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ORIGINAL ARTICLE

Determination of the Reference Intervals of Clinical Biochemistry Tests by Direct and Indirect Methods: a Multicentric Study

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SUMMARY

Background: **The aim of this study was to determine the reference intervals of 14 clinical biochemistry tests in healthy individuals aged 18 - 65 years. The reference intervals determined by using direct and indirect methods were compared with each other and the manufacturer's RI in terms of gender.**
Methods: **Blood was collected from 302 reference subjects selected on the basis of admission and exclusion criteria based on the procedures set out in document C28-A3, and 14 clinical chemistry tests were performed using the analytical systems available in our laboratory. The analyses were conducted using the MedCalc and SPSS20 programs in the direct method and the Bellview (1.2.6 Version) program in the indirect method, according to the Bhattacharya procedure.**
Results: **Nine biochemical tests showed statistically significant differences according to gender ($p < 0.05$). These tests include alkaline phosphatase, lactate dehydrogenase, high-density cholesterol, low-density cholesterol, urea, uric acid, triglycerides, total cholesterol, and inorganic phosphate.**
Conclusions: **The direct method was the first method used to obtain the reference intervals. The indirect method can be used as an alternative to the direct method for AMLY and UA tests for the general population. According to the manufacturer's RI, lower and upper limits of HDL, LDL, Ca, and Mg were compatible with indirect RI in two genders. Lower and upper limits of ALP, LDH, and ALB were compatible with manufacturer's RI in female.**
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KEYWORDS

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INTRODUCTION

The reference interval was defined as the predicted interval containing the test results obtained from healthy individuals (reference individuals). The clinical interpretation of the values measured in clinical biochemistry laboratories using reference intervals and the reliability of these values in the diagnosis, treatment, and follow-up of diseases enable an accurate assessment of the patients' clinical conditions. Reference intervals vary depending on the educational level, economic con-

ditions, and geographical differences among communities. Therefore, each laboratory should determine its reference values for that region, which reflect the community in the region it serves, using standard methods.

The Clinical and Laboratory Standards Institute (CLSI), in its EP28-A3c guide, identified the establishment of reliable reference intervals as an important task for manufacturers of clinical laboratories and diagnostic test kits and published a set of clauses on rules and methods to be followed in this process [1]. This guide describes the criteria for the selection of reference intervals, sampling procedures, requirements for the verification and validation of the analytical method, and statistical methods used to establish the reference interval. However, another method used to generate reference intervals is the indirect method, in which the reference interval is generated by using the laboratory data of the individuals who apply to the hospital [1,2]. This method can be used as the first choice in cases where sampling healthy individuals is difficult. Although this approach is relatively simple and inexpensive, the filtration criteria must be well defined when obtaining laboratory data.

This study aimed to identify the reference interval for 14 clinical biochemistry tests in individuals aged 18 - 65 years. A total of 302 volunteer reference individuals were included in this study to determine a direct reference interval. To determine the indirect reference interval, the patient-reported test results in the hospital information system were used as data. These values were also evaluated according to gender. The individuals involved in both methods were mostly those living in Istanbul. In this regard, when comparing different regions, it was assumed that these individuals reflect the Istanbul region.

MATERIALS AND METHODS

This study is multicentric and has been approved by the Ethics Board for Clinical Research at Bakirkoy Dr. Sadi Konuk Education and Research Hospital (07-18-2022 until 09-14-2022). The reference individuals, which will be the main reference method, were selected voluntarily from those who applied to the Haseki Educational and Research Hospital and the Bakirkoy Dr. Sadi Konuk Educational and Research Hospital. The tests were conducted at the Haseki Educational and Research Hospital. The test data source for the indirect method was the Biochemistry Laboratory at Haseki Educational and Research Hospital. The selection of volunteers was based on the admission and exclusion criteria set out in the questionnaire in accordance with the IFCC standards. A total of 302 volunteers were involved in the study, including 152 men and 150 women in different age groups ranging from 18 to 65 years. The ages and numbers of participants are presented in Table 1.

Blood samples were collected by experienced nurses in the blood collection division of the Haseki Center for Education and Research Hospital in two 7.5 mL biochemical tubes of the Sarstedt Monovette. The tubes were turned up and down at least four times. The biochemical tubes were protected from light and allowed to rest at room temperature for 20 - 30 minutes to create fibrin networks. The samples were then centrifuged at 3,500 g and +4°C for 10 minutes. Each resulting serum sample was divided and labeled with approximately 3 - 4 Eppendorf (1 mL). Portioned samples were stored at -20°C until working day.

