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Research Article

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Reference Intervals for Potassium, Sodium and Chloride among Libyan Healthy People

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Abstract

Background: *In Libya, all laboratories use reference intervals (RIs) derived from other country's populations, which differ from Libyan population in many aspects such as daily habits and race. Due to these differences, it is assumed that those RIs are not applicable to Libyans.*

Aim: *The aim of this study was to establish RIs of sodium, potassium and chloride in serum of Libyan people.*

Materials and Methods: *Two hundred and fifty-seven blood specimens of healthy people (males and females) were collected using venipuncture untreated tubes. The specimens were centrifuged for 15 minutes and the obtained sera were analyzed for their content of sodium, potassium and chloride using direct potentiometry method.*

Results and Discussion: *The non-parametric percentile method was applied to establish the RIs of sodium, chloride and potassium, which were found to be: 135-143.3; 103-110; 3.7-5.2 mmol/L, respectively. There were no considerable differences in lower limits and/or upper limits of all established RIs between males and females, except that the upper limit of Cl^- for females was slightly higher than that for males.*

Conclusion: *As there are evident regional differences in RIs, the established RIs in this study will be more favorable in Libya than those listed in the manufacturer's kit or those adopted from other population-based references.*

Keywords: Reference Intervals; Sodium; Potassium; Chloride.

Introduction

Common electrolytes in human body include chloride (Cl^-), sodium (Na^+) and potassium (K^+) [1]. These electrolytes affect many body functions; balance the amount of fluid inside and outside of cells throughout the body and play a vital role in muscle contraction and heart function [2]. Without a correct balance of the concentration of these electrolytes, body cells would lack the essential electrical conductivity necessary for cellular energy production. Also, electrolyte imbalance can lead to life-threatening cardiovascular conditions [3].

Electrolytes play a vital role in maintaining homeostasis within the body; chloride, the anion of chlorine, along with sodium and potassium are responsible for osmotic pressure and acid-base balance. In addition to that, chloride has a passive role in electrolyte balance, and it is required for the production of gastric hydrochloric acid secreted from the parietal cells of the gastric mucosa in the stomach [4, 5]. Sodium plays a major role in maintaining blood volume and blood pressure by attracting and holding water, also it is important in cellular osmotic pressure, transmitting nerve impulses and maintaining a constant pH. Last of all, potassium also has its vital role as an important mineral that helps to regulate fluid balance, nerve signals and muscle contractions [6].

A correct interpretation of any biochemical test, including electrolyte tests, requires comparing the result with accurate reference intervals (RIs) derived from an appropriate population [7]. The reference interval (RI) for any biochemical in the human body is defined as the values encompassing the central 95% of specimens; equating to 2 standard deviations on either side of the mean, and it is established by analyzing specimens from a sample group of people who meet carefully defined criteria [8, 9]. Obtaining RIs for a general population is a major challenge, as it requires selecting the appropriate reference population and recruiting individuals who represent relevant demographic groups that meet the inclusion criteria**;** collecting, processing and testing specimens; and finally, calculating RIs [10].

The currently used RIs in Libya vary from one clinical laboratory to another because those laboratories use values, which have been derived from other countries' populations. These RIs are not considered as accurate values for Libyan population due to many variables including race, daily habits, lifestyle, and food type. Consequently, without using Libyan-based RIs, there could be considerable misinterpretations of results of any biochemical test and incorrect diagnosis due to using inaccurate RIs to assess these results. Thus, the objective of this study was to establish RIs of sodium, potassium and chloride in Libyan people serum to provide accurate RIs of theses three ions, therefore Libyan ministry of health could accredit these RIs and incorporate them into the clinical laboratory reporting system. To establish RIs of those electrolytes, blood specimens were collected from a group of healthy Libyan people and their serum were analyzed for sodium, potassium, and chloride. The results were then statistically treated to create RIs for these electrolytes.

