Reference Physiological Ranges for Serum Biochemical Parameters among Healthy Cameroonians to Support HIV Vaccine and Related Clinical Trials

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SUMMARY

Background: A valid scientific evaluation of the efficacy of HIV vaccines or antiretroviral drugs includes measurement of changes in physiological parameters of subjects from known established baseline reference ranges. This study was designed to establish reference ranges for biochemical parameters among healthy adult Cameroonians to support planned HIV Vaccine clinical trials and scaling up of ARV drugs among AIDS patients.
Methods: After informed consent, blood and urine samples were collected from a total of 576 adult Cameroonians and analyzed for the presence of underlying pathologies that may affect biochemical parameters. Samples from 501 of them were found eligible for the determination of reference biochemical parameters. After complete assay, the data were subjected to both parametric and non parametric statistics for analyses with 2.5 and 97.5 percentiles considered as the lower and upper limits of reference ranges.
Results: There were 331(66.1%) males and 170(33.9) females, with 359(71.7%) and 142(28.3) of them residing in the urban and rural areas respectively. Statistically significant differences ($P<0.05$) were observed in the following biochemical parameters between urban and rural participants: AST, ALT, alkaline phosphatase, creatinine, total protein, albumin, triglyceride, total cholesterol, and the bilirubins. When the data were regrouped into sex, there were statistically significant differences ($P<0.05$) in the following parameters between males and females: AST, ALT, creatinine, albumin, triglyceride, total cholesterol, and direct bilirubin.
Conclusion: The present study shows that sex and geographic location have significant impact on reference physiological biochemical parameters of healthy, adult Cameroonians; hence this should be taken into consideration when monitoring participants either during HIV Vaccine clinical trials or on antiretroviral (ARV) drugs treatment.

Key Words: Normal Biochemical Ranges, Health Adult Cameroonians.

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Introduction

The human immunodeficiency virus (HIV) constitutes a major public health problem in Cameroon with a prevalence rate of 5.5% [1]. Furthermore, Cameroon has been identified worldwide as the country with the highest HIV heterogeneity; in fact the HIV-1 group N has been reported only among Cameroonians [2, 3]. In addition, it is suspected that the virus was initially transmitted from chimpanzees to humans in Cameroon, where the emergence of the AIDS pandemic occurred [4]. Therefore current international efforts to develop an effective and affordable preventive HIV Vaccine will necessary pay attention to Cameroon in choosing appropriate populations for clinical trials. Indeed, Cameroon is at the moment one of the African countries that is building capacity for HIV Vaccine trials. It has gone into partnership with leading

international organisations such as the United States based Centers for Disease Control and Prevention (CDC), the Department of Defence (DoD), and the WHO/UNAIDS-AAVP in efforts to bring this initiative to fruition. Such vaccine trials require a platform for clinical evaluation of safety and efficacy. Further, since the start of antiretroviral drug use in Cameroon, there has been a great need for a platform for the evaluation of the efficacy of treatment efforts. This will be timely particularly at this point when the Cameroon National AIDS Control Program is scaling up antiretroviral drug treatment among AIDS patients nationwide.

This platform for scientific and clinical validation of HIV Vaccines and antiretroviral drugs in Cameroon would be based on reference ranges for given parameters of clinical interest. Such reference ranges are defined as the concentration of these parameters in a group of clinically healthy persons [5,6]. Under normal physiological conditions, haemostatic mechanisms keep these parameters within a certain limit [6]. In healthy individuals they vary considerably in different populations [5,7]. In body fluids, and in the absence of diseases, they are influenced by several factors such as age, sex, dietary habits of the people, geographical location and climate [8,9,10,11,12,,13,14,]. Further, several of these parameters show diurnal and seasonal variations [2]. They may also change as a result of variation in techniques used by different laboratories [15,16,11,17].

The parameters can also vary following pathological conditions that affect major body organs and systems that produce, secret or store them such as the Liver, Pancreas, Kidney, Bone marrow and the Immune system [18,19,20]. Administration of drugs or vaccines for therapeutic purposes or clinical trials can also cause significant variations [2]. The timely measurement of these parameters is therefore absolutely necessary in the evaluation of either disease state or prognosis following therapeutic or clinical interventions.