Routine biochemical tests were performed in this study. These were: albumin (ALB), alkaline phosphatase (ALP), amylase (AMLY), high-density lipoprotein-cholesterol (HDL-COL), low-density lipoprotein-cholesterol (LDL-CHOL), calcium (Ca), phosphate (P), magnesium (Mg), total cholesterol (CHOL), lactate dehydrogenase (LDH), triglyceride (TG), urea (URE), and uric acid (UA) and lipase (LIP).

Obtaining indirect patient data

In this study, test results from the Clinical Biochemistry Laboratory of patients (outpatients and inpatients) admitted to the Haseki Training and Research Hospital between April 2022 and September 2022 were used to obtain indirect reference intervals. The biggest disadvantage in calculating the reference interval indirectly from hospital data is that there are many abnormal results for patients in the datasets. To minimize this effect, hospital data were filtered. The criteria used were as follows:

1) Data from patients aged 18 - 65 years who were treat-ed between April 2022 and September 2022 were used. These data were assessed according to gender sub-groups.
2) Emergency care, intensive care, nephrology, gastroenterology, oncology, endocrinology, and hematology polyclinics were not included.
3) The initial results of patients with more than one test request per year during the study period were used.
4) Non-numeric data (>, <, etc.) were excluded.
5) Patient data without gender or age information were excluded.
6) Patient data with more than three interval results for the same test request were not included in the study.
7) Patients with lipemia, hemolysis, and jaundice-positive test results were excluded.
8) Data from pregnant women were not used. The data were divided into three groups: women, men, and general population aged 18 - 65 years.

The Bhattacharya method was used to determine the reference interval values for each group. Patient samples were studied by using the Cobas 8000 biochemical autoanalyzer (Roche Diagnostics, GmbH, Mannheim, Germany) used in our hospital's biochemistry laboratory. The patient data used to obtain the indirect reference interval were obtained from the Cobas 8000 biochemical autoanalyzer (Roche Diagnostics, GmbH, Mannheim, Germany) used in our hospital's biochemistry laboratory. General maintenance and technical inspections of the Cobas 8000 autoanalyser were performed. All 14

routine biochemical analyses were performed using the original kits from Roche Diagnostics in an autoanalyzer. A commercial multi-calibrator (CFAS 56499500) was calibrated, including enzymes, in accordance with the specified procedures. The CFAS Lipid Calibrator (lot number: 57408400) calibrated the HDL-CHOL and LDL-CHOL tests.

Statistical analysis of reference intervals

Three general statistical methods, nonparametric, parametric, and robust, are described in the CLSI EP28-A3c guide [1]. The nonparametric method makes no special assumptions regarding the probability distribution of the observed reference values. Moreover, it remains the recommended procedure for establishing the reference intervals. The most important issue in developing reliable reference intervals is to select appropriate reference individuals, test a sufficient number of subjects, and avoid preanalytical errors, rather than the statistical method used to estimate reference intervals from observed data [1]. The parametric method estimates the Gaussian (i.e., "normal") probability distribution of observed values or some mathematical transformation of those values. Because the reference values of many analytes do not follow a Gaussian distribution, the parametric method requires that they be transformed in order to "normalize" them. This requires selecting the most appropriate transformation (e.g., logarithmic, power, or other functions) and testing on whether the transformed reference values fit a Gaussian distribution [3].

The robust method can be considered an intermediate form between the parametric and nonparametric methods. The robust method is used in various situations where the available sample size is less than 120 but the underlying population does not follow a Gaussian distribution [4].

Different techniques have been defined to calculate the reference interval using an indirect method. Hoffmann observed that the distribution of routine test results, regardless of the analyte, had a central smooth-looking peak that could be assumed to represent “normal” values and approximate a Gaussian distribution [5]. However, a problem with this method is that the assumption is always Gaussian, without considering other distribution models [6]. Bhattacharya [7] developed another graphical method for identifying one or more Gaussian peaks in a dataset. This method has been applied to laboratory data by assigning the largest peak to represent the reference population and deriving reference intervals. In this study, the Kolmogorov-Smirnov test was applied to ensure that the data conformed to a normal distribution.

