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Original Article

Estimation of Reference Values of Biochemical Parameters Exploring the Renal Function in Adults in Ngaoundere, Cameroon

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Clinical examinations are accompanied by biological analyzes to guide or confirm the clinical diagnosis. The results of these analyzes are interpreted by comparison with reference values. Studies on the biological norms of Africans are rare if not quasi-nonexistent. The aim of this study was to establish population-specific reference values for biochemical indices serving as renal function biomarkers in adults living in Ngaoundere, Cameroon. 205 presumed healthy subjects (118 women and 87 men) residing in the city of Ngaoundere aged 18 to 50 years from various socio-cultural backgrounds, were included in this study. Blood urea and creatinine were tested under standard conditions. Reference intervals varied between 10-44 mg/dl and 0.6-1.4 mg/dl for urea and creatinine, respectively. Urea mean values (27.93± 8.15 mg/dl) were higher in males in comparison with females (23.56± 7.14 mg/dl) (P= 0.02). Similarly, creatinine mean values (1.11 ± 0.13 mg/dl) were higher in males in comparison with females (0.82± 0.11 mg/dl) (P= 0.04). Also, an increase in uraemia was observed with age. The gender differences for creatinemia and uraemia may be related to anthropometric features differences such as the muscle mass. The increase of uraemia with age could be explained by the increase of poor hygiene, notably the consumption of alcohol, tobacco and meat, which is mostly a male habit. The results of this study may help to a better interpretation of the biochemical indices of African subjects, and avoid errors of appreciation by excess or by default.

Keywords: Biochemical parameters, urea, creatinine, Cameroon

Biochemistry is the science that studies the structure of living molecules, their concentration in each cell or biological fluid, their mode of formation (anabolism, catabolism). Therefore physiological mechanisms of metabolic regulation allow healthy subjects to maintain the biochemical constituents of biological media within the limits of concentrations considered normal or usual values. Thus, qualitative and even quantitative variations of these biological parameters may be indicative in pathological states. This is one of the very aims of clinical biochemistry, which is to assess a pathological condition by measuring the degree of qualitative or quantitative modification of a biochemical parameter.

Clinical examinations are accompanied by biological analyzes to guide or confirm the clinical diagnosis. The results of these analyzes are interpreted by comparison with reference values of the European populations provided by the manufacturer. However, European or North American standards do not necessarily reflect those

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of other ethnicities (1-2). Other factors such as altitude, environmental, gender and genetics can also affect on laboratory parameters (3-5). Studies on the biological norms of Africans are rare if not quasi-nonexistent. Studies by Yapo in Ivory Coast (6), Boum and Tantchou in Cameroon (7), Acker in Congo (8) have shown that there are differences between the mean values of some biological parameters between African and European populations. These differences may be due, among other things, to nutritional and environmental variations. If one adds the notion of intra- and inter-individual biological variations, it is clear that the reference values cannot be transposed indifferently from one country to another. Thus, in the course of an international co-operative study on transferability of reference values, Vincent-Viry et al. concluded that reference values should be adapted to geographical origin and the ethnic factor should be taken into account in Africa (9). The objective of the present study was to contribute to the improvement of the interpretation of laboratory results in Cameroon, particularly in Ngaoundere, by establishing population-specific biochemical indices reference values.

The biochemical parameters were determined in Ngaoundere (city of Cameroon, Central African country) with reagents supplied by European laboratories. The results of these analyzes were interpreted by comparing them with reference values of European populations supplied by the manufacturer.

Materials and methods

Subjects

The study population was composed of 205 presumed healthy subjects (118 women and 87 men) residing in the city of Ngaoundere aged 18 to 50 years from various socio-cultural backgrounds, selected rigorously using well-defined criteria (6). Relatively, a suspected healthy subject was a non-afflicted subject who did not take medication, alcohol, tobacco, was not in a particular physiological condition and did not have risk factors (obesity). The homogeneity by weight and size of the population was verified using the Fischer-Snedecor F-test. In order to search for age-related variations, we divided our population into three homogeneous sub-samples (A: 18-30 years, B: 31-40 years, C: 41-50 years). This distribution takes into account the evolution of size and weight according to sex and the age reported by Henny et al. (10). Sub-sample A was composed of 101 individuals including 40 men and 61 women; Sample B counted 54 individuals including 24 men and 30 women; Sample C also included 50 individuals composed of 21 men and 29 women. A preliminary interview to explain the purpose of the study, the expected results and their potential usefulness has been carefully carried out in order to obtain informed consent from each subject.

Biochemical analyzes

Subjects who have been resting for at least 30 min have undergone a venous blood sampling at the elbow bend between 7:00 am and 10:00 am to account for nycthemeral variations. Blood was collected in tubes without anticoagulant, centrifuged at 5000 rpm within 30 min of collection according to serum biochemical parameters determination recommendations (11). The serum was aliquoted and stored at -20 ° C until assayed. Blood creatinine levels were determined by kinetic Jaffe reaction using Kinetic creatinine kit (SGM Italia), while urea levels were determined by a modified Berthelot reaction using Urea kit S 180 (SGM Italia).