Reference ranges for Biochemical parameters are non-existent in Cameroon where, like in many other countries of the third world, it is common practice to use reference ranges established for Western populations for interpretation of laboratory results. There is therefore need for baseline reference laboratory ranges with which to monitor not only physiological or pathological changes following administration of various drugs and vaccines to healthy persons in Cameroon, but also disease states following infectionwith various pathogens. The reference ranges we have established here set the stage for the development of a framework to meet these goals.

Methods

Study Design and Population

This was a cross sectional study that recruited participants from both urban and rural communities with diverse backgrounds. The rural participants were recruited at the Shamka Foundation health unit located in the rural village of Ekorezok in Nkolbisson some 15 kilometres from Yaounde. This village holds a population of about 30,000 inhabitants. The staple food is mainly cassava leaves (nqwem), groundnuts and cassava itself in different forms. The area is surrounded by water and inhabitants depend on the mud-fish from these streams for supplementing their diet. Inhabitants of this locality who responded to the Foundation's health unit call for people to come for HIV/AIDS voluntary counselling and testing during the December 2006 World AIDS week, were consented and included in the study. The urban study samples were collected from the city of Yaounde. This is the capital of Cameroon. It has a population estimate of about one and a half million inhabitants. As a capital city, it hosts populations from diverse backgrounds, mostly of the higher and middle income earning bracket with typical life styles and nutritional habits different from those in the rural areas. Participants were identified from apparently healthy people who came to donate blood at the blood transfusion unit of the University of Yaounde Teaching Hospital (CHU). All consenting participants between the ages of 18 and 59 years from both the urban and rural areas were included in the study. A lower age of 18 years was used because this is the minimum age of independent consent in Cameroon. We limited the upper age at 59 years because above this age, body physiological parameers start deviating; further, people above this age would not be good candidates for clinical trials.

Laboratory Procedures:

After appropriate informed consent and a structured interview that solicited information on socio-demographic and medical history, about 10mls whole blood was collected from overnight fasted participants by trained phlebotomists. This was aliquoted into various blood collection tubes as follows: a portion was put into the K-EDTA tube for detection of HIV, Malaria parasite and Hepatitis B. The remaining portion was put in sterile plain tubes and allowed to clot for 4 – 6 hrs before the serum was prepared by centrifugation at 3000 rpm for 30 minutes. The serum samples were aliquoted and stored at – 20°C until

used for measurement of biochemical parameters. For urine collection, participants provided the samples in appropriately labelled containers given to them by us.

Screening for Exclusion from the study:

HIV serology was done using two rapid test kits, Determine (Abbott Laboratories, Abbott Park, IL) and OraQuick (Ora-Sure Technology Inc). Discordant samples were further tested using a tie-breaker; CAMSTIC (MINRESI, Yaounde); such samples that were positive with CAMSTIK were considered positive while those tested negative were considered negative. The thick blood film method was used to diagnose infection with malaria parasites; pregnancy and glucose were detected in urine by dipstick (Accurate and Medi – Test Combi 9). Hepatitis B surface antigen was detected by serology (Murex Abbott). Participants with the following outcomes that could affect the serum biochemical parameters were excluded from the study: pregnancy, HIV-positive status, Hepatitis B/C infection, Malaria and Urine glucose. Those diagnosed HIV-positive were orientated to Care and Treatment Centers after appropriate post test counselling and follow-up.

Biochemical Estimation of Reference Ranges

All Serum biochemical parameters were assayed by spectophotometry using kits from various manufacturers as indicated: Serum Aspartate aminotransferase (Orgernon, S.A, Spain), Serum Alanine aminotransferase (Quimica Clinica Aplicada, S.A, Spain), Alkaline phosphatase (Quimica Clinica Aplicada, S.A, Spain), Total and direct bilirubin (Human, Germany), Creatinine (Quimica Clinica Aplicada, S.A, Spain), Total and high density lipoprotein cholesterol (Quimica Clinica Aplicada, S.A, Spain), Triglycerides (Elitech, France), Albumin and Total protein (Human, Germany).

Quality Assurance/Quality Control

To ensure the reproducibility and repeatability of the test results, all pre-analytic, analytical and post-analytical precautions were taken into consideration. For example, all Standard Operating Procedures (SOPs) were reviewed for accuracy, all guidelines for sample collection, processing, storage and handling were strictly adhered to; instruments, personnel and procedure validation were carried out through an internal quality control (QC) program in which several samples were assayed through intra and inter daily laboratory runs with the calculation of standard deviations (SD) and coefficient of variations (CV) before the start of the study. Daily sample runs were usually done with blinded samples whose values were known only to the quality control officer.

