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International Journal of Health Research, September 2009; 2(3): 259-266

Available at http://www.ijhr.org

Original Research Article

Open Access
Online Journal

Reference Ranges for Some Biochemical Parameters in Adult Kenyans

Received: 23-Feb-09 **Revised:** 13-May-09 **Accepted:** 31-May-09

Abstract

PURPOSE: To establish the reference ranges of some biochemical parameters for adult Kenyan population.
METHODS: In a prospective involving 1100 healthy blood donors (age: 18-55 yr) in Kenyatta National Hospital, Kenya reference ranges of some biochemical analytes were constructed by using the parametric methods to estimate 2.5 and 97.5 percentiles of distribution.
RESULTS: The reference ranges of the analytes were: alanine aminotransferase (ALT) [males (0-39) U/L, females (0-34) U/L]; aspartate aminotransferase (AST) [males (6-40) U/L, females (3-37) U/L]; alkaline phosphatase (ALP) [males (13-201) U/L, females (5-227) U/L]; albumin (ALB) [males (29-52) g/L, females (28-50) g/L]; protein (PROT) [males (57-89) g/L, females (56-88) g/L]; creatinine (CREAT) [males (59-127) µmol/L, females (54-122) µmol/L]; glucose (GLU) [males (2.8-6.8) mmol/L, females (2.6-7) mmol/L]; phosphorus (PHOS) [males (0.5-2.0) mmol/L, females (0.2-2.4) mmo/L]; potassium (POT) [males (3-5.3), females (3.1-5.1) mmo/L]; sodium (SOD) [males (111-153) mmol/L, females (117-151) mmol/L]; Blood urea nitrogen BUN [males (1.5-5.9) mmol/L, females (1.2-6.0) mmol/L] and Uric acid (UA) [males (120-458) µmol/L, females (89-415) µmol/L]. Age differences in the established reference ranges were observed in ALT, ALB, CREAT, ALP and UA in males and in ALT, ALB, and CREAT in females. Gender differences were observed in ALT, AST, ALB, CREAT and UA in the 18-28 yr old, ALT, AST, ALB, SOD and UA in 29-39 yr old and AST, ALB, and UA in 40-50 yr old.
CONCLUSION: Age and sex specific reference ranges of some biochemical parameters were established some of which were different from those reported in literature. There therefore the need for each clinical chemistry laboratory to establish its own ranges.

Keywords*:* Reference range, Biochemical parameter, Adult Kenyan, Kenyatta National Hospital*.*

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Introduction

A reference range of a clinical chemistry parameter is a set of values used in the interpretation of a clinical chemistry report. There are two types of reference ranges categorized as subject based and group based. When doing a follow up on patients, a clinician often use a subject-based reference range to determine the progress made in the management of a pathological disorder. To establish whether a patient has a certain pathological disorder however, group-base reference range is used in the interpretation of laboratory report [1].

In clinical management of patients, physicians rely on blood chemistry analytes for accurate diagnosis, proper treatment and follow-up of patients. Correct interpretation of the results from these analytes presupposes that the clinician and the laboratory medicine physician have good reference information. Published reference ranges in literature do not sometimes represent adequately the specific population from which the patient comes from based on age, sex, genetics, diet, and altitude. In addition, reference ranges produced by reagent manufacturers are determined from analysis of blood samples of a few health workers who do not represent the general population. Reference information is often the weakest data provided by clinical laboratories even though such data is very useful for the correct and proper interpretation of laboratory results. It is therefore recommended that each clinical chemistry laboratory establish its own reference range for biochemical parameters [2].

There are no published reference ranges for biochemical parameters for Kenyan adult population. Clinical Chemistry Laboratory of Kenyatta National Hospital relies on reagent manufacturers' published reference ranges which may not adequately represent Kenyan adult population. The objective of this study was therefore to establish reference ranges for some biochemical parameters for adult Kenyan population.

