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ESTABLISHMENT OF ADULT REFERENCE VALUES FOR SOME BIOCHEMICAL ANALYTES IN A RWANDAN POPULATION
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ABSTRACT

***Objectives*: To establish the reference values for some routinely performed biochemical analytes in CHUK.**
***Design*: Cross-sectional descriptive study.**
***Settings*: National Center for Blood Transfusion, Rwanda and Kigali University Teaching Hospital, Laboratory Department. This study was conducted during the period between 15th September 2014 and 23rd February in 2015.**
***Subjects*: Blood donors donating blood at National Center for Blood Donation, and recruited by mobile teams across the country.**
***Results*: Median (Reference values:2.5th and 97.5th percentiles) for male and female respectively: Bilirubin Direct,3.9(2-6.9) and 3.9(2.6-6.5) μmol/L; Bilirubin Total,10.3(4.8-21.6) and 10.4(5.9-17.3) μmol/L; Aspartate Aminotransferase, 27.8(16.1-49.2) and 26.7(16.8-45.1) U/L;Urea,3.2(1.3-5.8) and 3.1(1.4-5.2)mmol/L; Glucose, 5.0(3.2-7.7) and 4.6(3.1-6.7) mmol/L; Total Proteins, 76.8(68.2-87.7) and 76.9(66.6-85.7)g/L; Albumin, 46.4(39.7-55.5) and 46.7(40-54.5) g/L; Alanine Aminotransferase, 17.1(7.2-36.2) and 16.0(7.3-33.9) U/L; Gamma Glutamyltransferase, 20.3(8-75.6) and 21.1(7.1-63.3) U/L; Alkaline Phosphatase, 74.3(43.8-145.7) and 73.5(50.3-135.4) U/L; Creatinine, 84.4(65.2-107.1) and 81.1(62.5-98.6) μmol/L; Sodium, 139.0(134.5-145.5) and 141.0(134.5-146.5) mmol/L; Potassium, .4.4(3.7-5) and 4.3(3.5-5.0) mmol/L; Chloride, 95.7(89.9-104.2) and 99.3(90.6-103.1) mmol/L; Magnesium, 0.9(0.7-1.0) and 0.9(0.7-1) mmol/L; Phosphate, 1.1(0.8-1.5) and 1.2(0.7-1.6) mmol/L.**
***Conclusion*: The results of our study on Clinical Chemistry parameters are similar to those published in other African countries, with variations due to the diet and geographical location. This study has shown that a strict adherence to reference ranges developed from industrialised countries could qualify many healthy Rwandans as pathological cases, and also exclude them from participating in clinical trials. Compared to other reference ranges established, reference values in our study presented remarkably low levels of urea which may be due to the diet low in proteins generally in Rwandan population.**

INTRODUCTION

In health-related fields, a reference range or reference interval describes the variations of a measurement or value in healthy individuals. It is a basis for a physician or other health professional to interpret a set of results for a particular patient (Saathoff *et al.*, 2008). The use of improper reference ranges may false or exclude otherwise volunteers who are eligible to participate in the research making the process of enrolment and execution more challenging(Karita et

al.,2009; Eller *et al*., 2008). On the other hand, as the biological and environmental characteristics vary between populations, it is imperative to establish local reference intervals for clinical laboratories, which makes it possible to judiciously interpret laboratory results using reference intervals obtained from the local population and in the same environmental background(Eller *et al*., 2008). The present study, therefore, aims to fill this gap by studying a set of serum biochemical parameters, chosen among the most relevant and the most commonly requested by our clinicians.

MATERIALS AND METHODS

Samples: The sites dispatched in five provinces of Rwanda have been used to collect samples: Central Province (Kigali), Northern Province, Southern Province, Eastern Province, and Western Province. This was a cross sectional prospective study.

Blood donors were recruited in rural area, schools and urban cities in five provinces during the study period.

Three hundred and eighty four blood donors, 93 in Northern, Southern, Western and Eastern provinces each, and 95 in Central province, all in rural areas, schools and urban cities were sampled for this study. Each blood donor who fulfilled all criteria as set by NCBT was given a questionnaire and a consent form for participation in this study.

