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ORIGINAL ARTICLE

Reference Values for Some Renal Function Parameters for Adult Population in North-Rift Valley, Kenya

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Abstract A population based, cross-sectional study was carried out at Moi Teaching and Referral Hospital in collaboration with the Regional Blood Transfusion Center, North Rift. 367 participants (211 males and 156 females) were involved in the renal function reference range establishment. Reference ranges were constructed using non-parametric methods to estimate 2.5 and 97.5 percentiles of distribution as lower and upper reference limits, respectively. Results showed significant sex and age specific reference values in some of the established renal function parameters. North Rift Kenyan population clinical chemistry reference ranges differ from the American values commonly used in Kenyan Hospitals. The renal function reference values established in this study some of which are sex and age specific can be adopted for the North Rift Kenyan population.

Keywords Reference values · Age · Sex · Renal function · Adults · North rift valley

Introduction

Measured laboratory parameters are influenced not only by individual factors such as age, sex, and lifestyle, but also by population and ecological factors such as ethnicity, climate, and altitude; they vary not only between individuals but also populations [1]. Laboratory reference values for populations from western countries are widely published in scientific literature [2], textbooks and on the worldwide web. However, there is a general lack of published data regarding laboratory parameters for populations living in tropical sub-Saharan Africa [3] and the few studies that have been undertaken have indicated differences in reference values of African populations compared to those derived from western populations [3–6]. These differences suggest the need for the development of locally derived reference values. Reference values are used to aid in interpreting results of laboratory measurements, clinical trials screening and as the basis of safety monitoring for trial participants [3, 5, 6]. These reference values are used to assess health in humans and are based on the effective performance of the major body organs such as the liver, pancreas, heart and kidney. Kidney function tests comprise a variety of individual tests and procedures that can be used to evaluate how well the kidney functions. These tests help to determine if the kidneys are performing their tasks adequately. The aim of this study was to establish reference values for renal function tests and to determine possible differences between published and local reference ranges.

Materials and Methods

Study Area

This study was undertaken at Moi Teaching and Referral Hospital (MTRH), Eldoret environs with collaborative arrangement of Regional Blood Transfusion Centre—North Rift. Eldoret is the capital of the North Rift Valley region of Western Kenya, a rich farming highland approximately 2100 m above sea level.

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Participants

The reference population consisted of healthy volunteer blood donors aged 18–50 years old who had been living in the North Rift valley region for at least the past 6 months. Participants came from different parts of the North Rift Valley region. After written informed consent, participants were interviewed through a questionnaire. Those who did not meet criteria for participation were excluded from the study.

Ethical Consideration

Ethical approval was obtained from Moi Teaching and Referral Hospital and Moi University Review and Ethical Committee and permission to use the regional blood donor facility granted by the Ministry of Health, Kenya.

Inclusion and Exclusion Criteria

Healthy males and females between 18 and 50 years who had stayed in North Rift Valley region of Kenya for not less than 6 months were included in the study. Donor serum samples from people on any form of medication, oral contraceptives, smokers and those with chronic diseases such as tuberculosis, diabetes mellitus, hypertension, chronic renal failure were excluded from the study. All donor serum samples which tested positive for HIV antibody, Hepatitis B surface antigen, Hepatitis C antibody and Syphilis were excluded from the study data set. Also excluded were donor serum samples from pregnant women and male and female blood donors who did not consent to participate in the study.

Initial screening for anti HIV-1 antibody was conducted using Genetic Systems rLAV ELISA (BioRad Laboratories, Redmond, WA). Reactive samples were repeated in duplicates using Vironostika HIV-1 Microelisa Systems (Organon Teknika, Durham, North Carolina). Samples repeatedly reactive by both ELISAs were tested using Genetic Systems Confirmatory Assay 3.0 (BioRad Laboratories, Redmond, WA). Screening for Hepatitis B surface antigen (HBsAg) was performed using the Genetic Systems HBsAg EIA 3.0 (BioRad Laboratories, Redmond, WA). Repeatedly reactive samples were confirmed using the Genetic System Confirmatory Assay 3.0 (BioRad Laboratories, Redmond, WA). Screening for anti-Hepatitis C antibody was performed using the Ortho HCV version 3.0 ELISA Tests System. Repeatedly reactive samples were tested in the Chiron RIBA HCV 3.0 SIA (Chiron Corporation, Emeryville, CA). Serum pregnancy testing was performed on all females using Wampole PreVue HCG cassettes (Wampole Laboratories, Inc Dist., Princeton, NJ). Syphilis testing was performed using the Wampole Laboratories Impact RPR (Wampole Laboratories, Princeton, NJ, USA).

