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REFERENCE VALUES FOR SERUM PROTEIN AND ELECTROLYTE STUDY FROM RWANDA

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## REFERENCE VALUES FOR SERUM PROTEIN AND ELECTROLYTE STUDY FROM RWANDA

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### ABSTRACT

**Objective:** To estimate reference values of serum proteins and electrolytes in a student population in Butare, Rwanda (altitude: 1768 m; barometric pressure: 629 mm Hg).

**Design:** A laboratory based cross-sectional study.

**Setting:** The units of physiology and clinical chemistry, department of medical biology, Butare University Hospital and Faculty of Medicine, National University of Rwanda, from February 2002 to May 2003.

**Subjects:** Young healthy adults were selected randomly from the students of the National University of Rwanda, using Epi-Info 6.04 software.

**Results:** The results mean and reference range (2.5<sup>th</sup>–97.5<sup>th</sup> percentile) in brackets – are as follows: total proteins: male: 7.3 (6.3-8.4) g/dL, female: 7.3 (6.5-8.5) g/dL; albumin: male: 4.3 (3.1-5.2) g/dL, female: 4.1 (3.2-5.0) g/dL; globulins: male: 3.1 (2.0-4.2) g/dL, female: 3.2 (2.1-4.2) g/dL; sodium: male: 138.5 (130 – 147) mmol/L, female: 139.8 (132 – 153) mmol/L; potassium: 4.0 (3.1 – 5.0) mmol/L both in male and in female; chloride: males: 104.4 (96-112) mmol/L, females: 106.1 (98-114) mmol/L; phosphates: male: 1.13 (0.65-1.59) mmol/L, female: 1.17 (0.71-1.52) mmol/L.

**Conclusion:** The values of serum proteins and electrolytes are comparable to classical sea level values; however there is a slight increase in serum chloride.

### INTRODUCTION

We carried out a reference values study in order to characterise the physiological adaptation to moderate altitude in Rwanda. We report here reference values of serum proteins (total proteins, globulins, albumin) and electrolytes (sodium, potassium, chloride, phosphates) in a student population of the National University of Rwanda in Butare, at an altitude of 1,768 m, barometric pressure 629 mm Hg. Local reference values are important for African hospitals because they take into account local variations due notably to environment and population factors.

### MATERIALS AND METHODS

This study was carried out in the laboratory of Physiology of the Faculty of Medicine of the National University of Rwanda, during the period from February 2002 to May 2003. The test subjects were selected randomly with Epi-Info 6.04 software from the student population of the National University of Rwanda in Butare. The objectives of the study, the nature and the conditions of sampling and the voluntary character of participation to the study were clearly explained. Selected test subjects were young healthy adults, age range 20–37 years for male and 20–35 years for female. They had a

normal arterial pressure, and had spent at least one year in Butare and had permanently stayed there for the four weeks preceding the sampling. Females were not pregnant, not in the menses period, not breastfeeding and not on contraception. All test subjects were non-smokers. There was no apparent disease on anamnesis and physical examination. The absence of malaria, infection, verminosis or use of drugs in the month preceding the sampling was checked during anamnesis. Most of test subjects were non-drinkers, some drank beer only occasionally. The test subjects were asked to do no physical exercise in the morning of the sampling. Venous blood for protein assay and ionogram was sampled in the morning on subjects in physical rest and sitting position since at least 10 minutes, after overnight fasting. Blood was collected from the cubital vein, in a dry clean glass tube without anticoagulant. After coagulation, the clot was separated from serum by centrifugation.

Serum total proteins and albumin were assayed by molecular absorption photometry with an Eppendorf spectrophotometer, model 1101 M or, for some samples, with a Cobas apparatus (MIRA Plus ABX Diagnostics). The assay of total proteins was based on the principle of the Biuret reaction while the determination of serum albumin was made by the Doumas method using bromocresol green in a succinate buffer at pH 4.2. The level of globulins was obtained by making the difference between total protein and albumin levels. Inorganic

phosphorus was assayed based on the reaction with molybdate, by molecular absorption photometry with the 1101 M Eppendorf spectrophotometer. Serum chloride level was determined either by molecular absorption photometry based on the mercurimetric assay, with the 1101 M Eppendorf spectrophotometer or by ion-selective electrodes with a Cobas apparatus (MIRA Plus ABX Diagnostics). Sodium and potassium levels were determined by flame photometry by means of an Eppendorf flame photometer or, for some samples, by ion-selective electrodes with a Cobas apparatus (MIRA Plus ABX Diagnostics). When using ion-selective electrodes, sodium, potassium and chloride were directly measured on undiluted samples (direct method). The equipments were suitably calibrated. Forty-eight samples were analysed with the Cobas apparatus (MIRA Plus ABX Diagnostics) for total proteins and albumin (absorbance measurement) and for sodium, potassium and chloride (direct ion-selective electrode method). All other samples were analysed with the methods described above, using an Eppendorf spectrophotometer or an Eppendorf flame photometer respectively. Due to technical problems, some samples were not analysed. This led to a particularly limited number of analysed samples for serum chloride. We removed outliers which were very distant from the closest data element on the minimum and maximum sides or which were out of the range of mean  $\pm$  3 SD, before