Statistical analysis

Statistical analyses were done using STATA version 9.2 (Statacorp., Texas, USA). Means and standard deviations were calculated for normally distributed continuous data. Means were compared using the t-test or the Kruskal Wallis test. Medians and, the 25th and 75th percentiles were also computed for the laboratory parameters. To compare the differences in the medians of sub-groups of the populations, Mann Whitney's U-test was performed. To further quantify the spread of the data for each of the laboratory parameters, the 2.5th and the 97.5th percentiles have been presented.

Ethical Approval

The study was approved by the Ethics committee of the Ministry of Public Health of Cameroon and the Institutional Review Board (IRB) of the Faculty of Medicine and Biomedical Sciences, University of Yaounde [1].

Summary of results

The mean ages, weights and heights of the entire population were 27.6 years, 71.5 kg and 1.7 meters respectively. The males constituted 66.1% (328) while the females constituted 33.9% (168) of the total population. Educational status of the participants was as follows: GCE A/L (25.7%), GCE O/L (32.6%), First school leaving certificate (21.8%), and Bachelors degree (21.0%) (Table I); 71.7 and 28.3% of them were recruited from the urban and rural areas respectively. A total of 576 participants were assessed for eligibility; 75 of them were excluded and 501 were retained for sample analyses for various biochemical parameters (Table II). Apart from HDL cholesterol levels, there were significant differences in biochemical parameters between the urban and rural participants (Table III). There were statistically significant differences between males and females in the following biochemical parameters; AST, ALT, creatinine, albumin, triglyceride, total cholesterol and direct bilirubin; however, values for alkaline phosphatase, total protein, HDL cholesterol and total bilirubin were not significantly different (Table IV). A comparison of ranges obtained in the present study and a similar study conducted in

Rwanda is presented in table V while table VI shows a proposed reference physiological biochemical ranges among adult, healthy Cameroonians.

Discussion

Reference ranges for biochemical parameters are non-existent in Cameroon. Although some of these data exist for several studies, the supposed "normal subjects" were not usually controlled for confounding factors and results were not adequately analyzed and presented in a format that was strong enough to assist various clinical and laboratory scientist when confronted with various problems that needed inferences to the normal population. To overcome these challenges, a sizeable representative population from both the urban and rural areas of Cameroon that met universally established inclusion criteria was used for the study. The demographic data collected (Table 1) truly mirrors information that was obtained during the 2004 nationwide Demographic and Health Survey (DHS) [1], hence we are confident that these reported reference ranges gives a true reflection of the entire adult population of urban and rural areas of Cameroon.

Table I: Demographics Characteristics of participants

Characteristics (N=501)	Mean(SD)
Age (years)	27.6 (8.0)
Weight (kilograms)	71.5 (11.6)
Height (meters)	1.7(0.1)
Sex	n(%)
Male	331(66.1)
Female	170(33.9)
Education	n(%)
General certificate of education, advance level (GCE A/L)	120(25.7)
Bachelors degree	92(21.0)
General certificate of education, ordinary level (GCE O/L)	152(32.6)
First school leaving certificate	102(21.8)
Residential areas	n (%)
Urban	359(71.7)
Rural	142(28.3)

Table II: Sampling

Assessed for eligibility	576
Excluded	75
Eligible for Biochemical analysis	501
Reasons for exclusion	
Pregnancy	11
HIV-positive	36
Hepatitis B/C	9
Malaria	15
Urine glucose	4

Several underlying pathologies were identified that led to exclusion of certain participants from the study; this highlights the need to develop and keep to strict criteria in the determination of normal or reference ranges of various populations (Table II). In the tropical context, there has been some disagreement in relation to the definition of a normal individual because of the fact that in this region, it would be impossible to find an individual who does not harbor at least a single parasite. In line with this, there is the need to look at the clinical pathological implications of these parasites in relation to the parameters in question. For example, an inclusion criterion for a study that seeks to establish normal reference ranges for hematological parameters needs to target parasites that affect the hematological pictures, likewise for biochemical parameters; however, inclusion of parasite density would give clinical laboratory meaning to the results obtained. We combined laboratory information with a good medical history of various participants to select individuals who were eligible for biochemical analysis.