The significance of the difference in terms of averages for men and women in parameters with a normal distribution was evaluated using the independent sample *t*-test, and the parameters with a non-normal distribution were evaluated using the Mann-Whitney U test. The Dixon D/R equation was used to exclude extreme values. In the direct and indirect methods, the 20th version of the licensed IBM SPSS (Statistical Package for the Social Sciences) program, the licensed MedCalc program, and the Bellview program were used to extract data, draw graphs, and analyze data.

CLIA 19 acceptable limits were used to assess, whether there was a difference between the RIs calculated by the two different methods and the manufacturer’s RIs. The difference between two reference values was considered compatible if it was less than 1 mg/dL for Ca, 15% for Mg, TG, and LDH, 10% or 0.3 mg/dL for P, 10% for AMLY, UA, and CHOL, 20% for HDL, ALP, and LDL, 8% for ALB, and 2 mg/dL for URE [8]. The difference between two reference values was considered compatible if it was less than 10% for LIP.

The importance of the amount of data in determining the reference interval

Although care is taken to ensure that reference individuals are healthy, there will always be some uncertainty in a given selection protocol due to the possibility that some of the selected subjects may actually have subclinical disease [9]. Therefore, the greater the number of data points, the more reliable the method used. According to some sources, it is recommended to use at least 400 reference individuals to calculate a statistically reliable reference interval [10].

The CLSI EP28-A3c guideline recommends determining reference intervals using a nonparametric method and that the sample size should consist of at least 120 values. If the dataset is below 120, the reference interval can be calculated using the robust [11] and bootstrap [12] methods. There are various opinions regarding the amount of data that should be used in indirect reference interval studies. A group in the Netherlands used the term "big data" in a study called the NUMBER Project, in which the reference interval was calculated using an indirect method [13].

RESULTS

The demographic data of working group individuals between the ages of 18 and 65 years were included in this study. A total of 1,557,800 test results were examined. After applying the exclusion criteria, 560,818 (35%) data points were included in the study; 330,910 (59%) of these data belong to female patients and 229,908 (31%) belong to male patients. Reference intervals and confidence intervals obtained by using the direct method for the 14 tests selected from routine biochemical parameters are shown in Table 2 in parametric and nonparametric formats.

The Asymp. Sig. (two-tailed) were examined. When p was greater than 0.05, there were no significant differences between men and women in the AMLY, Ca, Mg, ALB, and LIP tests. Independent sample *t*-test results, which show the significance of the difference between the male and female means of the normally distributed CHOL and P tests, are shown in Table 4. The difference

Table 1. Age and number of participants.

Gender	18 - 29 years	30 - 39 years	40 - 49 years	50 - 64 years	> 65 years
Male	67	43	27	15	-
Female	78	35	26	11	-

Table 2. Parametric and nonparametric reference intervals obtained by direct method.

				Parametric						Nonparametric			
Test	n	90% CI		RI		90% CI		90% CI		RI		90% CI	
ALP	302	0	6.2	1	125	119.9	130.3	24	34	29.5	107	102	130
LDH	302	56.2	68.8	62.5	213	207	219.6	47	67	57.1	205.8	200	215
AMLY	302	14.1	20.8	17.4	98.1	94.7	101.5	18	28	24.5	101.4	99	112
TG	302			0	217	208	227.2	26	37	31.1	247	227	399
CHOL	302	64	77	71	225	319	232	50	81	68	217	207	241
HDL-CHOL	302	17.3	21.5	19	66	66.9	71.1	16.8	22.5	18	71	67.6	75
LDL-CHOL	302	27.1	36.2	31	140	136	145	22	41	30	147	134	161
Ca	302	6.9	7.2	7.1	11	10.8	11.1	5.3	6.8	6.2	10.5	10.2	10.9
P	302	2.06	2.26	2.16	4.48	4.38	4.57	1.7	2.4	2.25	4.6	4.4	4.7
Mg	302			0	4.2	4.1	4.4	1	1.5	1.2	2.3	2.27	2.6
ALB	302	27	29.4	28.2	57.3	56.1	58.5	19.5	24.7	23.8	50.5	50.3	51.1
URE	302	10	21.4	11.2	39.7	38.5	40.9	12	16.2	14.9	43.7	41.3	48.2
UA	302	17	2.09	1.9	6.3	6.1	6.5	1.6	2.4	2.05	6.14	6.09	6.85
LIP	302	0.85	4.98	2.9	52.2	50.1	54.3	9	12	10.5	59.8	52	83

between the gender averages of the tests that did not show a normal distribution is shown in Table 4. According to these results, there was a significant difference between genders in the ALP, LDH, HDL, LDL, URE, UA, and TG tests ($p < 0.05$). An independent sample *t*-test was performed to evaluate whether there was a difference in the means of the normally distributed CHOL and P parameters. The difference in the CHOL and P parameters between the groups was found to be significant ($p < 0.05$).

