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Quantitative Reference Ranges for Fasting Profiles and Oral Glucose Tolerance Test for Healthy Adults in Metropolitan Region of Nairobi, Kenya

Abstract

Purpose: To establish quantitative reference ranges for fasting profiles and oral glucose tolerance test for healthy adults in metropolitan region of Nairobi.
Methods: A prospective study carried out on 871 healthy subjects from the metropolitan region of Kenya.
Results: The fasting profile parameters investigated were fasting blood glucose (FBG), total cholesterol (TC) triglycerides (TG), high density lipoprotein cholesterol (HDLC), low density lipoprotein cholesterol (LDLC) and TC/HDLC ratio. In addition, oral glucose tolerance test (OGTT) was also investigated. Eight hundred and seventy one (871) healthy study subjects were involved in the study. Established reference ranges were as follows: FBG (venous whole blood) (2.1 – 5.7) mmol/L, TC (2.9 – 6.4) mmol/L, TG (0.44- 2.44), HDL C (1.1 – 2.1) mmol/L, LDLC (1.1 – 4.3) mmol/L, TC/HDLC ratio (1.1 – 5.4). Established reference ranges for oral glucose tolerance test (OGTT) were as follows: baseline/fasting blood glucose capillary whole blood (3.2-5.4) mmol/L, half hour (4.7-8.9) mmol/L, one hour (4.4-9.8) mmol/L, one hour and half (4-8.1) mmol/L and two hours (3.4-7.2) mmol/L. Results for gender differences for the studied parameters were as follows: FBG (p=0.124), TC (p=0.205), TG (p=0.705) HDLC (p= 0.52), LDLC (p=0.417) and TC/HDLC ratio (p=0.359). On the other hand, the gender results for timed OGTT were as follows: 0 hour (p=0.123), half hour (p=0.479), one hour (p=0.412), one hour and half (p=0.596)) and two hours (p=0.630). Hence there were no gender disparities for the parameters in the studied adult Kenyan population.
Conclusion: Since the established reference ranges are a reflection of the Kenyan adult population our clinical chemistry laboratory reports interpretations will henceforth be independent of what has been quoted in literature. Likewise effective diagnosis and management of glucose and lipids pathological disorders will be achieved by the use of established adult Kenyan reference ranges.

Keywords: Reference ranges, lipid profile, fasting blood glucose, Adult Kenyan, Nairobi metropolitan city

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Introduction

Diagnosis and management of various pathological disorders is achieved through proper clinical history, physical examination and use of quality clinical laboratory results. Proper interpretation of qualitative laboratory results is based on the use of reference ranges established from healthy individuals. Several factors such as gender, age, sample type, analytical procedures, instruments and geographical location of the healthy individuals are know to influence the clinical laboratory parameters [1]. It is therefore recommended that clinical laboratories are used to establish reference ranges for laboratory parameters based on local healthy population [2].

Most clinical laboratories in developed countries are advanced in establishing reference ranges for laboratory parameters from their own local population. These reference ranges have been widely published in scientific journals [3], medical textbooks [4, 5] and internet. In the developing countries, especially Africa, the situation is changing since some regions have reported reference ranges in scientific literature. These regions include Tanzania [6], Rwanda [7], Kenya [8], Central African Republic [9], Ethiopia [10], and Nigeria [11]. The reported reference ranges from these regions are on routine clinical chemistry and immunohaematology parameters. There are no published reference ranges for fasting profiles including fasting blood glucose (FBG), fasting lipids (total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDLC), low density lipoprotein cholesterol (LDLC), total cholesterol/high density lipoprotein cholesterol ratio) and oral glucose tolerance test (OGTT) for Kenyan nationals. This situation presently leaves health providers in Kenya with no other alternative but to use literature quoted reference ranges in the diagnosis and management of cardiovascular diseases and diabetes mellitus.

The purpose of this study is therefore to establish reference ranges for fasting lipid profile, fasting blood glucose and oral glucose tolerance test for the adult Kenyans.