Statistical analyzes

The data were collected on the Excel 2013 file and the main statistical parameters, namely the mean (m), the standard deviation (s) at risk $\alpha = 5\%$ were determined by the R software version 2.13.0. The frequency was obtained according to the following formula: $F = ni / N$ multiply by 100. To determine the RI (reference interval), the parametric method of GAUSS at the risk $\alpha = 5\%$ ($RI = m \pm 1.96\ s$) was used. $P < 0.05$ was considered as statistically significant.

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Results

Reliability of analytical methods

The evaluation of the reliability of the methods used is shown in Table 1. According to the data corresponding to the accuracy and precision of the assay, it can be concluded that the methods used are reliable and allow us to exclude analytical biases.

Reference values

The estimation of the reference values of the urea and creatinine according to sex is shown in Table 2. Comparison of values by sex showed a significant increase in creatinine (P = 0.02) and uraemia (P = 0.04) in males. The study of reference values showed an increase in uraemia with age (Table 3).

Table 1. Evaluation of the reliability of the methods used

Constituents analyzed	Accuracy (n = 20)		Precision		
	True serum value of Xo Control	Average value X	Percentage of overlap	Intra-serial CV (n = 20)	Inter-series CV (n = 20)
Urea (mg/dl)	13.69	14	97.78	0.56	0.55
Creatinine (mg/dl)	0.8	0.82	97.56	1.35	1.45

CV: coefficient of variation; n: number of samples.

Table 2. Estimation of the reference values of urea and creatinine according to sex

Parameters	Male		Female		P
	m (s)	RI	m (s)	RI	
Urea (mg/dl)	27.93 (8.15)	[12 - 44]	23.56 (7.14)	[10 - 38]	0.02
Creatinine (mg/dl)	1.11 (0.13)	[0.9 - 1.4]	0.82 (0.11)	[0.6 - 1]	0.04

m: average; s: standard deviation; RI: reference interval; P: P value.

Table 3. Estimation of the reference values of urea and creatinine according to the age group

Parameters	[18 -30)		[31 - 40)		[41- 50)		P
	m (s)	RI	m (s)	RI	m (s)	RI	
Urea (mg/dl)	25.56 (7.09)	[12 - 40]	28.02 (8.07)	[12 - 44]	29.40 (9.10)	[12 - 47]	0.04
Creatinine (mg/dl)	1.14 (0.13)	[0.9 - 1.4]	1.1 (0.2)	[0.7-1.4]	1.02 (0.2)	[0.6 – 1.4]	0.03

m: average; s: standard deviation; RI: Reference Interval; P: P value.

Discussion

Recognizing precise reference values for biochemical markers is the most important step in clinical evaluation. Particular ethnicities need to be defined regarding their reference intervals before interpreting biochemical tests results. In order to find reference values in the studied population, the reliability of the analytical methods used in the present study were assessed. For accuracy, the comparison using the Student t-test showed that there was no significant differences between the target value of the serum and the average obtained at risk α = 5%. Intra-serial CVs (repeatability) and inter-serial CVs (reproducibility) were between 0.55 and 1.45; which makes it possible to state that the methods have acceptable precisions compared to the CVs reported by Vassault et al. (12).

The differences of the creatinine and urea levels between males and females found in the present study are in good agreement with the literature and could be related to various factors including anthropometric features. Thus, the higher

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muscle mass of males in comparison with females may be one of the factors at the origin of the differences observed. We also observed an increase of blood urea levels with age. This increase could also be explained by the fact that, with age, the poor hygiene of life increases, notably the consumption of alcohol, tobacco and the phenomenon of food from street to street represented by the consumption of meat, which is mostly a male habit. In general, our results are close to those reported by other studies reported in Ivory Coast and Cameroon (1, 2). We note that the values of uraemia and creatinemia with which we compared our results are values of countries located in humid tropical zone whose protein source is mainly represented by fish and meat. In Ngaoundere, on the other hand, the Sudano-Sahelian region, whose agricultural activity is dominated, livestock is the main source of protein.

It emerges from this study that the use of European reference values for interpreting the results of African subjects could lead to errors of appreciation by excess or by default. This could lead to a late diagnosis of pathologies. Moreover, with subnormal results, which cannot be decided, the clinician requests further examinations. This additional demand for examination leads to an inflation of the health benefits that are paid by patients without social insurance in Africa. Finally, this study suggests that while the fairly homogeneous standard of living in Europe allows us to talk about European benchmarks, this is not the case in Africa, where the level of living varies from one country to another and the same country between the city and the countryside raises the problem of transferability of reference values.

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Conflict of Interest

The authors declared no conflict of interest.

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