Clinical Chemistry Reference Intervals in a Rwandan Population

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ABSTRACT

Aim: To establish clinical chemistry reference intervals for the Rwandan population.

Study design: A population-based cross-sectional study.

Place and Duration of Study: The study was carried out in three blood transfusion centres: Buhanda, Ruhango and Nyaruteja, between August and December 2011.

Methods: Serum clinical chemistry tests were performed on a Cobas C311 automated chemistry analyzer.

Results: Results of 187 subjects (age range: 17-54 years) presented as median, with 2.5th-97.5th percentiles (95% reference interval) in brackets are as follows: For males: alanine aminotransferase: 25 (12-43) U/L; aspartate aminotransferase: 29 (16-47) U/L; gamma-glutamyl transferase: 22 (9-77) U/L; total bilirubin: 0.6 (0.2-1.7) mg/dL; direct bilirubin: 0.2 (0.1-0.4) mg/dL; creatinine: 0.8 (0.5-1.1) mg/dL; uric acid: 5 (3-7) mg/dL. For males and females: alkaline phosphatase: 71 (27-122) U/L; amylase: 144 (50-235) U/L; lactate dehydrogenase: 176 (114-237) U/L; triglycerides: 82 (32-172) mg/dL; high-density lipoprotein: 48 (29-86) mg/dL; glucose: 87 (70-114) mg/dL; total protein: 7.6 (6.5-8.5) g/dL; albumin: 4.4 (3.4-5.4) g/dL; sodium: 142 (137-147) mmol/L; potassium: 4.1 (3.3-5.0) mmol/L; chloride: 106 (100-112) mmol/L; phosphate: 1.16 (0.87-1.49) mmol/L.

Conclusion: The clinical chemistry reference values are in agreement with those reported in other African studies, with variations.

Keywords: reference intervals, clinical chemistry, moderate altitude, Rwanda
1. INTRODUCTION

Clinical chemistry parameters normally show intra- and inter-individual variations. Intra-individual variations are due to changes along time, such as the circadian rhythm. Inter-individual variations are related to physiological differences linked to sex, age, body mass index, etc (Ichihara et al., 2008). Another source of variation is environment, particularly altitude and diet.

As the biological and environmental characteristics vary between populations (Ichihara et al., 2008; Sundaram et al., 2008), 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. However, the usefulness of reference intervals in establishing a pathological condition and determining its gravity may be limited due to great intra- and inter-individual variations and imprecision of the measurement (Badrick et al., 2005).

Some reference interval studies have been carried out in the East African region (Eller et al., 2008; Karita et al., 2009; Kibaya et al., 2008; Zeh et al., 2011), but specific values for the population of the Southern province of Rwanda needed to be established. A study on a limited number of parameters was previously done among university students in the Southern province of Rwanda (Gahutu and Wane, 2006).

We report here the results of a study of clinical chemistry parameters among the Rwandan population at different sites at moderate altitude in the Southern Province. The aim is to establish specific clinical chemistry reference intervals for the local population. The findings will be resourceful for medical practitioners in the region.

2. SUBJECTS AND METHODS

A population-based cross-sectional study was performed from August to December 2011 among blood donors in the Southern Province of Rwanda (Buhanda centre, located in Ruhango District, altitude: 1,649 m, latitude: -2.23180, longitude: 29.66180; Ruhango centre, located in Ruhango District, altitude: 1,739 m, latitude: -2.2333, longitude: 29.7833; and Nyaruteja centre, located in Gisagara District, altitude: 1,667 m, latitude: -2.78056, longitude: 29.7117). The study participants were male and female adults in healthy condition and normal nutritional status on physical examination and without any history of disease in the preceding six months.

The national questionnaire for blood donation was used for the selection of participants among blood donor groups. Blood donors were aware of the requirements for blood donation, which were also used for study enrolment. Before enrolment, selection criteria were explained in detail, so that those who were not fulfilling them understood it and did not volunteer for the study. Malaria and infection during the preceding six months were ruled out during anamnesis. People under medication, females in the menses period, pregnant or breastfeeding women, and those using hormonal contraception were excluded from the study. Smoking and drug abuse were exclusion factors. Most of study participants were non-drinkers, some drank alcohol occasionally. A short physical examination was carried out, comprising inspection, radial pulse palpation, measurement of weight, height, body temperature and blood pressure, and cardiopulmonary auscultation. Any indication of disease reported for the preceding six months or observed during the physical examination excluded the individual from the study. Four blood donors (4/191, 2%) were excluded from the study, two of them because of anaemia and two because of impaired renal function.