Methods

Study Design

This study was carried out in Nairobi metropolitan region which covers the capital city and its environs between May 2008 and April 2009. Following ethical approved by Kenyatta National Hospital Ethical Research Committee (reference KNH-ERC/P342/11/2007), recruitment of the prospective study subjects was done in two phases. Phase one was for lipid profile and fasting plasma glucose whilst phase two was for oral glucose tolerance test and fasting blood glucose. Sensitization exercise was carried out through public lectures and advertisements in various institutions which included churches, universities and hospitals. Emphasis was laid on the need for prospective subjects to have fasted for 8-10 hours prior to specimen collection period. Sample size determination was done using Fisher *et al* (1998) formula with consideration to similar studies carried out elsewhere [6-11]. A total of 901 (483 females and 418 males) individuals between age 18-60 years were recruited in the two phases of the study. Five hundred and eighty subjects (320 females and 260 males) were recruited in phase one for lipid profile and plasma glucose. Three hundred and twenty one subjects (179 females and 142 males) were recruited in phase two for oral glucose tolerance test and fasting whole blood glucose. The included in the study were those Kenyan citizens who were 18-60 years of age, fasted for 8-10 hours prior to blood collection in the morning, and normotensive (systolic blood pressure of 115±15 mmHg and diastolic blood pressure of 75±15 mmHg). Subjects who were obese, hypertensive (systolic blood pressure >139 mmHg and diastolic blood pressure >90 mmHg), pregnant, involved in any excessive exercise, under any medication, taking any oral contraceptives, or uses alcohol and/or tobacco were excluded. All recruited subjects were requested to give consent for their blood to be used in the study. A questionnaire was administered to consenting study subjects to gather some sociodemographic data.

Blood specimen collection

Fasting blood samples, based on the type of test each subject was undertaking, was collected in the morning between 7.00 and 8.00 am. Capillary blood was collected from those tested for fasting blood glucose and oral glucose tolerance test while approximately 5 ml of venous blood was collected from each of those tested for fasting lipid profile and fasting whole blood glucose and divided into two volumes: 3 ml into a labeled plain tube and 2 ml into a labeled fluoride tube. A drop of capillary blood specimen was obtained from a sterilized fingertip area using a lancing device. Blood in the tubes was centrifuged at 3000g for 3 min. Separation of serum and plasma was done using an automatic pipette and transferred into specific labeled tubes in a rack ready for analysis.

Urine specimen collection

All study subjects for oral glucose tolerance test were instructed to collect urine into sterile urine containers immediately they arrived in the laboratory. Urine was also collected at 1 and 2 hr during the OGTT period.

Management of oral glucose tolerance test study subject

Study subject with no glycosuria and fasting capillary blood glucose less than 6.1mmol/l [12] was given 75 g glucose solution to drink and then allowed to sit quietly during the testing period of 2 hr.

Sample analysis

Lipid profile assays and plasma glucose were analyzed routinely on Olympus 640 auto-analyzer (Olympus Diagnostica GmbH, Hamburg, Germany) using established techniques summarized in Table 1. All the reagents were commercially prepared to fit the required volumes and concentrations. Capillary blood glucose analysis was performed on Accu-Chek glucose meter (Roche Diagnostics GmbH, Mannheim, Germany).For accuracy and precision of the analytical work, Data-Trol Multisera Normal and Accu-Chek Control 1 and 2 solutions were used during the entire study analytical period.

Table 1: Analytical methods for the analytes using Olympus 640 AU and Accu-Chek glucose meter

Analyte(unit)	Method
Glucose(mmo/l)	Enzymatic (glucose oxidase), timed endpoint reaction at 500nm.
*Glucose (mmol/l)	Glucometer (glucose oxidase)
Total cholesterol (mmol/l)	Enzymatic (cholesterol esterase/peroxidase), timed endpoint reaction at 540nm.
HDLC (mmol/l)	Direct enzymatic selective protection (HDLC protector), timed endpoint reaction at 540 nm
LDLC(mmol/l)	Direct enzymatic selective protection (LDLC protector), timed endpoint reaction at 540 nm

**Glucose method used in Accu-Chek glucose meter*

Data Analysis

The outliers were removed by testing the homogeneity of the study population by testing for Gaussian distribution of each analyte. A reference interval for each parameter was calculated from the arithmetic mean of ±1.96 SD to obtain the 2.5 % and 95% limits. Data comparison was achieved using Student's T-test. At 95% confidence interval, p values less than 0.05 were considered significant.

Results

The subjects used in the statistical analysis of fasting venous plasma glucose (FPG), fasting lipid profile (FLP) and oral glucose tolerance test(OGTT) were 567, 554 and 303, respectively. Study participants had a mean age 32.9 years. Gender difference for each analyte was determined to assess whether combined or gender–specific reference ranges should be established. P-values for the difference between male and female participants are presented in Table 2. The results for both male and female for the analytes were not statistically different.