In keeping with reports from similar studies that have been conducted in other countries, this study shows variations in several reference biochemical parameters between healthy Cameroonians by sex and by geographic location (Tables III, IV). As has been mentioned, several factors including sex, age, diet, and geographic location, significantly affect reference physiological parameters in humans [8,9,10,11,12,13,14]. The description of the two localities and their populations show that these are completely distinct entities, thus justifying the significant differences in biochemical parameters amongst the populations. As a quality assurance measure, all necessary pre-analytical, analytical and post-analytical precautions were taken to ensure that these data were not biased or thwarted by any factors. The present study underpins the importance of considering reference ranges in relation to geographic location and sex.

Table III: Analysis of Overall Biochemical Parameters by Residential Area (urban/rural)

Parameter	Mean (SD)		Median		2.5th, 97.5th percentiles		P-value
	Urban (N=359)	Rural (N=142)	Urban	Rural	Urban	Rural	
AST (U/L)	21.3 (7.9)	15.7(5.1)	20.1	14.8	9.6 to 43.6	7.9 to 27.9	0.001
ALT (U/L)	12.4(6.7)	10.6(3.9)	11.1	9.6	5.2 to 31.4	5.7 to 19.7	0.023
Alkaline Phos (U/L)	111.4(51.3)	136.4(62.6)	102.1	122.4	46.9 to 206.1	60.1 to 297.6	0.001
Creatinine (mg/dl)	0.7(0.6)	0.7(0.7)	0.6	0.5	0.2 to 2.1	0.2 to 2.8	0.027
Total Protein (g/l)	71.7(15.8)	79.3(12.6)	70.5	78.3	45.3 to 107.8	58.4 to 103.8	0.001
Albumin (g/l)	43.7(8.4)	51.3(7.8)	43	50.5	29.0 to 60.0	36.3 to 70.2	0.001
Triglyceride (g/l)	1.0(0.8)	1.6(1.0)	0.8	1.3	0.1 to 3.3	0.2 to 4.5	0.001
HDL Chol (mg/dl)	101.7(28.8)	102.3(30.6)	97.0	98.0	55.0 to 172.0	47.2 to 186.0	0.840
Total Chol (mg/dl)	180(50)	220(60)	170	210	90 to 300	120 to 380	0.001
Total Bil (mg/dl)	1.2(0.6)	1.9(0.9)	1.0	1.8	0.4 to 2.7	0.7 to 4.6	0.001
Direct Bil (mg/dl)	0.5(0.3)	0.6(0.3)	0.4	0.6	0.1 to 1.2	0.2 to 1.4	0.001

Table IV: Analysis of Overall Biochemical Parameters by Sex (males/females)

Parameter	Mean (SD)		Median		2.5th, 97.5th percentiles		P-value
	Males (N=331)	Females (N=170)	Males	Females	Males	Females	
AST (U/L)	20.9(7.1)	17.4(7.9)	20.1	15.7	9.6 to 39.9	7.9 to 44.3	0.001
ALT (U/L)	12.5(6.1)	10.7(5.9)	11.1	9.1	5.2 to 29.5	5.2 to 28.5	0.001
Alkaline Phos (U/L)	120.9(61.5)	113.7(42.4)	104.9	106.7	51.7 to 270.2	51.0 to 220.4	0.414
Creatinine (mg/dl)	0.7(0.5)	0.7(0.7)	0.6	0.5	0.2 to 2.2	0.2 to 2.3	0.004
Total Protein (g/l)	73.8(15.6)	74.0(14.9)	71.9	72.0	46.1 to 107.9	47.4 to 102.4	0.921
Albumin (g/l)	45.2(9.1)	47.1(8.4)	44.7	47.2	29.0 to 64.9	30.9 to 67.4	0.028
Triglyceride (g/l)	1.1(0.8)	1.3(1.0)	0.9	1.1	0.1 to 3.4	0.1 to 4.2	0.032
HDL Chol (mg/dl)	100.5(28.8)	104.5(30.1)	97.0	99.5	55.0 to 171.5	53.2 to 172.7	0.153
Total Chol (mg/dl)	180(50)	200(60)	180	190	90 to 310	90 to 350	0.001
Total Bil (mg/dl)	1.4(0.7)	1.3(0.8)	1.3	1.2	0.4 to 3.4	0.3 to 3.2	0.307
Direct Bil (mg/dl)	0.5(0.4)	0.5(0.3)	0.5	0.4	0.1 to 1.3	0.1 to 1.1	0.015