Blood donors have different living conditions: males and females, rural and semi-urban environment, with diverse occupation (peasants, shop keepers, nurses, technicians, teachers, students, craftsmen and civil servants).
The sampling was done in the morning after overnight fasting (two thirds of the study participants) or 4 hours after a light breakfast (one third of the study participants). Blood donors who volunteered for the study donated 5mL of venous blood, sampled in a dry tube without anticoagulant on the occasion of blood donation, the donor being in supine position, at complete physical rest. After clotting at room temperature, the clot was separated from the serum by centrifugation at 3000 rounds per minute during 10 minutes, after which the serum (supernatant) was immediately transferred in another dry tube. Clinical Chemistry tests were performed on the same day in the service of clinical chemistry of the laboratory of Butare University Teaching Hospital. Classical laboratory procedures were used on the Cobas C311 (Roche) automated chemistry analyzer. The new Cobas C311 equipment was installed in the laboratory in August 2011, just before the start of the study. Installation with complete verification was done by the supplier. Validation of the instrument was done with a Cobas MiraPlus chemistry analyzer and showed compliance between both equipments. The laboratory is in the process of strengthening laboratory management towards accreditation (SLMTA), an initiative of the World Health Organisation's Regional Office for Africa, which aims at leading African clinical laboratories to accreditation, in partnership with the Centers for Disease Control and Prevention (CDC Atlanta, GA, USA), the American Society for Clinical Pathology (ASCP) and the Clinton Foundation. External quality control by the National Reference Laboratory during the period of the study gave satisfactory results. Laboratory procedures and internal quality control were performed as per the instructions of the manufacturer. The Cobas C311 automated chemistry analyzer directly determined the values for total protein, albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase, amylase, lactate dehydrogenase (LDH), creatinine, uric acid, total bilirubin, direct bilirubin, high-density lipoprotein (HDL), triglycerides, glucose, sodium, potassium, chloride and phosphate.

We performed one-sample Kolmogorov-Smirnov test on rough data using SPSS Statistics 19. When the bilateral asymptotic significance was higher than .05, we considered the distribution as Gaussian. In case the bilateral asymptotic significance was lower than .05, we considered the distribution as non Gaussian.

The statistical data analysis was done with the Excel 2007 software for the determination of the median and the 95% reference intervals, which were determined as 2.5th-97.5th percentile intervals. Comparison was made between males and females using the Student's t-test.

3. RESULTS AND DISCUSSION

In total 187 subjects (136 males and 51 females) participated in the study. Before result analysis, we removed outliers (results that were too distant from other results or outside the range of mean ± 3 SD). Outliers were considered separately for each parameter as a distant result may be related to analytical imprecision or error and not necessarily to organ function impairment or variation that would influence a group of parameters simultaneously. The number of outliers, indicated in brackets for males and females respectively, was as follows: ALT and AST (4,0), gamma-GT (1,0), bilirubin, total and direct (5,0), alkaline phosphatase (6,1), amylase (6,3), LDH (4,1), glucose (8,2), total protein and albumin, considered together (8,4), triglycerides (4,2), HDL (3,0), sodium (5,1), potassium (2,0), phosphate (2,0), chloride (5,1). There was no outlier for creatinine and uric acid. In many cases, the total number of parameters tested on the sample depended on reagents available for the study, hence the great variation in number of cases. The sample size (n) for statistical analysis is specified in tables for the different tests. The radial pulse was in the normal range of 60-100 beats per minute, the blood pressure was normal (systolic pressure lower than 140 mm Hg and the diastolic pressure lower than
90 mm Hg) and the body temperature was in the normal range (36.2-37.5°C) for all recruited participants. The age range is 17-54 years. The mean age is 26 years (27 years for males and 24 years for females). The mean weight is 63.1 kg in males and 62.5 kg in females (range: 50-89 kg and 50-86 kg for males and females respectively).

Gaussian distribution was observed in males for alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatinine, triglycerides, uric acid, glucose, amylase and phosphate. The test is not reliable for small sample sizes, which was the case for the female groups.

The 95% confidence interval of the mean was calculated as mean ± 1.96 SEM (standard error of the mean) using the Excel 2007 software. When the 95% confidence interval of the mean for males overlapped with the one for females, we considered that there was no significant difference between males and females and a common 2.5th-97.5th percentile reference interval is presented, valid for males and females.