Materials and Methods

Selection of study sample

Following approved by the Kenyatta National Hospital Research and Ethical Committee (Reference KNH-ERC/01/12O6), 1100 randomly selected blood donors' (aged 18-55 yr) were recruited for the study. Sample size was determined as previously reported [3,7,9,13]. The inclusion/exclusion criteria were: Kenyan citizen, 18-55 years, not obese, not hypertensive (blood pressure was taken using sphygmomanometer and only those subjects with a BP <120/80 mm Hg were included in the study), not pregnant, not involved in any excessive exercise, not under any medication, not taking any oral contraceptives (female subjects), and none alcohol and tobacco users. Blood samples (5 ml) collected from the subjects were used for the screening of the subjects for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), hepatitis C virus and syphilis (VDRL) using standard procedures. These subjects were subsequently followed up and excluded from the study on detection of any abnormalities. Those who met the inclusion criteria were requested to give consent for their blood to be used in the study. A questionnaire was administered to consenting study subjects.

Determination of reference standards

Blood (200 ml) was allowed to flow down the pilot tube so as to clear any anticoagulant along the walls of the pilot tube. Five millilitres of blood for biochemical analysis was drawn from the pilot tube of the blood bag using a sterile needle connected to a 5 ml syringe and divided into two volumes: 3 ml was put into a plain bottle and the remaining 2 ml was put in a fluoride bottle for glucose analysis. The two specimens collected were centrifuged at 3000 rpm for 5 min and the serum (S) and plasma (P) obtained were separated and stored at –20 °C. Plasma separated from the fluoride bottle was used for glucose analysis. The

Table 1: Accuracy of the assay methods used in the determination of all the twelve analytes in the Quality Control (QC) sample (Multi Sera) using Beckman Synchron System CX5.

Analyte (unit)	Method	Assigned QC value Mean ± SD (coefficient of variation, CV %)	QC value Mean ± SD (CV%)
ALT (u/l)	Standard method IFCC (enzymatic rate), kinetic at 340 nm.	42 ± 7 (16.7)	36.4 ± 4.2 (11.4)
AST(u/l)	Standard method IFCC (enzymatic rate), kinetic at 340 nm.	39 ± 6 (15.4)	36.4 ± 3.2 (8.8)
ALP (u/l)	p-nitrophenol, AMP*, kinetic at 410 nm.	124 ± 19 (15.3)	134.3 ± 22.9 (17.5)
PROT (g/l)	Biuret, end point reaction at 560 nm.	58 ± 8.5 (14.7)	56 ± 5.1 (9.1)
ALB (g/l)	Bromocresol green(BCG), end point reaction at 600nm.	39 ± 6 (15.4)	38.5 ± 2.9 (7.6)
CREAT (µmol/l)	Modified rate Jaffe, end point reaction at 520 nm.	164 ± 25.2 (15.4)	173.1 ± 9.8 (5.6)
GLU (mmol/l)	Enzymatic (hexokinase), timed end point reaction at 340 nm.	6.0 ± 0.5 (8.3)	5.9 ± 0.3 (5.4)
PHOS (mmol/l)	Phosphomolybdate timed end point reaction at 340 nm.	1.47 ± 0.22 (15)	1.5 ± 0.17 (11.2)
SOD (mmol/l)	Ion selective electrode (ISE)	139 ± 21 (15.1)	139.5 ± 5 (3.6)
POT (mmol/l)	Ion selective electrode (ISE)	4.2 ± 0.63 (15)	4.2 ± 0.21 (5.0)
BUN (mmol/l)	Enzymatic (urease), kinetic at 340 nm.	6.45 ± 0.98 (15.2)	7 ± 0.79 (11.2)
UA (µmol/l)	Enzymatic (uricase/peroxidase), end point reaction at 520 nm.	330 ± 50 (14.9)	360 ± 24.9 (6.9)

Results are expressed as Mean ± standard deviation (SD), AMP(2-amino-2-methylpropanol*.

