International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: https://doi.org/10.7439/ijbar
CODEN: IJBABN **Original Research Article**

Reference interval of fasting plasma glucose and glycated haemoglobin in children in Port Harcourt Rivers State Nigeria

Frederick Igila Allison* and Aaron C Ojule

Department of Chemical Pathology, Faculty of Basic Health Sciences, University of Port Harcourt, Rivers State, Nigeria

Abstract

Background: Studies have shown an increase in the prevalence of diabetes mellitus in children and a significant difference in the age- specific means of fasting plasma glucose and this has made it necessary to determine the local reference intervals of fasting plasma glucose and glycated haemoglobin in children in Port Harcourt and compare this with the reference interval of adults.
Method: 160 apparently healthy children between the ages of 7-10 years were recruited for this study. 2ml of blood was collected into fluoride oxalate bottles and EDTA bottles. These were later analyzed for glucose and glycated haemoglobin using the glucose oxidase method and Boronate chromatographic method, respectively.
Results: Reference interval of fasting plasma glucose was 3.1-5.7mmol/L and that of glycated haemoglobin was 4.0-5.1%, while those of adults for this same locality was 3.4-5.8mmol/l for FPG and 4.0-5.9% for glycated haemoglobin.
Conclusion: From the reference intervals of glycated haemoglobin and fasting plasma glucose so determined in this study, it may be unnecessary to have age specific reference intervals for fasting plasma glucose while that of glycated haemoglobin may need to be reviewed.
Keywords: Glycated Haemoglobin, Chromatography, Reference Interval.

***Correspondence Info:**
Dr. Frederick Igila Allison
Department of Chemical Pathology,
Faculty of Basic Health Sciences,
University of Port Harcourt,
Rivers State, Nigeria

***Article History:**
Received: 19/10/2019
Revised: 29/11/2019
Accepted: 29/11/2019
DOI: https://doi.org/10.7439/ijbar.v10i11.5294

QR Code

How to cite: Allison F. I. and Ojule A. C. Reference interval of fasting plasma glucose and glycated haemoglobin in children in Port Harcourt Rivers State Nigeria. *International Journal of Biomedical and Advance Research* 2019; 10(11): e5294. Doi: 10.7439/ijbar.v10i11.5294 Available from: https://ssjournals.com/index.php/ijbar/article/view/5294

1. Background

The incidence of diabetes mellitus is on the rise globally [1] and Port Harcourt has one of the highest rates in Nigeria [2]. In the University of Port Harcourt Teaching Hospital, the number of children attending the diabetes and metabolic clinics is on the increase [3].

This therefore has created the need to improve the management of diabetes mellitus even in children. As part of this strategy, a local population- based reference interval for children may be necessary. The reference interval presently in use for fasting plasma glucose and glycated haemoglobin in children is the same as that used for adults. Some studies have shown age- related differences in the means of fasting plasma glucose [4].

This study was therefore designed to evaluate the reference interval of glycated haemoglobin and fasting plasma glucose in apparently healthy children in our locality and compare the values with those of adults for this population and those of other population.

2. Methods

This study was carried out in Port Harcourt, the capital city of Rivers state in Southern region of Nigeria. Ethical approval of the Ethics Committee of the University of Port Harcourt Teaching Hospital was sought and obtained before the commencement of the study. A cross sectional study design was used to select subjects between

the ages of 10 to 17 years because it was a lot easier to collect blood from this category of children. Subjects who were willing to participate, comply with instructions of study and sign the consent form were selected. Based on Reed's hypothesis [5], 160 secondary school subjects were selected for this study after getting authorization from the Principals of selected schools and consent from parents. Pre-test counseling was done for all participants with emphasis on the benefits of the study to the participants and the community. Relevant information was obtained from subjects with the help of a self administered questionnaire.

Height of subjects was measured using a height meter with the subject standing with feet together without shoes or headgear. The back and heels were applied 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 [6]. Weight was measured using a weighing scale. (Harson) Subjects were requested to wear light clothes and then stand erect on the scale. The weight was measured in kilograms. The BMI was calculated by dividing weight (kg) by the height in meters squared [6].

Subjects were told to eat their last meal of the day before 10 pm the previous night. Venous blood sample (2ml) each was taken between 7am and 10 am the next day into fluoride oxalate bottles and EDTA bottles for each subject. These were gently rocked to allow the anticoagulant in the bottle mix with the blood sample and kept in a rack at room temperature. Later, samples in fluoride oxalate were centrifuged at 3000 RPM for 5 minutes to separate plasma from blood cells. The plasma was transferred into a plain sample bottle and batch analyzed for fasting plasma glucose in duplicates same day using the glucose oxidase method by Randox laboratory.

2.1 Glycated haemoglobin

Samples in the EDTA bottles were also analysed same day using the Boronate chromatographic technique by Glover laboratory for glycated haemoglobin.

2.2 Quality Assurance

All standard guidelines for sample collection, processing, storage and handling were strictly adhered to. Instruments efficiency was checked through regular calibrations and re-calibrations.