Table 5 shows that the Bhattacharya and direct methods were found to be significantly consistent and compatible with each other in lower and upper limits for AMLY and UA in the general population. The upper limits of RIs determined was compatible except for LDH, the lower limits of RIs were not compatible except for AMLY and UA.

Table 6 shows that according to the manufacturer's RI, lower and upper limits of HDL, LDL, Ca, and Mg were compatible with indirect RI in both genders. Lower and upper limits of indirect RIs for ALP, LDH, and ALB were compatible with manufacturer's RI in females.

Lower and upper limits of ALP, HDL, and LDL and upper limits of Ca, ALB, UA, LIP, and P in direct methods were compatible with manufacturer's RI in both genders. Upper limit of TG was compatible in females and CHOL, Mg, and URE were compatible with manufacturer's RI in males.

DISCUSSION

Reference intervals are an important part of the clinical decision-making process. The reference intervals reported by laboratories are used by clinicians to interpret patient test results. Therefore, establishing reliable reference intervals is important for laboratories. These intervals used in the interpretation of biochemical tests may be different for each laboratory and region due to uncontrollable (age, gender, etc.) and controlled (fasting state, exercise, etc.) variables, ethnic factors, genetic factors due to geographical location, and methodological factors. For these reasons, the CLSI recommends in its EP28-A3c guidelines that each laboratory should determine its own reference interval values.

Although the process recommended by IFCC is a direct sampling method, it is difficult and costly in terms of the selection of reference individuals. It should be noted

Table 3. Gender averages of tests that do not show normal distribution.

Test	Gender	n	Parametric		Nonparametric		Mean	SD	Sig. (two-tailed)
			lower limit	upper limit	lower limit	upper limit			
ALP (IU/L)	male	152	0	147.4	27.7	121.3	66.9	41	0.02 *
	female	150	25.9	92.4	32.4	101.1	59.2	17	
LDH (IU/L)	male	152	52.8	208.4	53.3	208.1	130.1	40.2	0.002 *
	female	150	77.1	214.5	69.1	205.4	145.9	35.1	
AMLY (IU/L)	male	152	17.5	96.6	24.6	101.3	56.9	20.5	0.472
	female	150	18.1	99.2	24.7	102.2	58.7	20.7	
HDL (mg/dL)	male	152	18.4	58.9	16.9	57.6	38.6	10.4	< 0.001 *
	female	150	25.9	73.9	24.8	74.1	49.9	12.2	
LDL (mg/dL)	male	152	31	130.5	25.8	133.3	80.5	25.4	0.001 *
	female	150	34.9	148.8	34.8	152	91.9	29	
Ca (mg/dL)	male	152	6.7	11.1	6	10.6	8.97	1.12	0.231
	female	150	7.5	10.8	6.3	10.3	9.18	0.84	
Mg (mg/dL)	male	152	1.2	2.4	1.2	2.3	1.88	0.3	0.249
	female	150	0	5.3	1.4	2.2	2.05	1.66	
ALB (g/L)	male	152	25.7	58.6	23.5	50.7	42	8.6	0.562
	female	150	31.9	55.3	27.1	50.4	43.60	6.00	
URE (mg/dL)	male	152	13.9	42.9	16.8	47.4	28.6	7.4	< 0.001 *
	female	150	11.2	33.6	12.3	36	22.4	5.4	
UA (mg/dL)	male	152	2.4	6.8	2.3	6.8	4.63	1.11	< 0.001 *
	female	150	1.8	5.4	1.9	6	3.64	0.93	
LIP (mg/dL)	male	152	1.8	52.7	9.9	62.3	27.3	13.1	0.337
	female	150	4.1	51.5	11.8	57	27.8	12.1	
TG (mg/dL)	male	152	0	253.6	36.1	322.2	117.7	69.3	< 0.001 *
	female	150	9.2	166.9	28.3	189.9	88.1	40.2	

* - Tests that differ in terms of averages between men and women.

Table 4. Gender averages of tests that show normal distribution.