Table 2: Gender difference for the studied analytes

Analyte(unit)	Gender	Number	Mean	sd	P-value
*FPG (mmol/l)	M	284	4. 07	0.79	0.124
	F	283	4.16	0.8	
TC (mmol/l)	M	277	4.66	0.96	0.201
	F	277	4.56	0.89	
TG (mmol/l)	M	277	1.41	0.54	0.703
	F	277	1.39	0.51	
HDL-C (mmol/l)	M	277	1.59	0.27	0.527
	F	277	1.6	0.25	
LDL-C (mmol/l)	M	277	2.77	0.83	0.418
	F	277	2.71	0.81	
TC/HDL-C ratio (mmol/l)	M	277	3.2	1.08	0.354
	F	277	3.29	1.13	
OGTT (0 hr, mmol/l)	M	134	4.25	0.56	0.123
	F	169	4.31	0.52	
OGTT (0.5 hr, mmol/l)	M	134	6.82	1.06	0.477
	F	169	6.78	1.09	
OGTT (1 hr, mmol/l)	M	134	6.98	1.49	0.415
	F	169	7.06	1.27	
OGTT (1.5 hr, mmol/l)	M	134	6.05	1.16	0.596
	F	169	6.02	1.02	
OGTT (2 hr, mol/l)	M	134	5.14	0.9	0.639
	F	169	5.26	1.06	

*Fasting glucose using plasma specimen.

Table 3: Comparison of established fasting profiles reference ranges for the adult Kenyan population with adult populations quoted in literature

Analyte(unit)	Sex	KE	UG	NG	KW	ADA	Roche	Rk
*FPG (mmol/l)	M/F	2.1 – 5.7	-	-	3.8-6	4.1-5.9	3.1-6.4	4.1-5.9
TC (mmol/l)	M/F	2.9 – 6.4	2.7-5.2	3.4-5.5	3.3-5.4		<5.2	<5.2
TG (mmol/l)	M/F	0.4 – 2.4	0.6-4	0.6-1.6	0.3-2.1		<2.3	<2.6
HDLC (mmo/l)	M/F	1.1 – 2.1	0.6-2	0.9-2	0.7-1.9		>0.9	>1.6
LDLC (mmol/l)	M/F	1.1 – 4.3	1.3-3.6	1.7-3.5	2.6-3		<4	2.6-3.3
OGTT (0 hr, mmol/l)	M/F	3.2 – 5.4	3.1-7.6	-	-	<6.1	3.3-5.5	-
OGTT(0.5 hr, mmol/l)	M/F	4.7 – 8.9	-	-	-	-	-	-
OGTT (1 hr, mmol/l)	M/F	4.4 –9.8	-	-	-	-	-	-
OGTT (1.5 hr, mmol/l)	M/F	4 –8.1	-	-	-	-	-	-
OGTT (2 hr, mmol/l)	M/F	3.4 – 7.2	-	-	-	<7.8	-	-

*KE =Kenya, UG=Uganda **[13]**, NG=Nigeria **[11],** KW=Kuwait **[15]**, ADA=American Diabetic Association [16], Roche= Roche Diagnostics GmbH, Mannheim, Germany **[14]**, Rk = Manufacturer's reagent kit*

Therefore, the results for males and females for each analyte were used to construct common reference range. Reference ranges for the studied analytes are presented in Table 3 Significant glucose difference existed for capillary whole blood and venous blood ($p<0.001$). For the establishment of OGTT curve, the reference range upper limits for each half hour of the 2 hr testing period using capillary whole blood were: 0 hr (5.4 mmol/L), 0.5 hrs (8.9 mmol/L), 1 hr (9.8 mmol/L), 1.5 hrs (8.1 mmol/L) and 2 hr (7.2 mmol/L).

Accuracy and precision of the study results was ascertained by the use of day to day internal quality control whose results are shown in Table 4. The obtained mean quality control results for

Table 4: Quality control results for the entire study period

Analyte (unit)	*Assigned range	Mean value	Obtained range	Mean value
**GLU (mmol/l)	4.8-6.5	5.7	5-6.1	5.6
***GLU(mmol/l)	2.1-3.7	2.9	2.2-3.6	3
TC (mmol/l)	3.2-4.5	3.8	3.5-4.3	3.9
TG (mmol/l)	0.7-1.14	0.94	0.9-1.1	1
HDLC (mmol/l)	0.6-1.7	0.9	0.8-1.5	1.15
LDLC(mmol/l)	1.8-2.6	2.4	1.9-2.4	2.2

**Assigned quality control range given by Data-Trol Multisera Normal manufacturer*
***Glucose quality control results using OLYMPUS AU 640 autoanalyzer.*
***Glucose quality control results using ACCUCHEK glucometer*

each analyte were in close agreement with the assigned mean quality control value.

