Yesim Ozarda*, Kiyoshi Ichihara, Diler Aslan, Hulya Aybek, Zeki Ari, Fatma Taneli, Canan Coker, Pinar Akan, Ali Riza Sisman, Onur Bahceci, Nurzen Sezgin, Meltem Demir, Gultekin Yucel, Halide Akbas, Sebahat Ozdem, Gurbuz Polat, Ayse Binnur Erbagci, Mustafa Orkmez, Nuriye Mete, Osman Evliyaoglu, Aysel Kiyici, Husamettin Vatansev, Bahadir Ozturk, Dogan Yucel, Damla Kayaalp, Kubra Dogan, Asli Pinar, Mehmet Gurbilek, Cigdem Damla Cetinkaya, Okhan Akin, Muhittin Serdar, Ismail Kurt, Selda Erdinc, Ozgur Kadicesme, Necip Ilhan, Dilek Sadak Atali, Ebubekir Bakan, Harun Polat, Tevfik Noyan, Murat Can, Abdulkerim Bedir, Ali Okuyucu, Orhan Deger, Suret Agac, Evin Ademoglu, Ayşem Kaya, Turkan Nogay, Nezaket Eren, Melahat Dirican, GulOzlem Tuncer, Mehmet Aykus, Yeliz Gunes, Sevda Unalli Ozmen, Reo Kawano, Sehavet Tezcan, Ozlem Demirpence and Elif Degirmen

A multicenter nationwide reference intervals study for common biochemical analytes in Turkey using Abbott analyzers

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Abstract

Background: A nationwide multicenter study was organized to establish reference intervals (RIs) in the Turkish population for 25 commonly tested biochemical analytes and to explore sources of variation in reference values, including regionality.

Methods: Blood samples were collected nationwide in 28 laboratories from the seven regions (≥400 samples/region, 3066 in all). The sera were collectively analyzed in Uludag University in Bursa using Abbott reagents and

***Corresponding author: Yesim Ozarda,** MD, Department of Medical Biochemistry, Uludag University School of Medicine, Bursa, Turkey, Phone: +90 224 29 53917, Fax: +90 224 29 50019, E-mail: yesim@uludag.edu.tr

Kiyoshi Ichihara and Reo Kawano: Faculty of Health Sciences, Department of Laboratory Sciences, Yamaguchi University Graduate School of Medicine, Ube, Japan

Diler Aslan and Hulya Aybek: Department of Medical Biochemistry, Pamukkale University School of Medicine, Denizli, Turkey

Zeki Ari and Fatma Taneli: Department of Medical Biochemistry, Celal Bayar University School of Medicine, Manisa, Turkey

Canan Coker, Pinar Akan and Ali Riza Sisman: Department of Medical Biochemistry, Dokuz Eylul University School of Medicine, Izmir, Turkey

Onur Bahceci: Servergazi State Hospital, Denizli, Turkey

Nurzen Sezgin: Baskent University, Adana Research Center, Adana, Turkey

Meltem Demir: Medicalpark Hospital, Antalya, Turkey

Gultekin Yucel, Halide Akbas and Sebahat Ozdem: Department of Medical Biochemistry, Akdeniz University School of Medicine, Antalya, Turkey

Gurbuz Polat: Department of Medical Biochemistry, Mersin University School of Medicine, Mersin, Turkey

Ayse Binnur Erbagci and Mustafa Orkmez: Department of Medical Biochemistry, Gaziantep University School of Medicine, Gaziantep, Turkey

Nuriye Mete and Osman Evliyaoglu: Department of Medical Biochemistry, Dicle University School of Medicine, Diyarbakır, Turkey

Aysel Kiyici, Husamettin Vatansev and Bahadir Ozturk: Department of Medical Biochemistry, Selçuk University School of Medicine, Konya, Turkey

Dogan Yucel, Damla Kayaalp and Kubra Dogan: Ankara Teaching and Research Hospital, Ankara, Turkey

Asli Pinar: Department of Medical Biochemistry, Hacettepe University School of Medicine, Ankara, Turkey

Mehmet Gurbilek and Cigdem Damla Cetinkaya: Department of Medical Biochemistry, Necmettin Erbakan University School of Medicine, Konya, Turkey

Okhan Akin: Keçiören Teaching and Research Hospital, Ankara, Turkey

Muhittin Serdar and Ismail Kurt: Department of Medical Biochemistry, Gulhane Military School of Medicine, Ankara, Turkey