3. Results

A total of 160 subjects (71 females and 89 males) were recruited into this study. The data generated was analyzed using Statistical Package for Social Sciences version 20.0 (SPSS 20.0). The nonparametric method for the determination of reference interval was used since the data was skewed. The Data was ranked and mean calculated and outliers were eliminated using the Dixon outlier statistic [7]. The 2.5^{th} and 97.5^{th} percentile was also determined and used as the lower and upper reference limits respectively [8]. Multiple regression analysis was used to determine the association of age and FPG with glycated haemoglobin and the level of significance was set at a p-value < 0.05. The results are as shown in table 1, 2 and 3. The mean age of the study population was 15.58 years, while the mean glycated haemoglobin and fasting plasma glucose were 4.38% and 4.16 mmol/l, respectively.

The minimum and maximum values of fasting plasma glucose were 3.0 mmol/l and 6.0 mmol/l and for glycated haemoglobin, they were 4.0 and 5.5% respectively. Reference intervals of fasting plasma glucose and glycated haemoglobin were 3.1-5.7mmol/l and 4.0- 5.1%, respectively.

Table 1: Demographic and blood glucose parameters of study subjects

	FPG	HbA1c
Number of study subjects (n)	160)	160
Female	71(44.4%)	71(44.4%)
Male	89(55.6%)	89(55.6%)
Mean age(yrs)of study subjects	15.6	15.6
Mean(study subjects)	4.16mmol/l(0.052)	4.38%(0.026)
Mean value for males	4.23(0.07)	4.40(0.04)
Mean value for females	4.06(0.07)	4.35(0.03)
Lowest value	3.0mmol/l	3.6%
Highest value	6.0mmol/l	5.5%
Skewness	0.314	0.324
Ref interval	mmol/l	%
Study population	3.1- 5.7	4.0- 5.1
RI (male)	3.0-5.5	4.0-5.3
RI (female)	3.1-5.7	4.0-5.2

Table 2: Comparison of Mean (SD) of FPG and HbA1c between Males and Females

Parameters	n = 160	F/male mean	Male mean	t-test	p-value
Fasting Plasma Glucose (mmol/L)		4.06 (0.07)	4.23 (0.07)	1.63	0.11
HbA_1C		4.35 (0.03)	4.40 (0.04)	1.02	0.31

Table 3: Test of association between HbA1c and other factors using multiple linear regressions

Unstd beta		Std error	Std beta	t-test	p-value
PCV	0.017	0.007	0.196	2.44	0.02
FPG	0.094	0.039	0.187	2.41	0.02
BMI	0.003	0.007	0.029	0.36	0.72

4. Discussion

The increase in prevalence of diabetes mellitus among children, the positive association of age and fasting plasma glucose in previous studies [3] and the need to improve diabetes management as a whole, and especially in children, made it necessary to determine reference intervals of both fasting plasma glucose and glycated haemoglobin in children and compare same with that of adults in our locality.

The mean value of fasting plasma glucose and glycated haemoglobin in this study was found to be lower than that for adults in a previous study [9, 10] and this could be due to many factors such as lower cortisol value in children [10], increased physical activity in children [11] and since people tend to gain weight as they age, more adults are likely to be obese than children who are believed to be more active. Obesity has been shown to positively influence plasma glucose concentration [12].

The reference interval of fasting plasma glucose for children so determined in this study was found not to be statistically significant from a previous study for adults determined for this same population which was 3.1 – 5.7mmol/L [13]. That for glycated haemoglobin (4.0-5.1%) was statistically significantly different from that of adults for this region, which was 4.0-5.9% but clinically; the introduction of either one may have little or no effect in the management of diabetic patients regardless of age.

The data, when divided into male and female groups and the mean values of the male and female groups for both glycated haemoglobin and fasting plasma glucose were compared and found not to be statistically significantly different (Table 2). This finding was reflected when the reference interval for fasting plasma glucose for the male and female groups were determined, which was 3.0-5.5mmol/l for males and 3.1-5.7mmol/l for females, while that for glycated haemoglobin for male and female groups were 4.0-5.3% and 4.0-5.2% respectively. The reference interval for males and females were about the same in this study as in other Studies [13, 14]. From the findings in this study, it may be unnecessary to have an age or gender specific reference intervals for both fasting plasma glucose and glycated haemoglobin. Though the difference in the age related means may be significant as seen in some studies, [9, 15]

It was also noticed in this study that when the data was segregated into male and female groups, the mean values of the male group for both glycated haemoglobin and fasting plasma glucose were slightly higher than the female value (Table 1). This was again in agreement with an earlier study carried in an adults' population in Port Harcourt [12] and other studies [13, 14].

The non parametric method was used to calculate the reference intervals and this gave a reference interval of fasting plasma glucose as 3.1 – 5.7mmo/L and that of glycated haemoglobin as 4.0-5.1% (Table 1).