collected sera were analysed for sodium (SOD), potassium (POT), blood urea nitrogen (BUN), creatinine (CREAT), uric acid (UA), inorganic phosphorus (PHOS), total protein (PROT), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) using Synchron System CX5 at 37 ^{o}C using established techniques summarized in Table 1. To ensure accuracy and precision, calibration procedure for non-enzymatic chemistries was carried out using multicalibrator sera. Enzymatic chemistries were not calibrated since they are factory calibrated. Quality control checks were kept daily using Multisera normal (Randox Laboratories Ltd) and all values for all the analytes were within ± 2SD of their target mean for all the 33 days of laboratory analysis. Quality Control (QC) assayed Multisera normal was supplied in lyophilized form (sealed under vacuum). On the day of use, the vial was opened and reconstituted by adding accurately measured five milliliters of freshly distilled water at 20 – 25 ^{o}C.

Data Management and Statistical Analysis

Data were categorised by sex and age as appropriate and each parameter was then examined as a histogram and as a normal probability plot. Shapiro-Wilks W-test was performed with significance at p=0.05 level to examine the fit of the observed distribution. Since the results of the Shapiro-Wilks W-test were not significant ($p > 0.05$), the data was considered to have a Gaussian distribution. Age ranges chosen were 18-28; 29-39; and 40-50 years, respectively. A reference

interval for each parameter was calculated from the arithmetic mean ± 1.96 SD to obtain the 2.5% and 97.5% limits. Student's T-test was used to assess significance of means between sexes and ANOVA and post-ANOVA test was used for multiple comparisons of means among age ranges.

Results

Reference ranges for the biochemical analytes ALT, AST, ALP, ALB, CREAT, GLU, PHOS, POT, SOD, PROT, BUN and U.A were established separately for the 762 adult males and 338 adult females. Table 1 shows the accuracy of the assay methods used in the determination of all the twelve analytes in the quality control sample (MultiSera Normal) using Beckman Synchron system CX5. These values were in close agreement with the assigned value. Table 2 shows the established sex specific reference ranges for some biochemical parameters in adult Kenyans and their comparison with Kuwait, Rwanda and American populations quoted in literature. Results show statistically significant higher values for males than females for alanine aminotransferase (ALT) (p=0.000), aspartate aminotransferase (AST) (p=0.000), albumin (ALB) (p=0.000), creatinine (CREAT) (p=0.000) and uric acid (UA) (p=0.000) and lower values than those for females for alkaline phosphatase (ALP) (p=0.003).

The effect of age and sex on the biochemical parameters are giben in Table 3. Results shows that males in age range 18-28 yr have a significantly higher value for ALT than those in age range 29-39 yr (p=0.003), and both males (p=0.032) and females (p=0.019) in age ranges 40-50 yr have significantly higher values for ALT than those in age range 18-28 yr. In addition, males in age range 29-39 yr have significantly lower ALP value than those in age range 18-28 yr (p=0.001) while the males in age range 18-28 yr have a significantly lowered ALB value than those in age range 29-39 yr (p=0.019). Both males (p=0.041) and females (p=0.007) in age range 40-50 yr have a significantly lowered ALB value than those in age range 18-28 yr. Further more, the males in the age range 29-39 yr have a significantly increased creatinine value than those in age range 18-28 yr (p=0.013). Females (p=0.045) in age range 40-50 yr have significantly increased creatinine value than those of age range 18-28 yr. Males in age range 40-50 yr have significantly increased UA values than those for age ranges 18-29 yr (p=0.002) and 29-39 yr (p=0.007) respectively. Males have significantly higher biochemical values than females in the following age groups: ALT(18-28 yr (p=0.000)) and 29-39 yr (p=0.01), AST (18-28 yr (p=0.000) and 29-39 yr (p=0.01), ALB(18-28 yr (p=0.002), 29-39 yr (p=0.005), 40-50 yr (p=0.02), UA (18-28 yr (p=0.000), 29-39 yr (p=0.003), 40-50 yr (p=0.005) and CREAT(18-28 yr (p=0.000). On the other hand, males have significantly lower biochemical values than females in age group 29-39 yr (glucose (p=0.005), phosphate (p=0.039) and sodium (p=0.04).