Selda Erdinc and Ozgur Kadicesme: Kars State Hospital, Turkey

Necip Ilhan and Dilek Sadak Atali: Invitrolab Laboratory, Elazıg, Turkey

Ebubekir Bakan and Harun Polat: Department of Medical Biochemistry, Ataturk University School of Medicine, Erzurum, Turkey

Tevfik Noyan: Department of Medical Biochemistry, Ordu University School of Medicine, Ordu, Turkey

Murat Can: Department of Medical Biochemistry, Bulent Ecevit University School of Medicine, Zonguldak, Turkey

Abdulkerim Bedir and Ali Okuyucu: Department of Medical Biochemistry, Ondokuz Mayıs University School of Medicine, Samsun, Turkey

Orhan Deger and Suret Agac: Department of Medical Biochemistry, Karadeniz Teknik University School of Medicine, Trabzon, Turkey

Evin Ademoglu and Ayşem Kaya: Istanbul University, Instituent of Cardiology, Istanbul, Turkey

Turkan Nogay: Luleburgaz State Hospital, Kırklareli, Turkey

Nezaket Eren: Sisli Etfal Teaching and Research Hospital, İstanbul, Turkey

Melahat Dirican, GulOzlem Tuncer, Mehmet Aykus, Yeliz Gunes and Sevda Unalli Ozmen: Department of Medical Biochemistry, Uludag University School of Medicine, Bursa, Turkey

Sehavet Tezcan, Ozlem Demirpence and Elif Degirmen: Batman Region Hospital, Batman, Turkey

analyzer. Reference materials were used for standardization of test results. After secondary exclusion using the latent abnormal values exclusion method, RIs were derived by a parametric method employing the modified Box-Cox formula and compared with the RIs by the non-parametric method. Three-level nested ANOVA was used to evaluate variations among sexes, ages and regions. Associations between test results and age, body mass index (BMI) and region were determined by multiple regression analysis (MRA).

Results: By ANOVA, differences of reference values among seven regions were significant in none of the 25 analytes. Significant sex-related and age-related differences were observed for 10 and seven analytes, respectively. MRA revealed BMI-related changes in results for uric acid, glucose, triglycerides, high-density lipoprotein (HDL)-cholesterol, alanine aminotransferase, and γ-glutamyltransferase. Their RIs were thus derived by applying stricter criteria excluding individuals with BMI >28 kg/m^2. Ranges of RIs by non-parametric method were wider than those by parametric method especially for those analytes affected by BMI.

Conclusions: With the lack of regional differences and the well-standardized status of test results, the RIs derived from this nationwide study can be used for the entire Turkish population.

Keywords: biochemical parameters; common reference intervals; multicenter study; regional differences; Turkey.

Introduction

Reference intervals (RIs) for laboratory test results obtained from healthy reference populations have an important role in identifying diseased individuals and in monitoring disease changes. Careful determination of RIs by the laboratory for use in the served population is thus a very important task [1]. About 25 years ago, the International Federation of Clinical Chemistry (IFCC) published a series of six papers recommending that each laboratory produce has its own reference values and estimate the corresponding RIs according to defined procedures [2–7]. The Clinical and Laboratory Standards Institute (CLSI, previously NCCLS) published the C28-A3 Guideline in 2008 [8]. The following regulatory initiatives are driving renewed interest in the topic [9]. According to the directive on in vitro diagnostic medical devices of the European Union, diagnostic manufacturers are now requested to supply their clients with appropriate RIs for use with their assay platforms and reagents [10], and the International Organization for Standardization (ISO) 15189 standard for clinical laboratory accreditation states that each laboratory should periodically re-evaluate its own RIs [11]. Despite these requirements, the implementation of RIs in most clinical laboratories is still incomplete. The derivation of RIs on a national level by conducting a multicenter study that follows a common protocol, comprehensive standard operating procedures (SOPs), and secondary integration of the results on a global scale is probably the most effective way to seek globally applicable, or common, RIs [12]. The IFCC Committee for Reference Intervals and Decision Limits (C-RIDL) recently published two papers including a protocol and SOPs for multicenter RI studies [13], with indication of the utility of a panel of sera for the alignment of test results among laboratories in the multicenter studies [14].

There are a few reports on RIs for the Turkish population [15–19]. Recently, Turkey also participated in a worldwide multicenter study on RIs conducted by C-RIDL according to the protocol and SOPs for multicenter RI studies [13]. In parallel to this study, we organized and conducted a multicenter nationwide RI study for Turkey using the same protocol and SOPs [13]. The study was designed to: 1) explore possible regional differences in reference values of major analytes among the seven regions; and 2) define RIs to be used nationwide for as many analytes as possible. In this manuscript, we describe the data obtained from this first multicenter RI study in Turkey.