Table V: Comparison between Reference Biochemical Parameters of Cameroonians and Rwandans

Parameter	Site	Mean (SD)		Median		2.5th, 97.5th percentiles	
		Males	Females	Males	Females	Males	Females
AST (U/L)	CMR	20.9(7.1)	17.4(7.9)	20.1	15.7	9.6 to 39.9	7.9 to 44.3
	RWD	31(19.3)	22 (8.1)	26	21	16 to 19	13 to 43
ALT (U/L)	CMR	12.5(6.1)	10.7(5.9)	11.1	9.1	5.2 to 29.9	52.9 to 28.5
	RWD	31 (19.3)	21 (11.4)	22	19	9 to 58	8 to 48
Alkaline Phos (U/L)	CMR	120.9(61.5)	113.7(42.4)	104.9	106.7	51.7 to 270.2	51.0 to 220.4
	RWD	74 (17.6)	91 (33.8)	73	89	48 to 116	43 to 187
Creatinine (mg/dl)	CMR	0.7(0.5)	0.7(0.7)	0.6	0.5	0.2 to 2.2	0.2 to 2.3
	RWD	0.8 (0.2)	0.7 (0.2)	0.8	0.7	0.5 to 1.2	0.4 to 1.1
Total Protein (g/l)	CMR	73.8(15.6)	74.0(14.9)	71.9	72.0	46.1 to 107.9	47.4 to 102.4
	RWD	70 (7.3)	70 (7.9)	69	70	57 to 84	54 to 86
Albumin (g/l)	CMR	45.2(9.1)	47.1(8.4)	44.7	47.2	29.0 to 64.9	30.9 to 67.4
	RWD	43 (3.9)	42 (4.2)	42	41	36 to 50	35 to 52
Triglyceride (g/l)	CMR	1.1(0.8)	1.3(1.0)	0.9	1.1	0.1 to 3.4	0.1 to 4.2
	RWD	NA	NA	NA	NA	NA	NA
HDL Chol (mg/dl)	CMR	100.5(28.8)	104.5(30.1)	97.0	99.5	55.0 to 171.5	53.2 to 172.7
	RWD	NA	NA	NA	NA	NA	NA
Total Chol (mg/dl)	CMR	180(50)	200 (60)	180	190	90 to 310	90 to 350
	RWD	NA	NA	NA	NA	NA	NA
Total Bil (mg/dl)	CMR	1.4(0.7)	1.3(0.8)	1.3	1.2	0.4 to 3.4	0.3 to 3.2
	RWD	0.8 (0.5)	0.7 (0.3)	0.8	0.5	0.4 to 1.9	0.2 to 1.4
Direct Bil (mg/dl)	CMR	0.5(0.4)	0.5(0.3)	0.5	0.4	0.1 to 1.3	0.1 to 1.1
	RWD	0.2 (0.1)	0.2 (0.1)	0.2	0.1	0 to 0.6	0 to 0.4

CMR= Cameroon (Present study)
RWD= Rwanda (Kayitenkore et al, 2005)

Table VI: Proposed Reference Physiological Ranges for Plasma Biochemical Parameters among Adult Healthy Cameroonians