Same cannot be said when the means of male and female groups were compared in this study as they were found not to be statistically significant (Table 2). It is worthy to note that when the study population was divided into the male and female subgroups, the numbers for the male and female groups fell short of Reed's hypothesis for determining reference populations. This may have statistically affected our findings on determination of reference intervals of the sub groups. (Table 1) This is in keeping with other studies which also showed no gender effect in determining reference interval of fasting plasma glucose. Glycated haemoglobin was shown to have a positive association with fasting plasma glucose and subjects packed cell volume (PCV). This was expected as glycated haemoglobin is a reflection of subject's plasma glucose concentration over a period of about three months [16]. Glycation of haemoglobin is a non enzymatic reaction which occurs between glucose and amino terminal valine (N- terminal end) of the beta chain of haemoglobin. Therefore, the glycated haemoglobin value is usually affected by subjects' haemoglobin concentration [17].

5. Conclusion

The concentration of certain factors that help regulate blood glucose values like cortisol and progesterone are found to be higher in adults Studies have shown that BMI and age have a positive association with blood glucose concentration. Many factors such as obesity, physical activities, time of last meal, individual metabolic rate and so on determine plasma glucose value. Many studies have shown significant difference in age related means of fasting glucose level.

The reference interval of fasting plasma glucose and glycated haemoglobin for children in this study was found to be about the same with that of adults and similar to results from other studies of adults elsewhere. It would therefore be unnecessary to have age or gender specific reference intervals of fasting plasma glucose and glycated haemoglobin in our study area, as the differences were not clinically and statistically significant.

References

[1]. Rosenbloom AL, Joe JR, Young RS, Winter WE, Emerging epidemic of type 2 diabetes in youth. *Diabetes care* 1999; 22:345-354.

[2]. Nyenwe EA, Odia OJ, Ihekwaba AE, Ojule A. Babatunde S. Type 2 diabetes in adult Nigerians: A study of its prevalence and risk factors in Port

Harcourt, Nigeria. *Diab Res Clin Pract 2003*; 62:177-185.

[3]. Basu R, Breda E, Oberg AL, Powell CC, Dalla Man C, Basu A, et al. Mechanisms of the age-associated deteriaration in glucose tolerance: Contribution of Alterations in Insulin Secretion, Action and Clearance. *Diabetes* 2003; 52:1738-1748.

[4]. Kilpatrick ES, Dominiczak MH, Small M. The effects of ageing on glycation and the interpretation of glycaemic control in type 2 diabetes. *QJM* 1996; 89:307.

[5]. Solberg HE, A guide to IFCC recommendations on reference values. *J Int Fed Clin Chem*1993; 5:162-165.

[6]. Keys A, Fidanza F, Karvonen MJ, Kimura N, Taylor H. Indices of relative weight and obesity. *Int J Epidemiol* 2014;43(3):655-665

[7]. Horn PS, Feng L, Li Y, Pesce AJ. Effect of outliers and non healthy individuals on reference interval estimation. *Clin Chem* 2001; 47:2137-2145.

[8]. Walton RN. Establishing reference intervals: Health as a relative concept. *Seminars in Avian and Exotic Pet Medicine* 2001; 10(2):66-71.

[9]. Sapigni T, Astolfi G, Cavallini L, CremoniniF. Influence of age on blood glucose levels: percentile reference intervals determined on ambulatory patient. *Boll soc ital boil sper* 1981; 57:1254-1260.

[10]. Perrini S, Laviola L, Natalicchio A, Giorgino FJ. Associated hormonal declines in aging: DHEAS. *J Endocrinol Invest* 2005; 28(3):85–93.

[11]. Milanovic Z, Pantelic S, Trajkovic N, Sporis G, Kostic R, James N. Age related decrease in physical activities and functional fitness among elderly men and women. *Clin Interv Aging* 2013; 8; 549-556.

[12]. Ferrannini E, Natali A, Capaldo B, Lehtovirta M, Jacob S, Yki-Jarvinen H. Insulin resistance, hyperinsulinemia, and blood pressure: role of age and obesity. European group for the study of insulin resistance (EGIR). *Diabetes* 1996; 45:947–953.

[13]. Ejilemele AA, Ojule AC, Orluwene C. Plasma glucose in a reference population in Port Harcourt. *Port Harcourt Med J* 2008; 2 (3): 228-232.

[14]. Ghasemi A, Zahediasl S, Azizi F. Reference values for fasting serum glucose levels in healthy Iranian adult subjects. *Clin Lab* 2011;57(5):343-349

[15]. Suka M, Yoshida K, Kawai T, Aoki Y, Yamane N, Yamauchi K. Age and sex specific reference intervals for 19 health examination items; Mega-data from Japanese Health Sevice Association. *Rinsho byori* 2005;53:599-607

[16]. Singh R, Barden A, Mori T, Beillin L. Advanced glycation end-products; a review. *Diabetologia* 2001; 45: 129-146.

[17]. Connor GO, Molloy AM, Daly L, Scott JM. Deriving a useful packed cell volume estimate from haemoglobin analysis. *J Clin Pathol* 1994; 47: 78–79.