Materials and methods

A total of 3066 healthy individuals from the seven major regions of Turkey particiated in the study. The main target range of ages was 20–65 years. Blood samples were collected from healthy individuals selected according to the IFCC/C-RIDL protocol in 28 participating laboratories. The clinical laboratory in Uludag University (UU) in Bursa in the Marmara region met all requirements for a central laboratory [13] where 25 biochemical tests were to be collectively measured. Inclusion and exclusion criteria were set according to the IFCC/C-RIDL protocol [13]. A questionnaire comprising general health and lifestyle questions was completed and sent to the central laboratory. The birthplace and region of residence were recorded. The results of each analyte were classified according to the region of residence, and then by place of birth, including countries outside of Turkey, thus making eight groups for comparison.

The study protocol, the contents of the informed consent form, and the general health and lifestyle questionnaire were approved by the Ethics Committee of UU School of Medicine.

Preparation for sampling, sampling and sample processing procedues were conducted using the most recently published

IFCC/C-RIDL protocol [13]. In the sampling, 8 mL blood was collected into gel serum separator tubes (SST II, Becton-Dickinson). The time of sampling was set at 7–10 AM. At 30–60 min after sampling, the samples were centrifuged at 1200 *g* for 10 min at room temperature. The serum from the specimens was promptly divided into four aliquots of 1 mL each, using well-sealed freezing containers, and was immediately stored at −80 °C. Two samples, packed in dry ice, were sent to the central laboratory (Bursa) within 2 months.

Total protein (TP), albumin (Alb), urea nitrogen (UN), uric acid (UA), creatinine (CRE), total bilirubin (TBil), direct bilirubin (DBil), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphate (IP), glucose (GLU), total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL)-cholesterol (HDL-C), low-density lipoprotein (LDL)-cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyltransferase (GGT), creatine kinase (CK), and amylase (AMY) were measured in each serum sample. All of the tests were performed following the manufacturer's package inserts for Abbott reagents on an Architect 8000® analyzer (Abbott Diagnostics, IL, USA). The assay methods for the analytes and the Standard Reference Materials (SRMs) used to ensure traceability of the test results are listed in Table 1 of the Supplemental Data, which accompanies the article at http://www.degruyter.com/view/j/cclm.2014.52.issue-12/issue-files/cclm.2014.52.issue-12.xml.

As UU acted as a central laboratory of Turkey also for the multicenter worldwide study, the panel of 40 sera prepared from healthy individuals provided by the C-RIDL was measured for comparison of values with other countries [14]. In addition to this panel of sera and standard quality control materials supplied by manufacturers, the central laboratory prepared a mini-panel of five sera from healthy individuals and measured them in singleton on each day of measurement to monitor the stability of the assay [20]. The analytical coefficient of variation (CV_a) was computed for each analyte from the results of repeated measurements of the same panel measured in the central laboratory in Bursa [14]. The desirable limits for between- and within-day CVs were set as ½ of within-individual CV, as defined by Ricos et al. and reported in the Westgard website [21].

The magnitude of the standard deviations (SD) of test results attributable to between-region (SD-reg), between-sex (SD-sex), between-age (SD-age), and net between-individual (SD-btw_ind) variations were computed by three-level nested ANOVA. The regionality of test results was examined in two ways: one based on the information about birthplace and the other on the place of residence. The relative magnitude of each SD was expressed as the ratio of SD (SDR) over SD-btw_ind: SDRsex, SDRage, and SDRreg for SD due to sex, age, and region, respectively. An SDR greater than 0.3 was regarded as a guide to consider partitioning reference values by the factor [13, 21].

Multiple regression analysis (MRA) [22] was performed to identify factors possibly influencing the test results, including sex, age, BMI, level of cigarette smoking, daily alcohol consumption, and regular physical exercise with respective levels categorized into three, five, and eight grades as follows: none, ≤250, 250–500, 500< pack·year; none, ≤12.5, 12.5–25, 25–50, 50<g ethanol/day; none, 1–7 days/week. In the analysis dummy variables representing the Turkish regions, with Marmara set as the reference region, were also introduced in order to adjust for possible influence of either birthplace or place of residence on reference values (thus, the analysis was done in two ways). A given explanatory variable was considered to be of practical importance when its standardized partial regression coefficient, which corresponds to the partial correlation coefficient (r_p), was greater than 0.20.