Materials and Methods

Subjects and specimen's collection: A total of two hundred and fifty-seven healthy people, 120 males and 137 females, aged 1-89 years were included in this study, which was carried out at Bushra Medical laboratory, Tripoli, Libya, and lasted from March to August 2018. Each subject had normal blood pressure and was a non-smoker, non-alcohol abuser and non-drug abuser. A blood specimen was drawn carefully by venipuncture from the right arm of each subject, after 12 hours overnight fasting, and transferred into untreated (red-topped) tube (Labchem Sdn Bhd, Damansara Kim, Petaling Jaya, Selangor, Malaysia), which was filled to at least 2/3 of its total volume. The collected specimen was left standing for 30 minutes to allow clot formation, centrifuged for 15 minutes at 1500 rpm, and then the serum was transferred to a clean specimen tube and analyzed immediately for its content of Na^+, K^+ and Cl^-.

Ethics Approval: The study was approved by the biomedical ethics committee of Bushra Medical laboratory and informed consent was obtained from all subjects before blood specimen was drawn.

Instrumentation and analysis: All measurements of electrolyte concentrations were carried out using EasyLyte® analyzer (Medica Corporation, Bedford, MA, USA). The accuracy and precision of measurements were verified according to manufacturer's recommendation, and the measurement of the three ions concentrations in each sample was carried out according to the instruction manual of the instrument. The EasyLyte® analyzer is an automated, microprocessor-controlled analyzer for the measurement of sodium, potassium, chloride, calcium and pH in serum, plasma, whole blood and urine. The instrument uses ion-selective electrodes to measure the above-mentioned ions in each sample by direct potentiometry method. The instrument measures the potential of the standard solution and that of the sample then calculates the ion concentration solution in accordance with the Nernst equation (equation 1). The standard solution was purchased from the instrument's company and the concentration for each ion was 140.0 mmol/L Na^+; 4.0 mmol/L K^+; 125.0 mmol/L Cl^-. The analysis takes 55-60 seconds, and it requires only 100 µL of serum, plasma, or whole blood, or 400 µL of diluted urine.

$$E = E^{o} - \frac{0.05916}{n} \log Q \qquad (1)$$

Where E is the measured potential, E^o is the standard-state reduction potential, n is the number of electrons in the redox reaction, and Q is the reaction quotient.

Statistical analysis: Statistical analyses were done by Minitab 17 (Minitab Inc., State College, Pennsylvania, US), Microsoft Excel 2013 (Microsoft Corp., Seattle, WA, USA) and MedCal Software (MedCalc Software, Mariakerke, Belgium).

Results and Discussion

This study included 257 healthy subjects with ages from 1 to 89 years. The mean of age (M) and its standard deviation (SD) are shown in Table 1 as well as its skewness, Kurtosis, minimum (Min) and maximum (Max) values. The small negative value of skewness, shown in Table 1, indicated that age data was left-skewed or most values were higher than the age mean. On the other hand, the negative value of kurtosis revealed that age data was flatter than the normal distribution.

Table 1: Descriptive Statistics of Age

M	Min	Max	SD	Skewness	Kurtosis
49.28	1	89	20.83	-0.24	-0.81

Outliers Test for Ion Concentrations Data: As outliers can alter the reference interval significantly, Dixon's r22 ratio test was applied to test ion concentration data for outliers. Four values were detected as outliers at $P<0.05$ and were excluded [11, 12].

Descriptive Statistics of Ion Concentrations Data: The highest value for the coefficient of variance (CV%) was for potassium (Table 2) indicating it was more variable relative to its mean compared to sodium and chloride, which were found to have similar variability as their CV% values were close. The positive values of kurtosis of the three ions pointed out that the distributions of their concentrations were sharper than the normal peak. The skewness was negative for both of sodium and chloride concentrations data showing that their distributions were left-skewed or most values were higher than the mean, while the positive value of skewness in case of potassium concentration data indicating that its distribution was right-skewed or most values were lower than the mean. As long as kurtosis and skewness of concentrations data were far from zero, the data distribution could be not normal, thus a normality test was needed.