Whole blood was collected into two tubes, in 5 ml plain tube without anticoagulant for serum chemistry and in Sodium fluoride tube specifically for blood glucose levels determination.

Samples after collection were allowed to clot at room temperature and put in a cool box for transportation. After clotting at room temperature, the blood cells were separated from the serum by centrifugation at 5000 rounds for 5 minutes, and serum was put in code labeled serum vials, and analysed within 10 hours. Those which were not analysed were kept in a fridge at -21ºC and analysed the following day.

Laboratory analysis was performed at Kigali University Teaching Hospital, using Cobas Integra 400 Plus, a chemistry analyser (Roche Diagnostics Ltd, Switzerland). All tests were done according to the laboratory SOPs, equipment and reagent manufacturer's instructions.

Statistical analysis: Socio-demographic and medical information was obtained from the questionnaires and included gender, age, demographic location, and results of transmissible disease from blood donors files. Results from the laboratory tests were entered into a hard cover register and a password protected Microsoft Excel database. Samples were identified by a unique study number for confidentiality. Results were analysed using SPSS version 20 (IBM corporation, 2012). All calculations to determine the reference values were based on Clinical and Laboratory Standards Institute/International Federation of Clinical Chemistry(CLSI/IFCC)(The Former National Committee of Clinical Laboratory Standards) guidelines document on defining, establishing and verifying reference intervals in Clinical Laboratory(Edward A.et al., 2000).

2.5^{th} and 97.5^{th} percentiles were calculated non-parametrically, after removing identified outliers in each subgroup by using Reed-Dixon method of identifying the outliers(Dixon, 2013; Reed, Henry, & Mason, 1971).The extreme values were retained in the distribution if $D/R = 0.33$,where D is the absolute difference between the most extreme distribution and the next value and R is the range(maximum-minimum), as recommended by CLSI/IFCC guidelines document.

Differences between genders and age groups were evaluated using Wilkoxon-Mann-Whitney test and when $p = 0.05$, it was considered as a statistically significant difference between groups. All statistical analyses were carried out using SPSS v.20 (IBM Corporation, 2012).

Ethical consideration: This study was ethically approved by Rwanda Biomedical Center division of Medical Research Committee, Ethical approval from Rwanda National Ethics Committee, Ethical Review Committees of National Center for Blood Transfusion and Kigali University Teaching Hospital before excursion.

RESULTS

The subjects used in the statistical analysis were 467, with 333 males and 134 females (representing 71.3 % and 28.7%, respectively). The study participants had a mean age of 32.6 years.

Before analysing obtained data, outliers were removed. An outlier was any value distant from the other values or outside the range of ±3SD. An outlier may be a result of variability in measurement or an experimental error. All outliers were removed as recommended by Reed *et al*. and Dixon in CLSI essential guidelines for establishing reference intervals. The number of outliers removed is indicated in the brackets for males and females respectively as following: Albumin (0, 0), Alkaline Phosphatase (5, 1), Alanine Aminotransferase (1, 0), Aspartate Aminotransferase (1, 0), Creatinine (0, 0), Gamma Glutamyl Transferase (13, 2), Magnesium (0, 0), Phosphate (6, 1), Total Proteins (1, 0), Urea (1,

0), Sodium (1, 0), Potassium (0, 0), Chloride (0, 0), Bilirubin Direct (0, 0), Bilirubin Total (0, 0), Glucose (0, 0).

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 and age groups were statistically compared using Mann-Whitney test. P< 0.05 was considered statistically different. Serum concentration difference of the analyte was determined to assess whether age group specific reference ranges or reference ranges for the whole population regardless the age should be established. Results showed a statistically significant difference in gender, whereby males have significantly higher serum concentration values of Creatinine of 65.2-107.1 μmol/L against 62.5-98.6 μmol/L for females (p = 0.001), serum concentration values of Sodium higher in females with 134.5-146.5 mmol/L against 134.5-145.5 for males (p = 0.013) and serum concentration values of Chloride higher in females with 90.6-103.1 mmol/L against 89.9-104.2 for males (p = 0.028), since their p-values are less than 0.05(Table 1.,2.,3.). Comparing age groups, subjects aged of 30 years and below have a significantly lower serum concentration values of Total Proteins of 62.9-86.5 g/L against 68.6-87.7 g/L for subjects aged above 30 years (p = 0.002), serum concentration of Albumin of 37.1-55.2 g/L against 40.9-55.5 for subjects aged of above 30 years (p = 0.022), serum concentration of Alkaline Phosphatase of 43.3-133.1 U/L against 44.6-152.0 U/L for subjects aged of above 30 years (p = 0.001), serum concentration of Sodium of 134.5-146.0 mmol/L against 134.5-146.5 mmol/L for subjects aged of above 30 years (p = 0.015), serum concentration of Bilirubin Direct of 1.8-7.0 g/L against 2.3-6.8 μmol/L for subjects aged of above 30 years (p = 0.025)(Table 4.,5.,6.).