Specimen Collection

Blood from suitable blood donors was the specimen of choice and collection was done during the day between the months of August and December 2007. Three hundred milliliters of blood was allowed to flow into the blood bag to clear any anticoagulant along the wall of the pilot tube. Ten millilitres of blood was then sampled from the sampling pot along the pilot line of the blood bag during donation using vacutainer needles and plain vacutainer tubes. Once the specimens were acquired, they were labeled with the donor and study numbers. After bleeding each participant, about 5 ml of blood from the blood bag was collected and used to screen for human immunodeficiency virus (HIV), Hepatitis B surface antigen (HBsAg), Hepatitis C virus (HCV), Syphilis (VDRL) and pregnancy.

Specimen Transportation, Processing and Storage

Specimens were transported from the donation centre to the processing laboratory in ice packed cool boxes within 1 h. Once clotted, the blood specimens were centrifuged at 3000 revolutions per minute for 2 min and serum separated immediately into labeled cryovials in duplicate. Serum specimens were then stored at $-70°C$ awaiting laboratory analysis at AMPATH clinical laboratory at MTRH.

Quality Control (QC)

This study incorporated internal QC materials from Roche diagnostics; Precinorm and Precipath which were run daily and external QC materials from American Proficiency Institute (API) which monitors the performance and participation of MTRH and AMPATH laboratories were run twice during the analytical period according to the manufacturers' instructions and QC protocols. During the entire analytical period, everyday control value result and the standard deviation (SD) from the control target value were noted. All the daily QC runs were within $\pm 2SD$ from the target values.

Laboratory Management and Statistical Analysis

Nine renal function parameters; urea (BUN), creatinine (CREAT), sodium (NA), potassium (K), chloride (CL), calcium (CA), inorganic phosphorus (PHOS) and uric acid (UA), were determined on the sera specimens. All the assays were performed based on the standard operating procedures written and maintained in the AMPATH modular laboratory. Cobas Integra® 400 plus automatic

Chemistry Analyzer (Roche Diagnostics, Mannheim, Germany) was used.

The data was visually inspected for extreme values and ten values for single parameters that appeared physiologically impossible removed. Outliers in the remaining data were identified using Box plots, a procedure proposed by Horn and Pesce [7]. The first quartile (Q_{25}), the median ($Q_{.50}$) and third quartile ($Q_{.75}$) were calculated, that is, the interquartile range (IQR) by subtracting the first quartile from the third quartile ($Q_{.75}-Q_{.25}$). Any data observation which lay more than 1.5 × IQR lower than the first quartile or 1.5 × IQR higher than the third quartile was considered an outlier and manually deleted from the data. The above exclusions and missing results for some parameters led to different sample sizes for each parameter.

A reference interval is defined as the interval between and including two numbers, the upper and lower limit which are estimated to enclose a specified percentage usually 95%. Since more than half of the measured parameters did not follow a Gaussian probability curve according to Kolmogorov–Smirnov (a) and Shapiro–Wilk tests for normality, non parametric statistical methods were therefore used as per the CLSI guideline [1].

Statistical package for social sciences (SPSS) was used. Non-parametrically, reference ranges, and medians were directly obtained from the analyzed data separately for both males and females at 95% reference range (2.5th and 97.5th percentiles. *P*-values for the difference between male and female participants were estimated using the Mann–Whitney test where $P < 0.05$ were considered significantly different. Comparison of reference ranges for different age groups for each sex was compared using One-Way-ANOVA and Dunnetts Multiple Comparisons test where $P < 0.05$ were considered significantly different.

Results

Establishment of Reference Ranges for Adult Males and Females

Out of the 400 participants recruited for the study, only 367 were involved in the study; 211 males and 156 females. Of those whose data were excluded, 11 (33%) were HIV positive, 6 (18%) HBsAg positive, 5 (15%) HCV positive, 2 (6%) VDRL positive, 2 (6%) were lipeamic, 3 (9%) icteric, and 4 (12%) were hemolyzed. Reference ranges for nine renal function parameters were established for males and females with an age range of 18–50 years with median age of 25 and 27 for males and for females, respectively. The reference values were constructed using 2.5th and 97.5th percentiles as lower and upper limits at 95% confidence interval in accordance with CLSI (formerly NCCLS) guideline for determining reference intervals. The medians for males and females were statistically compared using Mann–Whitney test. $P < 0.05$ was considered statistically different.

Table 1 shows combined or sex specific reference ranges for each parameter based on the *P*-values for the difference between male and female participants. The table also indicate the number of combined and sex specific participants used for determining the reference values for each parameter which were all above the minimum sample size ($N = 120$) suggested by CLSI [1]. BUN showed no significant sex differences while CREAT, NA, K and CL showed significant sex differences ($P < 0.05$). Sex difference was also noted with UA among other substrates while CA and PHOS did not indicate any sex differences.