Table 2: Descriptive Statistics of Ion Concentration Data

	M	SD	CV%	Skewness	Kurtosis
Sodium	140.31	1.96	1.39	-1.17	2.15
Chloride	107.59	1.97	1.83	-0.85	2.49
Potassium	4.28	0.37	8.73	0.61	0.78

Normality test: As shown in figures 1, 2 and 3, the plotted points do not form straight lines suggesting that the distributions of ion concentration are not normal. This was confirmed by the results of the Kolmogorov-Smirnov test for normality, as the p-value was less than 0.01 for the three ions concentrations data. Thus, at this level, the null hypothesis was rejected for each set of ion concentration data and it was concluded that the population of the three ions was not normal [13].

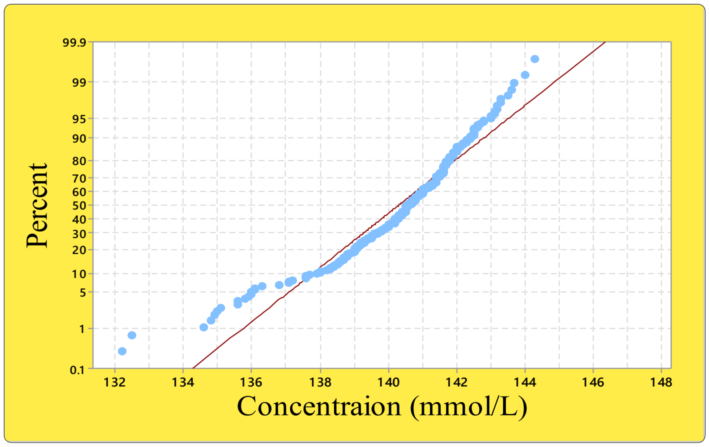

Figure 1: the probability plot of sodium concentration

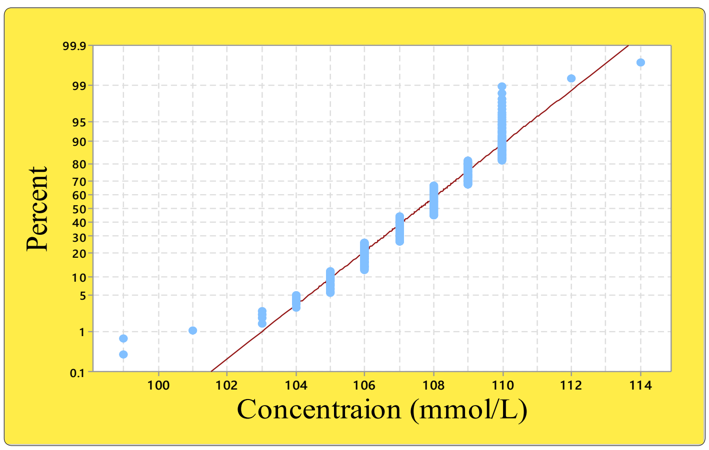

Figure 2: the probability plot of chloride concentration

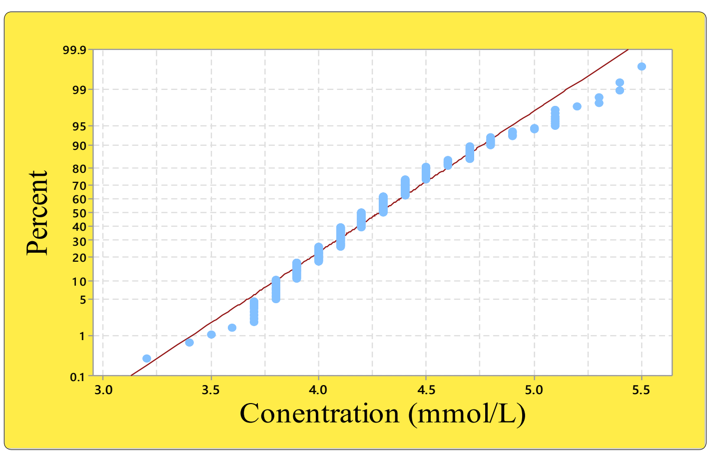

Figure 3: the probability plot of potassium concentration

Establishing the reference intervals: As the distributions for the concentration of the three ions were not normal, the non-parametric percentile method was applied to establish the RIs of the three ions by using MedCal Software (MedCalc Software, Mariakerke, Belgium). The software performs the operation according to the Clinical and Laboratory Standards Institute (CLSI) guidelines C28-A3 [14]. The method is simple and fast and it includes sorting all N values ascendingly, then assigning rank numbers to each value so that the lowest value has the rank l, and the highest value has rank N. After sorting and ranking the values, the rank number of the 0.025 fractal is calculated as 0.025* (N + 1) and that of the 0.975 fractal as 0.975 *(N+l). The lower reference limit is the reference value corresponding to the rank number of the 0.025 fractal, while the upper reference limit is equal to the value corresponding to the rank number of the 0.975 fractal [15]. Table 3 shows the RI for each ion as well as the confidence interval (CI) at 90% for lower and upper limits of RIs.