Table 1

Reference values of clinical chemistry analytes under study and differences in genders

Analytes (Unit)	Male				Female				All participants					
			Percentiles				Percentiles				Percentiles			
	N	Median	2.5th	97.5th	N	Median	2.5th	97.5th	N	Median	2.5th	97.5th	Z - Value	P - Value
Renal and Liver function tests														
Bil-D (μmol/L)	333	3.9	2.0	6.9	134	3.9	2.6	6.5	143	4.0	2.1	6.9	-0.186	0.852
Bil-T (μmol/L)	333	10.3	4.8	21.6	134	10.4	5.9	17.3	143	10.4	5.0	21.6	-0.849	0.396
AST (U/L)	332	27.8	16.1	49.2	134	26.7	16.8	45.1	436	27.6	16.4	49.2	-1.169	0.242
UREA (mmol/L)	332	3.1	1.3	5.8	134	3.1	1.4	5.2	436	3.1	1.3	5.8	-0.781	0.435
GLU (mmol/L)	333	5.0	3.2	7.7	134	4.6	3.1	6.7	143	4.8	3.1	7.7	-1.760	0.078
Liver function tests														
TP (g/L)	332	76.8	68.2	87.6	134	76.9	66.6	85.7	436	76.8	68.0	87.6	-0.532	0.595
ALB (g/L)	333	46.4	39.7	55.5	134	46.7	40.0	54.5	437	46.5	39.8	55.4	-0.472	0.637
ALT (U/L)	332	17.1	7.2	36.2	134	16.0	7.3	33.9	436	16.9	7.4	36.2	-0.824	0.41
GGT (U/L)	320	20.3	8.0	75.6	132	21.1	7.1	63.3	349	20.4	7.6	75.2	-0.350	0.726

ALP (U/L)	328	74.3	43.8	145.7	133	73.5	50.3	135.4	431	74.3	44.2	145.7	-0.593	0.553
Renal function tests and electrolytes														
CREA (μmol/L)	333	84.4	65.2	107.1	134	81.1	62.5	98.6	437	83.3	64.5	106.3	-3.258	0.001
Na^+ (mmol/L)	332	139.0	134.5	145.5	134	141.0	134.5	146.5	142	140.0	134.5	146.5	-2.472	0.013
K^+ (mmol/L)	333	4.4	3.7	5.0	134	4.3	3.5	5.0	143	4.4	3.7	5.1	-1.044	0.297
Cl- (mmol/L)	333	95.7	89.9	104.2	134	99.3	90.6	103.1	143	97.5	90.3	104.1	-2.199	0.028
Mg+ (mmol/L)	333	0.9	0.7	1.0	134	0.9	0.7	1.0	321	0.9	0.7	1.0	-1.270	0.204
PO_3^{2-} (mmol/L)	327	1.1	0.8	1.5	133	1.2	0.7	1.6	430	1.2	0.8	1.6	-1.237	0.216

Table 1. indicates renal and liver function tests and electrolytes for both genders while separate and combined. The values are given as both the median and the percentiles. Reference range is given as the range between 2.5 and 97.5 of the percentile. The number of subjects is indicated under the column labelled N. The bolded values indicate significance difference between sexes. The sex difference is significant at $p < 0.05$(Creatinine, Sodium and Chloride).