Reference Range Values Among Different Age Groups in Healthy Adult North Rift Valley Kenyan Population

The different age groups were categorized as: (a) Category 1 (18–28 years); (b) Category 2 (29–39 years); (c) Category 3 (40–50 years). Reference range differences between males and females were estimated within each age category. The determination of the differences in the three age categories was done by using One-Way-ANOVA and Dunnetts Multiple Comparisons test since all age categories did not have a minimum sample size of 120 required by CLSI [1]. *P*-values less than 0.05 were considered statistically significant.

In Table 2, among the renal function tests, age category 1 showed significant differences for CREAT, UA, NA, and K with males having greater mean reference values than their female counterparts. CREAT, UA and NA showed a significant sex difference in age category 2 while in age category 3, only UA showed a significant sex difference for all the renal function analytes. UA showed significant sex differences in all the age categories with males having higher values than females. Within females, reference ranges for CREAT for age category 3 were greater than those of age category 1. Males in age category 2 had lower mean reference CA values than in age category 1. Other renal function tests did not show any significant difference.

Discussion

The significantly higher values of the reference ranges for CREAT, NA, K, CL, and UA in males compared to females indicates sex differences in these kidney function test parameters. Similar findings have been reported in black populations of Kampala, Uganda; Kericho, Kenya; Mbeya, Tanzania; Rwanda; Rawalpindi, Pakistan and white USA populations [3–6, 8, 9]. The finding of

Table 1 The established reference ranges for BUN, CREAT, NA, K, Cl, UA, CA and PHOS for male and female adults from North rift valley, Kenya

Analyte (unit)	Sex	*N*	Median	Percentiles		Reference Value	IV	Difference between M&F	
				2.5th	97.5th			*Z*-value	Sig*
BUN (mmol/L)	M&F	366	3.3	1.9	5.9	1.9–5.9	4.1	−0.661	0.509
	F	156	3.3	1.8	5.8	1.8–5.8	4.0		
	M	210	3.2	1.9	6.1	1.9–6.1	4.2		
CREAT (μmol/L)	M&F	360	71	49	98	49–98	49	−10.981	0.001*
	F	154	62	48	85	48–85	37		
	M	206	78	56	99	56–99	43		
NA (mmol/L)	M&F	352	141.4	136.4	155.7	136.4–155.7	19.3	−0.984	0.004*
	F	151	141	136	153.5	136–153.5	17.5		
	M	201	141.7	136.5	156.7	136.5–156.7	20.2		
K (mmol/L)	M&F	360	4.1	3.4	5.3	3.4–5.3	1.9	−1.986	0.047*
	F	153	4.1	3.3	4.7	3.3–4.7	1.4		
	M	207	4.14	3.4	5.4	3.4–5.4	2.0		
CL (mmol/L)	M&F	357	104	97	114	97–114	17	−2.749	0.006*
	F	151	104.7	98	114	98–114	16		
	M	206	103.1	96	115	96–115	19		
UA (μmol/L)	M&F	357	292	169	434	169–434	265	−0.539	0.001*
	F	153	259	142	362	142–362	220		
	M	204	182	191	461	191–461	270		
CA (mmol/L)	M&F	355	2.43	2.16	2.66	2.16–2.66	0.50	−0.659	0.510
	F	151	2.40	2.14	2.66	2.14–2.66	0.52		
	M	204	2.43	2.16	2.70	2.16–2.70	0.54		
PHOS (mmol/L)	M&F	346	1.14	0.81	1.47	0.81–1.47	0.66	−0.863	0.388
	F	146	1.15	0.80	1.47	0.80–1.47	0.67		
	M	200	1.12	0.80	1.51	0.80–1.51	0.71		

Results are expressed as Median (Range). *The sex difference is significant at $P < 0.05$ by Mann–Whitney test; *Sig* significance; *N* number of subjects; *F* female and *M* males; *IV* interval value

significant sex differences for CREAT agrees with the well established fact that males have higher reference range values for CREAT than females due to the higher muscle and bone mass. Similar results have been reported from China and Asia [8–10].

Sex differences observed for NA, K and CL could be attributed to the differences in response to dietary salts due to effects of sex hormone patterns and sex- related genetic factors. Similar findings were noted in Rwanda [8]. Sex differences in UA have been previously documented by Roche Diagnostics, Eller et al., Saathoff et al. and Khan et al. [2, 4, 5, 9] which could be due to the influence of sex hormones resulting in differences in body mass between sexes. The general absence of sex differences for BUN, CA, and PHOS in this study agrees with those reported in the reagent manufacturer's reference range values [2]; the result is similar to studies done in Pakistan [10], an indication that these parameters are not influenced by sex hormones.