When 95% confidence intervals of the mean for males and females were not overlapping, we considered that the differences between both groups were statistically significant (p<.05). The differences between males and females were confirmed by use of Student’s t-test on Excel 2007. In the absence of statistically significant difference (p>.05, which was the case for ALP, amylase, LDH, glucose, total protein, albumin, globulins, triglycerides, HDL, sodium, potassium, chloride, phosphate) a common reference interval is presented. For ALT, AST, gamma-GT, bilirubin (total, direct, indirect), creatinine and uric acid, there was a statistically significant difference (p<.05) between male and female groups. As the female group has less than 120 subjects in total, only reference intervals for males (sample size >120) are presented for these parameters. The median and the 95% reference interval (2.5th-97.5th percentile interval) for the different parameters are presented in table 1.

To illustrate the difference between males and females, scatter plots of individual values of creatinine, ALT, AST, gamma-GT, total and direct bilirubin.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sex</th>
<th>n</th>
<th>Conventional units</th>
<th>SI units</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver function and enzymes</strong></td>
<td></td>
<td></td>
<td>Unit</td>
<td>Median (2.5\textsuperscript{th} - 97.5\textsuperscript{th} percentile interval)</td>
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<tr>
<td>ALT</td>
<td>M</td>
<td>125</td>
<td>U/L</td>
<td>25 (12-43)</td>
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<tr>
<td>AST</td>
<td>M</td>
<td>125</td>
<td>U/L</td>
<td>29 (16-47)</td>
</tr>
<tr>
<td>GGT</td>
<td>M</td>
<td>133</td>
<td>IU/L</td>
<td>22 (9-77)</td>
</tr>
<tr>
<td>Total bilirubin</td>
<td>M</td>
<td>131</td>
<td>mg/dL</td>
<td>0.6 (0.2-1.7)</td>
</tr>
<tr>
<td>Direct bilirubin</td>
<td>M</td>
<td>131</td>
<td>mg/dL</td>
<td>0.2 (0.1-0.4)</td>
</tr>
<tr>
<td>Indirect bilirubin</td>
<td>M</td>
<td>131</td>
<td>mg/dL</td>
<td>0.4 (0.1-1.3)</td>
</tr>
<tr>
<td>ALP</td>
<td>M&amp;F</td>
<td>164</td>
<td>IU/L</td>
<td>71 (27-122)</td>
</tr>
<tr>
<td>Amylase</td>
<td>M&amp;F</td>
<td>150</td>
<td>Somogyi units/dL</td>
<td>78 (27-127)</td>
</tr>
<tr>
<td>LDH</td>
<td>M&amp;F</td>
<td>126</td>
<td>U/L</td>
<td>176 (114-237)</td>
</tr>
<tr>
<td><strong>Glucose, proteins and lipids</strong></td>
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<td></td>
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</tr>
<tr>
<td>Glucose</td>
<td>M&amp;F</td>
<td>173</td>
<td>mg/dL</td>
<td>87 (70-114)</td>
</tr>
<tr>
<td>Total protein</td>
<td>M&amp;F</td>
<td>163</td>
<td>g/dL</td>
<td>7.5 (6.5-8.5)</td>
</tr>
<tr>
<td>Albumin</td>
<td>M&amp;F</td>
<td>163</td>
<td>g/dL</td>
<td>4.4 (3.4-5.4)</td>
</tr>
<tr>
<td>Globulins</td>
<td>M&amp;F</td>
<td>163</td>
<td>g/dL</td>
<td>3.3 (2.3-4.2)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>M&amp;F</td>
<td>172</td>
<td>mg/dL</td>
<td>82 (32-172)</td>
</tr>
<tr>
<td>HDL</td>
<td>M&amp;F</td>
<td>130</td>
<td>mg/dL</td>
<td>48 (29-86)</td>
</tr>
<tr>
<td><strong>Renal function, metabolic intermediates and electrolytes</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Creatinine</td>
<td>M</td>
<td>133</td>
<td>mg/dL</td>
<td>0.8 (0.5-1.1)</td>
</tr>
<tr>
<td>Uric acid</td>
<td>M</td>
<td>134</td>
<td>mg/dL</td>
<td>5 (3-7)</td>
</tr>
<tr>
<td>Sodium</td>
<td>M&amp;F</td>
<td>122</td>
<td>mEq/L</td>
<td>142 (137-147)</td>
</tr>
<tr>
<td>Potassium</td>
<td>M&amp;F</td>
<td>131</td>
<td>mEq/L</td>
<td>4.1 (3.3-5.0)</td>
</tr>
<tr>
<td>Chloride</td>
<td>M&amp;F</td>
<td>121</td>
<td>mEq/L</td>
<td>106 (100-112)</td>
</tr>
<tr>
<td>Phosphate</td>
<td>M&amp;F</td>
<td>157</td>
<td>mg/dL</td>
<td>3.6 (2.7-4.6)</td>
</tr>
</tbody>
</table>

M: Males; F: Females; ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase, HDL: high-density lipoprotein
As the study population consists homogeneously of young adults, there is no trend for the age. A decrease is normally seen in the elderly. However, serum creatinine values are significantly higher in males (mean ± SD: 0.82 ± 0.14 mg/dL) than in females (mean ± SD: 0.71 ± 0.12 mg/dL), p = .00001.