Test	Gender	n	Parametric		Nonparametric		Mean	SD	Sig. (two-tailed)
			lower limit	upper limit	lower limit	upper limit			
CHOL (mg/dL)	male	152	64.1	215.7	63.9	204.8	139.3	38.8	< 0.001 *
	female	150	83	232.5	79.9	234.5	157.8	38.1	
P (mg/dL)	male	152	1.9	4.5	1.7	4.6	3.21	0.64	0.001 *
	female	150	2.4	4.4	2.5	4.4	3.43	0.51	

* - Tests that differ in terms of averages between men and women.

that the indirect method may be preferable, because it is more practical. "Big data" should be used to determine the reference interval using the Bhattacharya method, and exclusion criteria created by certain procedures should be applied to these data. In the indirect sampling method, which is shown in the CLSI EP28-A3c guidelines as an alternative to the direct method for pediatric and geriatric groups where sampling is difficult, patient test results stored in the laboratory database are used to create a reference interval. Interest in this method has

Table 5. Comparison of reference intervals obtained by direct and indirect methods.

Test	Direct method			Bhattacharya procedure			Difference	
	n	LL	UL	n	LL	UL	LL	UL
ALP (IU/L)	302	29.5	107	38,440	39.8	123.2	25.8% *	15.1%
LDH (IU/L)	302	57.1	205.8	42,775	128.9	252.3	55.7% *	22.5% *
AMLY (IU/L)	302	24.5	101.4	22,143	23	94.7	6.5%	6.6%
TG (mg/dL)	302	31.1	247	29,010	1.2	219.2	2491.6% *	11.2%
CHOL (mg/dL)	302	68	217	29,378	108.6	239.9	37.3% *	10.5%
HDL-CHOL (mg/dL)	302	18	71	28,980	27.1	64.4	33.5% *	9.2%
LDL-CHOL (mg/dL)	302	30	147	27,854	44.8	145.7	33% *	0.8%
Ca (mg/dL)	302	6.2	10.5	82,368	8.4	10.3	2.2 (mg/dL) *	-0.2 (mg/dL)
P (mg/dL)	302	2.25	4.6	24,676	2	4.7	-12.5 * (mg/dL)	0.1 (mg/dL)
Mg (mg/dL)	302	1.2	2.3	27,324	1.6	2.5	25% *	8.6%
ALB (g/L)	302	23.8	50.5	54,248	39.2	51.3	39.2% *	1.5%
URE (mg/dL)	302	14.9	43.7	96,855	8.9	44.4	-6 * (mg/dL)	0.7 (mg/dL)
UA (mg/dL)	302	2.05	6.14	36,097	2.1	6.2	2.3%	0.9%
LIP (IU/L)	302	10.5	59.8	20,670	3.8	55.6	176.3% *	7%

* **- Exceeding acceptable limits. LL - lower limit, UL - upper limit.**

increased in recent years, because it is more practical and less costly [2,6,14,15].

There are many studies in our country in which the reference interval is calculated using direct, indirect, or both methods [6,14,16-18]. Ozarda Ilcol and Aslan calculated the reference interval of 40 routine biochemical parameters in Bursa using an indirect method. Individuals between the ages of 18 and 45 were included in the study, and they examined 422,919 laboratory data from patients who applied to Uludag University Faculty of Medicine Hospital Biochemistry Laboratory between 2004 and 2005.

The common point of these studies was that hospital data were used in all of them, and different methods were applied to filter the data. It is necessary to compare studies conducted in different regions to standardize the methods used to calculate the reference intervals. Outlier removal, selection of reference individuals, exclusion, subgrouping, and differences in the methods of obtaining reference intervals used in creating reference intervals make it difficult to make comparisons between studies. Despite this, common reference interval use can be achieved by carrying out verification and data transfer studies between laboratories or multicenter reference interval studies.

In this study, reference intervals were calculated separately for men and women upon clinical need, without considering the Harris-Boyd [19] method used for the decision to divide into subgroups. The significance of the difference in means between men and women was evaluated using an independent sample *t*-test. Owing to difficulties in determining the reference individual, subgrouping by age could not be performed. By comparing the different methods, we can say that the direct method is the preferred method for determining reference intervals. For the indirect method, the difficulty of standardization on the effect of preanalytical factors, biological variation, sufficient amount of data, criteria for subdividing, methods used to eliminate extreme values, data to be excluded, and the method to be chosen when calculating the reference interval show that studies on this subject are still insufficient. However, we believe that this method is a good alternative to the direct method, because it is easy and inexpensive.

