration of standard. This was done for all the samples, and each blood sample was measured in duplicate and the average value was taken to reduce errors.

The data generated were analysed using Statistical Package for Social Sciences version 20.0 (SPSS 20.0, IBM, USA). Outliers were eliminated using the Dixon outlier statistic[7] and the non-parametric method was used to determine the reference interval since the data were skewed and did not assume any particular distribution. This typically encompass the central 95% percentile of reference values and the 2.5th and 97.5th percentile as the lower and upper reference limit, respectively.[6] Multiple regression analysis was used to determine the association of BMI, age and FPG values for the total population, and the level of significance was set at $P < 0.05$.

All standard guidelines for sample collection, processing, storage and handling were strictly adhered to. Instruments were regularly calibrated and recalibrated, and the procedures were carried out with quality control samples in each run.

RESULTS

A total of 332 subjects were recruited into the study and this was made up of 172 (51.8%) males and 160 (48.2%) females, with a mean age of 25.83 years. The lowest and highest FPG values were 2.8 and 6.1 mmol/l, respectively, with a mean value of 4.4 mmol/l. When the reference subjects were divided into males and females, their mean FPG values were 4.4 mmol/l for males and 4.3 mmol/l for females [Table 1]. The FPG reference interval was 3.1–5.8 mmol/l using the non-parametric method. Multiple linear regression analysis showed a positive association of subject's age and BMI with FPG [Table 2].

DISCUSSION

Maintaining blood glucose concentration close to the reference interval will prevent or delay diabetic patients from developing complications. This would depend on correctly determined reference interval of FPG for a local population. Reference intervals may vary from population to population, and this may be due to diversity in the genetic constitution, nutritional habits and lifestyles, among others.

The reference interval obtained from this study (3.1–5.8 mmol/l) was different from that stated by the diagnostic kit manufacturer, which was given as

Table 1: Characteristics and fasting plasma glucose results of study subjects

Parameters	Results
Total subject (*n*)	332
Female, *n* (%)	160 (48.2)
Male, *n* (%)	172 (51.8)
Mean age	25.83
Lowest FPG value (mmol/l)	2.8
Highest FPG value (mmol/l)	6.1
Total subject (males+females)=all 332 subjects	4.35 (0.04)
Mean of male Subjects (SEM)	4.61 (0.05)
Mean of female Subjects (SEM)	4.27 (0.06)
Reference Interval of FPG males (mmol/l)	3.1-5.9
Reference interval of FPG females (mmol/l)	3.1-5.8
Reference interval of FPG study population (mmol/l)	3.1-5.8

FPG: Fasting plasma glucose

Table 2: Test of association between fasting plasma glucose and other factors using multiple linear regressions

Model	Unstandardised coefficients		Standardised coefficients (*β*)	*t*	Significant
	B	SE			
Constant	2.740	0.225	-	12.18	0.001
Ages	0.012	0.002	0.324	5.69	0.001
BMI	0.012	0.005	0.128	2.38	0.02
Alcohol	0.042	0.033	0.063	1.27	0.20

Dependent variable: FPG. FPG: Fasting plasma glucose, BMI: Body mass index, SE: Standard error

4.2–6.4 mmol/l (Randox glucose kit),[7] and was also different from but close to the results obtained from an earlier study done in Port Harcourt in 2008, which gave a reference interval of 3.0–5.7 mmol/l.[8] That study was also carried out using the glucose oxidase method and the same non-parametric method but with a reference population of 605 apparently healthy individuals. This was about twice the number used in the present study, which may be responsible for the slight and insignificant difference in the reference intervals. Currently, the reference interval in use in our laboratory is 3.9–6.4 mmol/l. The upper reference value of the earlier Port Harcourt study was 5.7 mmol/l while that for this study was 5.8 mmol/l, and this difference was not statistically or clinically significant. This may mean that more than 10 years after the first Port Harcourt study, the population demographics may have changed but not enough to produce a clinically significant change in the reference interval, or that increased awareness of diabetes in Port Harcourt over the last decade may have led to modified lifestyle and nutritional habits of individuals. Increased physical activities due to lifestyle modification will also lead to reduced plasma glucose concentrations,[9] which could be responsible for the clinically insignificant difference of the reference intervals observed in the two Port Harcourt studies.

Factors such as obesity, age or gender have been shown to affect blood glucose concentration in other studies.[10-12] This was also demonstrated in this study as there was a positive correlation of these factors with FPG, which could explain in part the variations in reference interval among different ethnic groups. To further strengthen this argument is the fact that other studies have shown an age-related increase in glucose values,[11] with significant differences in the mean values of the different age groups when compared.

In this study, when the data were divided into male and female groups, the mean plasma glucose level of the males (4.61) was slightly higher than those of females (4.27), but the difference in the mean values was not statistically significant. The reference interval for the male group was 3.1–5.9 mmol/l while that for the female was 3.1–5.8 mmol/l. These reference intervals and that of the study population were about the same. This is in keeping with other studies that showed that overall, gender had no significant effect on the FPG value.[13]

When the reference intervals from both Port Harcourt studies were compared with that of the kit's manufacturer which was derived from a different reference population, there was significant difference in the values. Diet, environment, genetic factors[15] among others influences blood glucose values which in a way affect the reference intervals of fasting plasma glucose. This may explain the differences in reference values among different ethnic populations, thus further emphasizing the importance of a local population-based reference interval of FPG, even when using the same assay method.[15]

An Iranian study and a study in Kumasi, Ghana, had reference intervals of 3.9–5.6[16] and 3.1–6.3 mmol/l,[17] respectively. Although the Iranian value was close to that determined in this study, the Kumasi value was significantly different from that in this study; however, both studies were done in Sub-Saharan West Africa. Most staple diets in West Africa are rich in carbohydrates such as corn, cassava, yam and coco yam, with different glycaemic indices,[18] and this is prepared in different ways and eaten in different proportions by different ethnic groups. This may influence the plasma glucose concentration, which could also be a reason for the significant difference in the reference intervals observed.

Since reference intervals vary from population to population, and sometimes significantly, there may be need to re-evaluate the universally accepted cut-off values for the diagnosis and management of diabetes mellitus in different populations, especially among populations with significantly low reference values, to further improve on the diagnosis, care and follow-up of diabetics in

these populations. This proposal might sound strange, considering the present state of knowledge in diabetology, but needs further consideration and evaluation by the international medical research community.

Limitation

Based on the Reed's hypothesis, a minimum of 120 subjects can be used for the determination of reference interval of a study population.[6] For this study, a reference population of 332 was used although a larger number would have been preferred.

CONCLUSION

The reference intervals of the two Port Harcourt based studies were about the same (3.1-5.7 mmol/l and 3.1-5.8 mmol/l) and different form reference interval presently in used in our laboratory (3.9-6.4 mmol/l) and that determined by the manufacturer of the Randox diagnostic kit (4.2-6.4 mmol/l). The use of this new reference interval in our laboratory will be necessary for better management of diabetic patients in our locality.

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Conflicts of interest

There are no conflicts of interest.

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