Discussion

Correct interpretation of biochemical data for patients is critical in the correct diagnosis of several illnesses. In adult Kenyans, accurate reference ranges of these biological data that closely relate to the patients under investigation has not been reported previously and diagnosis is often based on data obtained from subjects/patients outside Kenya. This study reports age and sex specific reference ranges for some routinely requested for blood chemistry analytes from healthy Kenyans aged 18 to 55 yr. The significantly higher reference range values for males compared to those of females in the established reference ranges for the Kenyan adult population for alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), creatinine (CREAT) and uric acid (UA) were also observed in the reference ranges in Kuwait population [3]. Higher reference ranges for males compared to those of females were also observed for ALT and UA for the American adult population quoted in literature [4-6], In Switzerland adult

Table 2: Established reference ranges for some biochemical parameters in adult KENYANS and their comparison with the Kuwait, Rwandan, and American adult population quoted in literature

Analyte (units)	Sex	N	Kenyann Mean±SD	Kenyann range (KR)	Kenyan Interval (KI)	Kuwaits Mean±SD	*[1]Kuwaits Range (KR)	*[1]Kuwaits Interval (KI)	Rwanda Mean±SD	Rwanda Range (RR)	Rwanda Interval (RI)	American Range (AR)	American Iinterval (AI)
ALT (U/L)	M	760	18.9±10.5*	0 – 39	39	32±36*	0.0-102.6	102.6				10-40	30
	F	338	15.9±9.1	0 – 34	34	19±10	0.0-38.6	38.6				7-35	28
AST (U/L)	M	760	23.5±8.6*	6 – 40	34	23±5*	13.2-32.8	19.6				10-42	32
	F	338	19.8±8.4	3 – 37	34	21±5	11.2-30.8	19.6				10-42	32
ALP (U/L)	M	762	107.3±47*	13 – 201	188	64±16	32.6-95.4	62.8				32-92	60
	F	337	116.6±55.6	5 – 227	222	64±16	32.6-95.4	62.8				32-92	60
PROT (g/L)	M	762	72.5±8.0	57 – 89	32	71±4	63.2-78.8	15.6	73±5.6	62-84	22	64-83	19
	F	338	72.1±8.1	56 – 88	32	72±4	64.2-79.8	15.6	73±6.1	61-85	24	64-83	19
ALB (g/L)	M	762	40.7±5.8*	29– 52	23	42±3*	36.1-47.9	11.8	43±4.6	34-52	18	35-50	15
	F	338	39.4±5.5	28 – 50	22	40±3	34.1-45.8	11.7	41±4.6	32-50	18	35-50	15
GLU (mmol/L)	M	746	4.8±1.0	2.8 – 6.8	4.0	4.83±0.59	3.7-6.0	2.3				2.7-8.3	5.6
	F	318	4.8±1.1	2.6 – 7.0	4.4	4.91±0.58	3.8-6.0	2.2				2.7-8.3	5.6
PHOS (mmol/L)	M	751	1.21±0.38	0.5 – 2.0	1.5	1.13±0.14	0.9-1.4	0.5	1.13±0.23	0.67-1.59	0.92	0.83-1.48	0.65
	F	330	1.28±0.55	0.2 – 2.4	2.2	1.14±0.14	0.9-1.4	0.5	1.17±0.18	0.82-1.52	0.70	0.83-1.48	0.65
POT (mmol/L)	M	762	4.1±0.58	3.0 – 5.3	2.3	4.15±0.33	2.5-4.8	2.3	4.0±0.5	3.0-5.0	2.0	3.6-5.0	1.4
	F	338	4.1±0.5	3.1 – 5.1	2.0	4.20±0.37	3.5-4.9	1.4	4.0±0.5	3.0-5.0	2.0	3.6-5.0	1.4
SOD (mmol/L)	M	762	132.3±10.8	111-153	43	141±3	135.1-146.9	11.8	139±4	131-147	16	135-145	10
	F	338	134.0±8.6	117 – 151	34	140±3	134.1-145.9	11.8	140±7	126-154	28	135-145	10
CREAT (µmol/L)	M	762	93.0±17.0*	59 – 127	68	89±13*	63.5-114.5	51				53-115	62
	F	338	88.0±17.0	54 – 122	68	74±12	50.5-97.5	47				53-115	62
BUN (mmol/L)	M	750	3.7±1.1	1.5 – 5.9	4.4	5.03±0.85	3.4-6.7	3.3				2.5-6.4	3.9
	F	320	3.6±1.2	1.2 – 6.0	4.8	4.51±1.09	2.4-6.7	4.3				2.5-6.4	3.9
UA (µmol/L)	M	761	289.0±86.0*	120 – 458	338	322±52*	220.1-423.9	203.8				155-428	273
	F	338	252.0±83.0	89–415	326	269±58	155.3-382.7	227.4				120-360	240