CONCLUSION

Reference intervals that guide clinical diagnosis and treatment may vary depending on the population characteristics and the characteristics of the device used for analysis. When we compare our own reference interval values with studies conducted in different regions of our country, we conclude that reference interval values vary depending on factors such as geographical region, ethnicity, socioeconomic level, gender, age, diet, and differences in analytical methods. Therefore, each laboratory should establish its own reference interval values within its means.

Although the process recommended by IFCC is a direct sampling method, it is a difficult and costly process in terms of the selection of reference individuals. It should be noted that the indirect method may be preferable as it is more practical. "Big data" should be used to deter-

Table 6. Comparison of reference intervals with manufacturer RI.

Test	Direct method nonparametric			Indirect method		Manufacturer RI		Differences between indirect and manufacturer RI		Differences between direct and manufacturer RI	
	Gender	LL	UL	LL	UL	LL	UL	LL	UL	LL	UL
ALP (IU/L)	male	27.7	121.3	12.6	116.8	40	129	68.5% *	9.4%	9.5%	5.9%
	female	32.4	101.1	38.5	111.4	35	104	-10%	-7.1%	2.5%	2.7%
LDH (IU/L)	male	53.3	208.1	127.3	263	135	225	5.7%	-16.8% *	36.3% *	7.5%
	female	69.1	205.4	125.5	220.1	135	214	7%	-2.8%	30.7% *	45
AMLY (IU/L)	male	24.6	101.3	17.6	103.3	28	100	59% *	-3.1%	13.8% *	-1.2%
	female	24.7	102.2	21.6	97.62			29.6% *	2.4%	13.3% *	-2.1%
TG (mg/dL)	male	36.1	322.2	43.1	156.2	< 200			28% *		-61% *
	female	28.3	189.9	39.4	138.7				44.1% *		5%
CHOL (mg/dL)	male	63.9	204.8	105.6	241	< 200			-17% *		-2.4%
	female	79.9	234.5	117.1	239.8				-16.5% *		17.2% *
HDL-CHOL (mg/dL)	male	16.9	57.6	25	58.3	> 55			-5.6%		-4.7%
	female	24.8	74.1	31	68.1	> 65			-4.5%		-14%
LDL-CHOL (mg/dL)	male	25.8	133.3	41.7	154.8	< 155			0.1%		14%
	female	34.8	152	46.6	140.1				10.6%		1.9%
Ca (mg/dL)	male	6	10.6	8.1	10.5	8.6	10	0.5	0.5	2.6 *	0.6
	female	6.3	10.3	8.4	10.3			0.2	0.3	2.3 *	0.3
P (mg/dL)	male	1.7	4.6	1.9	4.7	2.5	4.5	0.6 *	0.2	0.8 *	0.1
	female	2.5	4.4	2.3	4.7			0.2	0.2	0	0.1
Mg (mg/dL)	male	1.2	2.3	1.5	2.5	1.6	2.6	6.6%	4%	33.3% *	13
	female	1.4	2.2	1.5	2.3			6.6%	13%	14.2%	18.1% *
ALB (g/L)	male	23.5	50.7	21.3	43	39.7	49.4	86.3% *	14.8%	68.9% *	-2.5%
	female	27.1	50.4	37.6	50.3			5.5%	-1.7%	46.4% *	-1.9%
URE (mg/dL)	male	16.8	47.4	14.7	44.7	16.6	48.5	1.9	3.8 *	0.1	1.1
	female	12.3	36	9.7	35.1			6.9 *	13.4 *	4.3 *	12.5 *
UA (mg/dL)	male	2.3	6.8	2.5	7.8	3.4	7	36% *	-10.2% *	47.8% *	2,9%
	female	1.9	6	2	5.7	2.4	5.7	20% *	0%	26.3% *	-5%
LIP (IU/L)	male	9.9	62.3	10.3	51.8	13	60	26.2% *	15.8% *	31.3% *	-3.6%
	female	11.8	57	12.9	45.3			0.7%	32.4% *	10.1% *	5.2%

* - Exceeding acceptable limits. LL - lower limit, UL - upper limit.

mine the reference interval with the Bhattacharya method, and exclusion criteria created by certain procedures should be applied to these data. Outlier removal, selection of reference individuals, exclusion, subgrouping, and differences in the methods of obtaining reference intervals used in creating reference intervals make it difficult to make comparisons between studies. Despite this, common reference interval use can be achieved by carrying out verification and data transfer studies between laboratories or multi-center reference interval studies.

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Declaration of Interest:
The authors declare that they have no conflicts of interest.

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