Serum ALT values show a steady increase between 18 and 35 years and stabilize thereafter. The values are significantly higher in males (mean ± SD: 26.3 ± 9.4 U/L) than in females (mean ± SD: 19.0 ± 6.0 U/L), p = .000001.
Figure 3. Profile of serum AST concentration as a function of sex and age

Serum AST values show a steady increase between 18 and 35 years and stabilize thereafter. The values are significantly higher in males (mean ± SD: 30.7 ± 9.4 U/L) than in females (mean ± SD: 22.7 ± 5.6 U/L), p = .0000001.

Figure 4. Profile of serum gamma-glutamyl transferase concentration as a function of sex and age

Serum gamma-glutamyl transferase values show a steady increase between 18 and 43 years and stabilize at lower levels thereafter. The values are significantly higher in males (mean ± SD: 27.8 ± 17.4 U/L) than in females (mean ± SD: 16.5 ± 10.1 U/L), p = .00002.
Serum total bilirubin concentration shows high levels around 20 years and stabilizes at lower values thereafter, both in males and in females. Total bilirubin values are significantly higher in males (mean ± SD: 0.71 ± 0.45 mg/dL) than in females (mean ± SD: 0.54 ± 0.36 mg/dL), p = 0.01.
Figure 6. Profile of serum direct bilirubin concentration as a function of sex and age

Serum direct bilirubin concentration shows high levels around 20 years and stabilizes at lower values thereafter, both in males and in females. Direct bilirubin values are significantly higher in males (mean ± SD: 0.21 ± 0.09 mg/dL) than in females (mean ± SD: 0.15 ± 0.08 mg/dL), p = .0004.

The higher values of creatinine in the males as compared to females and the stable creatinine levels in the age range studied, as seen on figure 1, are in line with the findings of other studies (Pottel et al., 2008). The differences observed between males and females on the scatter plots of individual data for ALT, AST, and gamma-GT (figures 2-4), with higher values in the males are in agreement with classical patterns for these enzymes (Dufour et al., 2011).

In a previous study (Gahutu and Wane, 2006), we reported protein and electrolyte values in a Rwandan student population. The findings of this study carried out in a rural population compare well with previous results from a student population. This may be related to the fact that blood donors have a similar diet as the urban or semi-urban population. However, the findings indicate that common reference intervals can be used for rural and urban populations. The chloride concentration in the present study is higher than levels in individuals living at sea level (Kratz et al., 2004; Lehmann and Henry, 2001); this is related to the moderate altitude. The previously reported slight chronic respiratory alkalosis, with complete metabolic compensation and decreased bicarbonate concentration (Gahutu et al., 2005), results in an increase in chloride concentration, which ensures electrical neutrality. At a higher altitude of 3,500 m, Siquès et al. (2007) found an increase in triglyceride levels after 8 months sojourn at altitude. At the moderate altitude of 1,768 m, at which there is no hypoxemia (Gahutu et al., 2005) our results do not show any increase in the lipid profile.

There are variations with findings of other studies in the region (Karita et al., 2009; Zeh et al., 2011) and at sea level (Kratz et al., 2004), which may be due to differences in the age of the study population and to different environment.
At a slightly higher altitude of 2,042 m, Kibaya et al. (2008) found values similar to ours except that in our study the values for hepatic enzymes in males are higher. Electrolyte values compare well with our study and the increase in chloride concentration is similar to the one observed in our study. Our results compare very well with those of Eller et al. (2008), from a population of blood donors in Uganda. Specifically, there is no statistically significant difference (p>0.05) between the two series concerning gamma-glutamyl transferase and creatinine (for males and females), direct bilirubin and total protein (for males) and triglycerides and albumin (for females).

4. CONCLUSION
The results of our study on clinical chemistry parameters in different population groups and at different sites in the Southern Province of Rwanda compare well with other African studies, with variations due to moderate altitude. The reference intervals from this study will be resourceful for Rwandan medical practice. Further sampling and laboratory tests need to be done for a complete scope of reference intervals.

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COMPETING INTERESTS
The author has declared that no competing interests exist.

CONSENT
The author declares that written informed consent was obtained from the study participants prior to the sampling. Copies of the written consent are available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL
The research project was approved by the ethics committee of the Faculty of Medicine of the National University of Rwanda.

REFERENCES