Discussion

Current study presents reference ranges for fasting profiles for Kenyan adult population which have not been reported previously in this region of Africa. Studies reported have been on analytes investigated using random specimens. With the large number of study participants of both male and female, the study did not find any gender differences on the studied analytes.

Due to the differences between capillary and venous blood glucose, the study established two different types of fasting glucose reference ranges. This was necessary since the two type of glucose analyses are commonly adopted in routine laboratories [17]. A study by Roche Diagnostics has fasting capillary blood glucose reference range for adult as 3.3-5.5 mmol/L [14] which was similar with the current study reference range of 3.2-5.4 mmol/L. The current study reference ranges of capillary whole blood differs substantially from that of Ugandan population given as 3.11-7.55 mmol/l [13], despite the proximate of the two populations. Also, the fasting plasma glucose reference range established by Roche Diagnostic (3.1-6.4 mmol/L) differs from the current study reference range of 2.1-5.7 mmol/L. The two studies carried out on different populations are in agreement that fasting capillary blood glucose differs from fasting plasma glucose, with the former being higher [17,18] as in the case with their corresponding reference ranges. Other documented reference ranges for fasting plasma glucose which differs from that established in this study are those carried out among Kuwaitis (3.75-6.03 mmol/L) [15], Americans (4.1-5.9 mmol/L) [16] and Israelitis (2.7-5.5 mmol/L).

All along the clinical laboratories in Kenya have been using the WHO recommended reference ranges [17]. This is the first study on OGTT reference ranges to be carried out here in Kenya. The usefulness of OGTT as a gold standard for diagnosing diabetes mellitus (type 1 and 2) has been drop by the American Diabetes Association (ADA) who only recommends its use for diagnosing gestation diabetes mellitus. Due to these different recommendations by WHO and ADA [16], it was therefore important to establish Kenyan based OGTT reference ranges. The fact that glucose concentrations during an OGTT in capillary blood are significantly higher than those in venous blood by 20-25% or a mean difference of 1.7 mmol/l [17,18], is an important factor to consider in comparing the current study findings with what has been published in literature. Despite the fact that the current study did not use venous blood specimen to establish OGTT reference values, it is obvious that the values would be lower than what has been produced using the capillary blood specimens. For example, the 2 hr OGTT capillary blood value of 8 mmol/l translates to 6-6.4 mmol/l venous blood glucose which is lower than WHO recommended value of 7.8 mmol/l. Considering these venous blood glucose values would mean that the impaired glucose tolerance phase for the Kenyan adult population is greater, therefore avoiding under diagnosis of diabetes and impaired glucose tolerance.

On lipid profile, the current study did not find any gender differences thus the reference ranges established are common in both male and female, which is in consistent with other reports in literature [11, 13]. The lipid profile parameters reference ranges upper limits of the current study are higher than manufacturer's reference values that are currently in use in Kenyan's clinical laboratories, apart from the triglycerides value. For example the reference range upper limit of total cholesterol in the studied Kenyan population (6.4 mmol/L) is greater than the Manufacturer's reference range upper limit of 5.2 mmol/L. These differences suggest that Kenyan population have always been over diagnosed on hyperlipidaemia and its related pathological conditions such as cardiovascular diseases. Studies done on fasting lipid profile in other populations in different geographical locations exhibits the same trend of lower parameter values than the Kenyan population

Conclusion

For the first time in Kenya, reference ranges for fasting profiles have been established. The study recommends the usage of reference ranges reported in this study independently of what has been quoted in literature. It is evident from this study that different geographical regions have different reference ranges of the studied fasting profiles.

There is a great need for reference ranges of other parameters not included in this study to be established. The finding of this study opens an avenue for similar studies to be carried out in other geographical regions.

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Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by us. SKW, ENMN and JJNN conceived and designed this study. Data collection and analysis was carried out by SKW, DM, BC, LN and WG. The manuscript was prepared by SKW, ENMN and JJNN. All authors approved the manuscript before it was submitted for publication.

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