The observed significant increase of some renal function tests and decrease of others in one or both sexes as age progressed is an indication that these analytes are age dependent. The decrease in serum CA for males with progression of age from the second to the third decade agrees with the observations of Olusi and Al-Awadhi [11]. The rise of CREAT with progressing age noted for females in this study could be attributed to reduction in glomerular filtration rate and increased degradation of the muscle mass resulting in a decrease in muscle mass and is similar to that reported by Verma et al. [12].

The North Rift Kenya values for urea were lower than those of America and Kuwait [2, 11], comparable with the Kampala, Uganda group [4] but slightly higher than those for Mbeya, Tanzania and Kericho, Kenya [3, 5]. Male creatinine values for the current study were lower than those for America, Kericho and Kuwait groups [2, 5, 11], greater than those for Tanzanian population [3] and comparable with those of the Uganda group [4]. North Rift female creatinine values were higher than the America, Mbeya, and Kampala [2–4] but lower than those for Kericho populations [5]. Among the electrolytes, NA and

Table 2 Comparison of reference ranges for male and female in different age categories for BUN, CREAT, Na, K, CL, UA, CA and PHOS

Analyte (unit)	Sex	Age categories					
		N	18–28 years	*N*	29–39 years	*N*	40–50 years
BUN (mmol/L)	M	134	3.23 ± 0.91	53	3.45 ± 1.11	23	3.61 ± 1.67
	F	91	3.21 ± 0.96	44	3.51 ± 0.81	21	3.53 ± 0.89
CREAT (μmol/L)	M	134	76.87 ± 10.21*	52	80.85 ± 11.23*	20	74.35 ± 13.36
	F	90	61.71 ± 9.85	43	64.67 ± 7.99	21	68.00 ± 7.97[b]
NA (mmol/L)	M	131	143.48 ± 5.52*	49	144.49 ± 5.64*	21	143.89 ± 6.97
	F	88	141.96 ± 4.38	44	142.39 ± 4.46	19	141.11 ± 3.76
K (mmol/L)	M	133	4.23 ± 0.52*	52	4.10 ± 0.52	22	4.22 ± 0.53
	F	89	4.03 ± 0.33	43	4.10 ± 0.32	21	4.13 ± 0.34
CL (mmol/L)	M	132	103.59 ± 5.85*	51	103.45 ± 14.22	23	105.87 ± 5.78
	F	88	104.87 ± 3.75	43	106.21 ± 3.50	20	104.33 ± 4.22
UA (μmol/L)	M	132	312.63 ± 59.08*	50	337.02 ± 62.61*	22	332.32 ± 63.19*
	F	90	257.40 ± 55.63	43	259.16 ± 56.52	20	275.10 ± 51.19
CA (mmol/L)	M	131	2.46 ± 0.13	51	2.39 ± 0.14[a]	22	2.42 ± 0.11
	F	89	2.43 ± 0.20	41	2.40 ± 0.12	21	2.41 ± 0.13
PHOS (mmol/L)	M	128	1.13 ± 0.18	49	1.11 ± 0.16	23	1.18 ± 1.16
	F	87	1.14 ± 0.18	40	1.14 ± 0.17	19	1.09 ± 0.30

Results are expressed as Mean ± standard deviation (SD) of the number of subjects shown in columns labeled N. *represents significant sex difference in each age category where $P < 0.05$ by *t*-test

[a] represents significant specific sex difference between age category 1 and 2 where $P < 0.05$ by One-Way ANOVA and Dunnetts Multiple Comparison test

[b] represents significant specific sex difference between age category 1 and 3 where $P < 0.05$ by One-Way ANOVA and Dunnetts Multiple Comparison test

CL upper limits were higher than those for all the other groups [2–5, 11]. K values varied in all the comparison populations [2–5, 11]. Differences were also seen with UA, CA and PHOS [2–5, 11]. The observed variation in renal function test reference values developed in this study compared to reference range values for the same parameters from other locations suggest variations in analytical methods in addition to ethnic composition and ecological parameters as stated by Saathoff et al. [4, 13]. The higher reference range value for CREAT compared to those of other locations could be due to genetic factors and environmental factors. The higher reference range values for CA, NA, CL, PHOS and K in this study compared to those of other locations could be due to variations in dietary intake and local climate of these populations; this is in agreement with findings of a study in Tanzania [4, 13]. The different lifestyles and genetic composition of the two populations could also explain the differences. These differences have also been reported from other countries [4, 5, 11, 13].

The findings of this study provide adult sex and age specific renal function reference values to be used in North Rift valley region, Kenya and show that reference values obtained from America and other regions are unsuitable for use in the North Rift valley population. This will ensure better evaluation and interpretation of renal function results, leading to good clinical practice and proper research thus improving quality of healthcare in the region.

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