Table 2

Reference values of clinical chemistry analytes under study and differences in age groups

Analytes (Unit)	<=30				31+				All participants					
		Percentiles				Percentiles				Percentiles				
	N	Median	2.5th	97.2th	N	Median	2.5th	97.2th	N	Median	2.5th	97.2th	Z-Value	P-Value
Renal and liver function tests														
BIL-D (μmol/L)	193	3.6	1.8	7.0	274	3.9	2.3	6.8	467	4.0	2.1	6.9	-2.244	0.025
BIL-T (μmol/L)	193	9.7	4.2	21.4	274	10.7	5.8	23.0	467	10.4	5.0	21.6	-1.458	0.145
UREA (mmol/L)	193	2.9	1.3	5.8	273	3.0	1.4	4.8	466	3.1	1.3	5.8	-1.075	0.283
AST (U/L)	192	26.7	17.1	45.6	274	28.3	16.1	49.3	466	27.6	16.4	49.2	-1.347	0.178
GLU (mmol/L)	193	4.7	3.1	6.7	274	4.8	2.6	8.2	467	4.8	3.1	7.7	-1.701	0.089
Liver function tests														
TP (g/L)	192	75.9	62.9	86.5	274	77.4	68.6	87.7	466	76.8	68.0	87.6	-3.035	0.002
ALB (g/L)	193	45.9	37.1	55.2	274	46.8	40.9	55.5	467	46.5	39.8	55.4	-2.288	0.022
ALT (U/L)	193	17.4	6.9	37.4	273	16.8	7.9	34.2	466	16.9	7.4	36.2	-0.153	0.878
GGT (U/L)	189	19.5	7.6	70.7	263	22.2	6.1	76.1	452	20.4	7.6	75.2	-1.542	0.123
ALP (U/L)	189	68.8	43.3	133.1	272	76.6	44.6	152.0	461	74.3	44.2	145.7	-3.250	0.001
Renal function tests and electrolytes														
CREA (μmol/L)	193	83.7	66.3	104.6	274	82.7	62.7	105.9	467	83.3	64.5	106.3	-0.846	0.397

Na+ (mmol/L)	192	139.0	134.5	146.0	274	141.0	134.5	146.5	466	140.0	134.5	146.5	-2.425	0.015
K+ (mmol/L)	193	4.3	3.6	5.1	274	4.4	3.7	5.1	467	4.4	3.7	5.1	-1.426	0.154
Cl- (mmol/L)	193	95.4	89.2	103.5	274	98.0	90.5	105.1	467	97.5	90.3	104.1	-1.636	0.102
Mg+ (mmol/L)	193	0.9	0.7	1.0	274	0.9	0.7	1.0	467	0.9	0.7	1.0	-0.521	0.603
PO32- (mmol/L)	190	1.2	0.7	1.5	270	1.2	0.8	1.2	460	1.2	0.8	1.6	-0.862	0.389

Table 2. indicates renal and liver function tests and electrolytes for both age groups while separate and combined. The values are given as both the median and the percentiles. Reference range is given as the range between 2.5 and 97.5 of the percentile. The number of subjects is indicated under the column labelled N. The bolded values indicate significance difference between age groups. The age group difference is significant at $p < 0.05$(Bilirubin Direct, Total Proteins, Albumin, Alkaline Phosphatase and Sodium).

DISCUSSION

Reference ranges are used by the clinicians in the interpretation of clinical laboratory data. They are defined as a set of a measured quantity of an analyte obtained from a group of individuals or an individual in a defined state of health. International Federation of Clinical Chemistry (IFCC) recommends that the reference ranges be constructed from 95% of a reference population of healthy individuals. This study was aimed at establishing the reference ranges for the biochemical parameters in adult Rwandan population to serve as standards for the interpretation of laboratory results during screening and follow-ups in clinical trials and routine healthcare.

This study provides the established clinical chemistry reference ranges for adults 19-58 years for both males and females in Rwanda derived from healthy individuals. Out of 467 participants recruited, the number of males (333) was quite high comparing to that of females (134); each group exceeded the minimum of 120 participants for nonparametric estimates required for 95% reference interval determination as recommended by National Committee for Clinical Laboratory Standard (NCCLS, 2000). Emphasis was laid on external and internal quality control methods which ensured accuracy and precision in addition to following all the set standard operating procedure of Kigali University Teaching Hospital.