Table 3: Reference Intervals (mmol/L) and Confidence Intervals of Three Ions

Chloride	**RI**	**103-110**
	90% CI for a lower limit	101-104
	90% CI for an upper limit	110-110
Potassium	**RI**	**3.7-5.2**
	90% CI for a lower limit	3.5-3.7
	90% CI for an upper limit	5.1-5.4
Sodium	**RI**	**135-143.3**
	90% CI for a lower limit	134.6-136
	90% CI for an upper limit	143-143.7

RIs for laboratory parameters provide important data for interpreting clinical and research findings, thus the accurate determination of RIs by a laboratory is exceedingly important as a guide in clinical management of patients [16]. Because there is a paucity of RI data in Libya, practicing physicians, laboratory professionals and researchers often depend on western-derived RIs. Several studies have highlighted apparent regional differences in electrolytes values [8, 17-20]. Hence, studies should be carried to establish locally derived reference intervals that may improve the quality of clinical care provided to patients and to perform accurate clinical researches. As far as we know, there have been almost no reports in the literature about RIs derived from population-based study on electrolytes in Libyan population, thus this study provides the first electrolytes RIs for healthy Libyan people. The importance of our data regarding electrolyte ranges lies in that the number of males and females in the study populations were statistically enough to determine meaningful 95% RIs for both genders together (Table 3) and separately (Table 4), with ≥120 participants per group.

The findings of this study revealed that there were no considerable differences in lower and/or upper limits of all established RIs between males and females, except that the lower limit of Cl^- for females was slightly higher than that for males (Table. 4), which was almost similar to those recorded in Turkey by Bakan *et al.* [21]. As shown in Table 3, the RIs obtained for Na^+ and K^+, and the upper limit for Cl^- in this study were lower than consensus measurements from a study conducted by Kibaya *et al.* in Kenya [17]. In a similar way, RIs upper limits for Na^+ and K^+ were higher, while their lower limits, besides lower and upper limits for Cl^- were slightly similar when compared to those reported in subjects from Denmark [22]. Compared to a study in Botswana by Segolodi *et al*., the lower limits for RIs of the three ions in our study were higher, whereas the upper limits for Na^+ and K^+ were dramatically lower [10]. In the previously mentioned Turkish study, the RI upper limit for Na^+ was found to be higher, while lower and upper limits for Cl^- were lower, but RI lower and upper limits for K^+ were nearly similar when compared to our study [21]. However, the outcomes of this study were almost similar to those of a study conducted among the Saudi population by Borai *et al*, that might due to the participation of culture, religion, lifestyle and food type, which might need further studies to determine the influence of these factors on RIs [23]. In contrast, the observed differences in lower and/or upper limits of RIs of the studied electrolytes may reflect regional characteristics of the population, and nutritional and environmental factors.

Table 4: RIs (mmol/L) According to Gender

	Males	Females
Sodium	135 - 143.1	134.9 - 143.6
Chloride	101 - 110	104 - 110
Potassium	3.7 - 5.1	3.6 - 5.2

Conclusion

The evident regional differences in RIs reflects the usefulness and importance of establishing RIs for local populations. The established RIs in this study will be more favourable in Libya than those listed in the manufacturer's kit or those adopted from other references, and these RIs could be used by the Libyan Ministry of health in establishing Libyan population-based RIs for standardization of laboratory diagnosis. Other studies are needed to test the role of age on RIs of Na^+, K^+ and of Cl^-, as well as to establish RIs for other biochemicals for Libyans in urine, blood and other body fluids.

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