Table 1

*Mean values and reference ranges for serum proteins and electrolytes*

	Total Proteins (g/dL)		Albumin (g/dL)		Globulins (g/dL)		Sodium (mmol/L)		Potassium (mmol/L)		Phosphates (mmol/L)		Chloride (mmol/L)	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
No.	145	86	145	86	145	86	161	78	140	73	156	88	63	16
Mean	7.3	7.3	4.3	4.1	3.1	3.2	138.5	139.8	4.0	4.0	1.13	1.17	104.4	106
SD	0.5	0.5	0.6	0.5	0.7	0.6	4.3	4.9	0.5	0.5	0.24	0.21	4.1	3.8
2.5 th –	6.3-	6.5-	3.1-	3.2-	2.0-	2.1-	130-	132-	3.1-	3.1-	0.65-	0.71-	96-	98-
97.5th	8.4	8.5	5.2	5.0	4.2	4.2	147	153	5.0	5.0	1.59	1.52	112	114
Percentile														

M = males; F = females; No. = number of subjects; SD = standard deviation

making the definite statistical analysis. The test of normality of distributions was done on the SPSS (Statistical Package for the Social Sciences), version 10.1.3, using the One Sample Kolmogorov-Smirnov test. Statistical analysis was done on Microsoft Excel software, using the statistical function to calculate the mean, the standard deviation and the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles. As an estimation of analytical quality, the coefficient of variation (CV), i.e. the standard deviation (SD) of the set of results divided by the mean results expressed as a percentage was calculated, using mean and SD values (Table 1).

## RESULTS

The mean age was 26 years for males and 25 years for females. The weight (mean  $\pm$  SD) was 63.7  $\pm$  7.3 kg in males and 59.9  $\pm$  8.2 kg in females.

All distributions were Gaussian (bilateral asymptotic significance  $>0.05$ ), except the distribution of serum potassium level in males (bilateral asymptotic significance  $<0.05$ ). Considering the high sensitivity of the test, a non-Gaussian result is still convenient, particularly for a sample size  $>120$ . Some parametric statistics are presented, notably the mean and standard deviation. However, we present the reference ranges based on non-parametric statistics: the 2.5<sup>th</sup> – 97.5<sup>th</sup> percentile interval, convenient for both Gaussian and non-Gaussian distributions. Table 1 presents results as mean, standard deviation and 2.5<sup>th</sup> – 97<sup>th</sup> percentile (reference range).

The rounded off results for the coefficient of variation (CV) are as follows: total proteins: 7%; albumin: 12%; globulins: 18%; sodium: 3%; potassium: 12%; chloride: 4%; phosphates: 18%.

## DISCUSSION

The values of serum total proteins and albumin are comparable to classical sea level reference values (1,2). This is probably due to the fact that the studied population consists of well-nourished students, while deviations from classical ranges, with a higher proportion of globulins, would be expected in rural under-nourished populations, with high prevalence of infectious diseases. Our reference ranges, particularly for total protein level are wider than other published reference ranges. This may be due

to differences in the precision of analytical methods and in sample size. Indeed, with a greater sample size, the standard deviation decreases, leading to a narrower reference range, when based on the mean  $\pm$  2 SD. Serum sodium, potassium and phosphate values are comparable to classical sea level reference values (1,2). For sodium distributions, the lowest data elements did not seem distant from the closest data and fell in the range of mean  $\pm$  3 SD. Therefore, no data element was removed as outlier, which may have led to a low 2.5<sup>th</sup> percentile for sodium. Different authors have studied the effect of altitude on plasma protein (3-5) and electrolyte (3-8) levels. These studies concern mainly adaptive mechanisms for lowlanders coming to moderate and high altitude and show slight serum protein and electrolyte level variations, mostly due to dehydration and increased natriuresis during acclimatisation, but also to hormonal effects. Our series is different since it concerns moderate altitude highlanders. A slight increase in serum chloride is observed in our series and ensures electrical compensation for the decrease in mean plasma bicarbonate level observed in the same population: 21.6 mmol/L in male and 20.4 mmol/L in female (9). A large-scale study is needed to establish local reference ranges.

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