*Results are expressed as means (X) ± standard deviation (SD). The reference range for each analyte was calculated as the minimum and maximum value of 95% of the study subjects using the formula mean (X) ± 1.96 standard deviation (SD). *Reference range for biochemical parameters in American healthy adult population[4] ; Kuwait's adult population [3]; Rwandan adult population [8]*

Table 3: Age and sex specific reference ranges for Kenyan adults

Analyte (unit)	Age Ranges (yr)						
	18-28			29-39		40-50	
	Sex	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
ALT(iu/L)	M	401	17.6±9.6*	268	20.2 ± 0.6[a]*	80	20.1 ± 10.3[b]
	F	182	14.6±8.1	110	17.1 ± 10.0	46	18.4 ± 9.4[b]
AST(iu/L)	M	401	23.6±8.9*	268	23.4 ± 8.4*	80	23.2 ± 7.7*
	F	182	19.7±7.9	110	19.8 ± 8.9	46	20.1 ± 9.4
ALP(iu/L)	M	401	11l.9±46	268	98.7 ± 44[a]	80	101.2 ± 38.6
	F	182	119.1±57	110	112.5 ±52.6	46	112.3 ±46.4
ALB(iu/L)	M	401	41.5±5.5*	268	40.1 ± 5.6[a]*	80	40 ± 5[b]*
	F	182	40±5.1	110	39.4 ± 5.5	46	37.9 ± 4.5[b]
CREAT	M	401	91.2±16.5*	268	94.9±16.6[a]	80	95.5±19.1
(µmol/L)	F	182	85.5±15.5	110	89.3±18.6	46	92.1±16.9[b]
GLU(mmol/L)	M	401	4.8±1.0	268	4.7±1.1*	80	4.7±0.89
	F	182	4.8±1.0	110	4.8 ± 1.1	46	5.1 ± 1.4
PHOS(mmol/ll	M	401	1.25±0.41	268	1.2± 0.34*	80	1.2± 0.36
	F	182	1.25±0.32	110	1.3 ± 0.34	46	1.2 ± 0.37
POT(mmol/L)	M	182	4.1±0.59	268	4.2 ± 0.54	80	4.1± 0.5
	F	182	4.1±0.48	110	4.1± 0.58	46	4.2± 0.5
	M	401	132.4±10.5	268	132.3± 8.8*	80	133.4± 7.9
SOD(mmol/L)	F	182	133.9±8.6	110	134.3 ± 8.1	46	133.8 ± 9.5
	M	401	73.1±6.7	268	72.3 ± 7.8	80	72.2 ± 7.2
PROT(g/L)	F	182	72.7±7.4	110	72.2 ± 7.8	46	71.6 ± 5.0
	M	401	3.7±1.1	268	3.8 ± 1.1	80	3.8 ± 1.1
BUN(mmol/L)	F	182	3.5±1.1	110	3.7 ± 1.1	46	3.8 ± 1.3
	M	401	284±76*	268	288 ± 93*	80	322 ± 99[bc]*
U.A(µmol/L)	F	182	244±82	110	257 ± 85	46	273 ± 80