	Urban		Rural	
	Males	Females	Males	Females
Parameter	2.5th to 97.5th percentiles	2.5th to 97.5th Percentiles	2.5th to 97.5th Percentiles	2.5th to 97.5th percentiles
AST (U/L)	10.5 to 30.5	7.9 to 49.9	8.3 to 28.8	7.9 to 24.4
ALT (U/L)	5.2 to 31.6	5.1 to 36.5	5.5 to 18.1	5.1 to 36.6
Alkaline Phos (U/L)	49.4 to 233	41.4 to 183	60.1 to 297.6	60.1 to 297.6
Creatinine (mg/dl)	0.2 to 3.4	0.2 to 2.4	0.2 to 3.4	0.2 to 2.4
Total Protein (g/l)	45.2 to 108	42.9 to 102	58.4 to 103.8	58.4 to 103.8
Albumin (g/l)	29.0 to 61.7	30.1 to 58. 8	36.0 to 76.4	37.7 to 67.5
Triglyceride (g/l)	0.1 to 3.4	0.1 to 2.4	0.1 to 5.0	0.2 to 4.7
HDL Chol (mg/dl)	54.5 to 172.0	54.5 to 172.0	54.5 to 172.0	54.5 to 172.0
Total Chol (mg/dl)	90 to 290	80 to 320	110 to 390	130 to 460
Total Bil (mg/dl)	0.4 to 2.8	0.3 to 2.4	0.6 to 5.1	0.7 to 3.6
Direct Bil (mg/dl)	0.1 to 1.2	0.3 to 2.4	0.1 to1.5	0.1 to 1.2

The liver participates in a variety of metabolic activities such as detoxification and excretion of vaccines and drugs. Hence, any abnormal concentrations or constituents of these substances given to subjects will affect the liver's architecture and be presented in the serum or plasma as abnormal levels of its metabolites such as enzymes and bilirubin. Statistically significant differences were observed in the serum levels of the liver aminotransferases (AST, ALT), alkaline phosphatise and bilirubin by sex and geographic located (Table III, IV). These are very important markers for assessing the functional integrity of the liver [7] hence the interpretation of their levels in the context of drug administration or vaccines should be done accordingly. Creatinine, a serum metabolic product of muscle creatine phosphate is the principal indicator of renal function. Being a product associated with the muscles, it usually shows sex differentials with women having lower levels than men due to lower body mass indexes [5,8,13]. The present study confirms this trend with both urban and rural males having significant higher levels of serum creatinine. Serum total proteins has in general been used to assess the state of hydration and nutritional status of individuals, hence its variations will usually reflects peoples' lifestyles and habits [12,13]. The significantly higher protein levels observed in rural as compared to urban participants (Table III) are probably due to differences in their dietary habits with rural inhabitants having the opportunity of eating their traditional foods that may contain more proteins than the usual expensive artificial urban diets. A similar pattern was observed for serum albumin (Table III), a protein with a half life of 19 days usually used to differentiate cases of acute and chronic liver failures.

Albumin also serves an additional function as a binding and transport protein for several substances including bilirubin and hormones [22]; hence its serum levels needs to be interpreted in relation to the concentrations of these binding substances. Rural participants had higher mean serum bilirubins (Table III) than their urban counterparts, thus confirming the observed serum albumin patterns. Several factors will influence changes in serum lipid levels. These include diet, sex, age, and lifestyle [8,9,10,11,12]. These could partly explain the significant and specific differences observed in both triglyceride and total cholesterol levels in the present study; however, the High Density Lipoprotein (HDL) cholesterol levels were similar in all categories (Tables VI).

A literature search shows that there have been a lot of limitations on data generated in similar studies from other African countries. For example, sample sizes have been very limited, inclusion and exclusion criteria were poorly defined, and sex and residential identities were sometimes not highlighted. A very important weakness of several of these studies has also been the statistical analyses. Several biochemical parameters particularly the enzymes and lipids do not show Gaussian distribution; hence skewed data from these parameters can only be analysed and presented using non-parametric statistics. This has not been the case for several studies which imply that either data were poorly collected or several acceptable variables were erroneously eliminated in order to conveniently use parametric statistics in presenting the results. The only available study with similar methodology to the present study and whose results could be compared

within the African regions is that of Kayitenkore et al [13] from Rwanda (Table V). The data so presented shows similar patterns to those of the present study with variability in ranges that could be ascribed to differences in geographic region with particular underlying physiological factors that could influence their reference biochemical ranges. Other studies though not strongly comparable have also shown variations between reference ranges [15, 23,24].

Proposed reference ranges for biochemical parameters among healthy adult Cameroonians based on data generated from this study are presented in table VI. These ranges should be applied for various studies and research activities in Cameroon that apply strictly to these populations.

It is concluded that sex and geographic location have significant impact on reference physiological biochemical parameters of healthy, adult Cameroonians. Differences exist in reference ranges among adult, healthy Cameroonians and populations from similar Africa countries. There is need for similar studies that target the Paediatrics, Adolescent and Geriatric populations in Cameroon and other African countries.

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