The results of this study shown that measured biochemical parameters Albumin, Alkaline Phosphatase, Sodium, Potassium, Magnesium, Phosphate serum concentrations are comparing with the ranges established by consensus by Roche Diagnostics Company, Germany.

Bilirubin Direct, Bilirubin Total, Aspartate Aminotransferase and Creatinine are slightly higher than the limits established by consensus by the Roche Diagnostics. Alanine Aminotransferase, Urea and Chloride are below the established limits by consensus by the manufacturer.

The results of this study confirmed the results reported in a study conducted in Northern Province (Gahutu, 2013), and shown the similar results as the previous study (Gahutu & Wane, 2006) in Rwandan students in Northern Province.

The significantly higher values of the reference values for Creatinine and Chloride in male compared to female, and the higher values of the reference values for Sodium in female compared to male indicates sex differences in these clinical chemistry parameters. Sex differences in Creatinine have been known to exist due to differences in muscle mass. Similar findings have been reported in adult black populations of Kampala, Uganda; Kericho, Kenya; Mbeya, Tanzania; Huye, Rwanda; Kintampo, Ghana and adult white USA populations (Eller et al. 2008; Gahutu & Wane 2006; Kibaya et al. 2008; Roche Diagnostics, 2015; Saathoff *et al*. 2008; Dosoo *et al*. 2012).

The slight differences due to gender (sex) in the reference range for total protein and albumin could be attributed to the size; however, this difference may not have any clinical significance. The sex difference in the reference range values for serum total protein observed in this study are in contrast to that reported for the American population (Roche Diagnostics, 2015) where males and females have common reference range values but agrees with the findings of a Rwandan study (J. Gahutu & Wane, 2006).

Serum concentration of Bilirubin Total and Bilirubin Direct, Total proteins and Albumin show high values in age of 30 years and below comparing to that of above 30 years individuals in accordance with Gahutu 2013 and Wane 1985.

Sex differences in the total bilirubin and direct bilirubin values could be partly due to influence of sex hormones. These findings are in agreement with those of similar studies done in Uganda (Eller et al. 2008) for adults.

Differences in values of liver enzymes higher compared to our study for both males and females have been reported in Kenya(Kibaya *et al*., 2008). Bilirubins, Glucose, electrolytes values compare well

with the reported values in our study, except low values in serum concentration of Chloride in our study.

This study shows low levels of urea in females compared to that in males, and shows a slight decrease in age, this confirming a study done in Congo by Wane 1985. Serum concentration of urea in this study is low compared to the results published in other studies. This may due to a diet low in animal proteins common in Rwanda.

Overseas similar results have been reported with differences in higher serum concentrations of Bilirubin Total, Creatinine, Urea, Chloride and Alkaline Phosphatase for both males and females(Sluss et al., 2008).

Variations have been observed in other studies in the region (Karita et al., 2009; Palacpac et al., 2014; Saathoff et al., 2008) which may result in differences in age and living environment.

In conclusion, the results of our study on Clinical Chemistry parameters are similar to those published in other African countries, with variations due to the diet and geographical location.

This study has shown that a strict adherence to reference ranges developed from industrialized countries could qualify many healthy Rwandans as pathological cases, and also exclude them from participating in clinical trials.

Compared to other reference ranges established, reference values in our study presented remarkably low levels of urea which may be due to the diet low in proteins generally in Rwandan population.

Our study not only defined the reference ranges of healthy Rwandans attending NCBT in CHUK, but also enhanced laboratory capacity of Clinical Chemistry service as it was conducted under the guidelines of Good Clinical and Laboratory Practice, thus preparing the site to Clinical trials as a center of research.