*Results are expressed as mean±SD for number of subjects indicated in the column labelled N. * stands for significant differences between male and female subjects in the same age range. [a] represents significant difference of each analyte between age range 18-28 yr and 29-39 yr for same sex; [b] represents significant differences of each analyte between age range 18-28 yr and 40-50 yr for same sex; [c] represents significant differences in each analyte between age range 29-39 yr and 40-50 yr for same sex*

population, BUN data (male mean (SD): 6.3(1.2) mmol/L, reference range 3.9-8.7mmol/L; female mean (SD): 5.2(1.2) mmol/L, reference range 2.8-7.6mmol/L); and CREAT (male mean (SD): 88(15) µmol/L, reference range 58.6-117.4 µmol/L; female mean (SD): 74(9.5) µmol/L, reference range 55.4-92.6 µmol/L) have been reported [7] Higher reference ranges for males compared to those of females were also observed in Rwandan adult population for phosphates while lower values were observed for sodium [8]. Established adult Kenyan male reference ranges also differs from those of Saudi male adult population (reference range for: ALT 0-63 U/L; AST 11-46 U/L; ALP 3.4-14.9 U/L; PROT 62-80g/L; ALB 43-57g/L; GLU 3.3-7.1mmol/L; CREAT 53-124 µmol/L; BUN 1.2-3.7 mmol/L; UA 214-500 µmol/L) [9]. Observed differences of these established reference ranges of Kenyan adult population compared to those of other adult population could be due to differences in either the lower reference limit or the upper reference limits or both [4]. Differences in the lower and upper reference limits could be due to differences in the geographical location, methods and equipments used, sample size, posture, race, regional differences in the

dietary intakes of foods rich in these analytes, and genetics [5, 10-12].

Significantly higher reference range of creatinine in males relative to that of females with increasing age range in this study agrees with similar observation of Gardner and Cott [13]. Increase in creatinine with the advancement of age for both sexes could be due to muscle degradation with age. Higher creatinine levels in males than in females may be due to the greater muscle mass in males than females [14]. The higher UA in males relative to females could be explained by the higher clearance rate in females than in males [15]. Significant increase in UA in the fourth decade in males relative to the second decade could be explained by the loss of estrogen [13]. Lower ALP levels observed in males compared to females indicate that this enzyme varies with sex [16]. That this differs with the similar values for males and females observed with the Kuwait [3] and American adult population [4] could suggest that ALP value depends on the laboratory method used (temperature and substrate used in the test reaction) (Sacher et al., 1991). Decrease of ALP with advancing age for both sexes indicates that ALP levels are age dependent [16]. Lower ALB levels in females compared to males indicate that the synthesis of ALB is sex dependent. This lower level of ALB in females compared to males in the Kenyan population has also been observed with the Kuwait [3] and Rwandan adult population [8] but not in the American adult population [4] which has similar values for ALB for both sexes. ALB decrease with increase in age suggests a decrease in its synthesis in the liver with advancement with age. ALB is involved in the transportation and storage of cortisol, sex hormones, and calcium which decrease with age [17]. Increase of ALT with advancing age in both males and females suggests that ALT levels are age dependent. This age dependence of ALT was not demonstrated in the Kuwait adult population [3].

Conclusion

The study has established reference ranges for twelve routinely analyzed biochemical parameters for the adult Kenyan population. These reference ranges are different from those quoted in literature for other geographical regions. It is hoped that the results of this study will stimulate the establishment of reference ranges for other biochemical parameters in Kenyan population.

Acknowledgements

Study was financed by Kenyatta National Hospital (KNH). We thank the Director of KNH and the Head of Department of Laboratory Medicine KNH, for their support. We gratefully acknowledge the contribution made by Moses Karanja, Leonard Njagi, Wilfred Gatua, Gladys Wanja Mugo, Mercy Muriuki, Jacob Wekoto.and Dr Simon Kanga.

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