Table 3
Comparison of established reference ranges to those in literature

Analytes (unit)	Gender	Established reference ranges		Northern province-Rwanda		Central Province-Rwanda		Central part of Ghana		Ugandan adult blood bank donors		Kericho, Kenya		Case records of Massachusetts General Hospital		Mbeya, Tanzania	
		Lower	Upper	Lower	Upper	lower	upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
BIL-D (μmol/L)	Male	2.0	6.9	2	7	0.4	8.8	0.9	4.1	1.71	8.5	1.3	9	1.7	5.1	0.93	8.43
	Female	2.6	6.5	-	-	-	-	0.8	4	0	6.8	0.8	6.7	-	-	0.70	5.83
BIL-T (μmol/L)	Male	4.8	21.6	3	29	2.9	37	3.8	32	6.8	44.4	5.6	41.9	5.1	17	6.0	42.0
	Female	5.9	17.3	-	-	-	-	2.7	26.6	5.1	32.5	4.4	26.8	-	-	4.5	31.3
TP (g/L)	Male	68.2	87.6	65	85	58	88	46.7	86.4	65	89	-	-	55	80	67.2	85.2
	Female	66.6	85.7	-	-	-	-	55.2	86.9	68	90	-	-	-	-	65.8	85.5
ALB (g/L)	Male	39.7	55.5	34	54	35	52	32.7	49.8	39	55	36.9	48.5	35	55	37.06	50.72
	Female	40.0	54.5	-	-	-	-	33.5	50.4	37	52	34.4	47.5	-	-	35.57	49.27
ALP (U/L)	Male	43.8	145.7	27	122	48	164	101	353	42	159	-	-	-	-	45.4	170.4
	Female	50.3	135.4	-	-	-	-	82	293	47	160	-	-	-	-	45.3	155.0
ALT (U/L)	Male	7.2	36.2	12	43	8	61	8	54	7.2	43.3	10.8	53.9	0	35	9.1	55.3
	Female	7.3	33.9	-	-	-	-	7	51	5.3	39.9	8.6	47	-	-	6.7	44.9
AST (U/L)	Male	16.1	49.2	16	47	14	60	17	60	13.2	35.9	14.9	45.3	0	35	15.2	53.4
	Female	16.8	45.1	-	-	-	-	13	48	11.4	28.8	13.1	38.1	-	-	13.5	35.2
GGT (U/L)	Male	8.0	75.6	9	77	-	-	9	71	8.7	70.7	-	-	-	-	9.3	120.8
	Female	7.1	63.3	-	-	-	-	6	53	8	41.3	-	-	-	-	7.3	51.8
CREA (μmol/L)	Male	65.2	107.1	44	97	47	109	56	119	53.1	106.2	62	106	0	133	48	96
	Female	62.5	98.6	-	-	-	-	47	110	44.2	79.6	51	91	-	-	40	81
UREA (mmol/L)	Male	1.3	5.8	-	-	-	-	0.9	6.2	-	-	-	-	3.6	7.1	-	-

	Female	1.4	5.2	-	-	-	-	0.9	5.4	-	-	-	-	-	-	-	-
Na+ (mmol/L)	Male	134.5	145.5	137	147	-	-	135	151	136	148	141.8	152.1	136	145	133.6	143.1
	Female	134.5	146.5	-	-	-	-	135	150	135	146	140.3	155.3	-	-	133.4	142.3
K+ (mmol/L)	Male	3.7	5.0	3.3	5	-	-	3.6	5.2	3.4	4.8	3.9	5.8	3.5	5	3.8	55
	Female	3.5	5.0	-	-	-	-	3.4	5.1	3.4	4.8	3.8	5.8	-	-	-	-
Cl- (mmol/L)	Male	89.9	104.2	100	112	-	-	101	115	96	104.6	100.4	110.8	98	106	97.9	108.3
	Female	90.6	103.1	-	-	-	-	103	113	97.4	104.5	101.1	113.4	-	-	97.8	107
Mg+ (mmol/L)	Male	0.7	1.0	-.	-	-	-	-	-	0.4	1	-	-	0.8	1.2	0.67	0.97
	Female	0.7	1.0	-	-	-	-	-	-	0.4	1	-	-	-	-	0.71	0.95
P3+ (mmol/L)	Male	0.8	1.5	-	-	-	-	0.742	2.032	-	-	-	-	-	-	0.7	1.48
	Female	0.7	1.6	-	-	-	-	0.806	1.806	-	-	-	-	-	-	0.72	1.52
GLU (mmol/L)	Male	3.2	7.7	-	-	-	-	-	-	3	5.6	4.2	6.4	4.2		2.88	5.3
	Female	3.1	6.7	-	-	-	-	-	-	3.2	5.7	-	-	-	-	3.3	5.06

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