RIs derived by the parametric method after normalizing the data by use of the modified Box-Cox power transformation method [22] were compared with those derived by the non-parametric method in both sexes and in each decade of age. The latent abnormal value exclusion (LAVE) method [22] was applied at the time of computing RIs as a method for secondary exclusion. The method has been developed to exclude possibly abnormal results hidden within the reference values. However, it does not judge how extreme a given test value might be in isolation. Instead, this method looks at other concurrently measured test results. The method is a type of iterative approach for derivation of multiple RIs simultaneously, in which no exclusion of values is made in the initial computation of RIs. The algorithm then uses those initial values of RIs to judge the abnormality of each individual's record by counting the number of abnormal results in tests other than the one for which the RI is being determined [22]. The 90% confidence intervals (CI) of the lower and upper limits of the RIs (LRLs and URLs) were calculated by use of the bootstrap method, both for the parametric and non-parametric method, through random resampling of the same dataset for 200 times.

Recalibration of RIs was performed based on test results of SRMs according to the scheme employed in the worldwide study. When there was only a single SRM specimen, the ratio of its assigned value to the average of measured values (with nine replicates) was used for recalibration of the RI. When there were two or more specimens in a SRM, the least-square regression line was derived using the assigned values (x) and all measured values (y), each specimen in nine replicates, and the regression line was used for recalibration of the RI.

Results

Age and sex distributions of the participants in the seven regions of Turkey are shown in Table 1. The male to female ratio was close to 1. The majority of participants (2696; 88.8% of the total) were between 20 and 59 years old (Table 1). The within- and between-day CVs for all analytes, listed in Table 1 of the Supplemental Data, did not exceed the desirable limits reported in the Westgard website [21]. Recalibration was performed for AST, ALT, CK, TC, HDL and LDL by using regression coefficients (intercepts and slopes; see Table 2 of the Supplemental Data). Recalibration was not performed other analytes as there was excellent agreement of test values with the assigned values.

By use of three-level nested ANOVA, sex-related differences (SDR>0.3) were observed for 10 analytes (UA, UN, TBil, CRE, TG, HDL-C, ALT, AST, CK, and GGT), and age-related differences for seven analytes (ALB, UN, TG, LDL-C, TC, GLU and ALP), as shown in Table 2). No significant regional difference was observed in any of the analytes on the basis of either birthplace or place of residence in both males and females (Table 2).

Table 1 Age and sex composition of the volunteers from the seven regions of Turkey.

Sex, n	Region	20–29 years	30–39 years	40–49 years	50–59 years	60–69 years	70–79 years	Total
Male (1584)	Aegean	40	56	48	35	16	9	204
	Black Sea	41	65	54	38	12	2	212
	Central Anatolia	48	60	45	36	12	13	214
	Eastern Anatolia	43	49	45	29	23	13	202
	Marmara	60	70	67	53	25	13	288
	Mediterranian	37	39	40	25	18	5	164
	Southeastern Anatolia	58	74	63	40	26	9	270
Female (1578)	Aegean	42	46	42	28	18	6	182
	Black Sea	38	54	47	23	9	6	177
	Central Anatolia	55	51	49	37	8	9	209
	Eastern Anatolia	48	51	52	42	20	7	220
	Marmara	58	70	77	48	32	6	291
	Mediterranian	29	41	41	26	15	7	159
	Southeastern Anatolia	59	75	70	39	23	8	274
	Total (n)	656	801	740	499	257	113	3066

Table 2 The results of three-level nested ANOVA for sex, age, birth place and region.

Analyte	SDR-sex	SDR-age (M, F)	SDR-birth place (M, F)	SDR-region (M, F)
TP	0.07	0.15 (0.11, 0.18)	0.17 (0.19, 0.14)	0.18 (0.16, 0.20)
ALB	0.3	0.40 (0.43, 0.37)	0.13 (0.16, 0.10)	0.09 (0.08, 0.10)
UN	0.32	0.46 (0.33, 0.56)	0.14 (0.19, 0.06)	0.06 (0.07, 0.05)
UA	0.94	0.20 (0.03, 0.30)	0.09 (0.09, 0.09)	0.12 (0.10, 0.13)
CRE	1.01	0.24 (0.14, 0.37)	0.23 (0.25, 0.18)	0.15 (0.16, 0.14)
TBIL	0.44	0.04 (0.05, 0.02)	0.19 (0.17, 0.21)	0.19 (0.13, 0.23)
DBIL	0.3	0.14 (0.10, 0.17)	0.11 (0.11, 0.11)	0.13 (0.13, 0.11)
GLU	0	0.38 (0.37, 0.39)	0.17 (0.08, 0.23)	0.12 (0.09, 0.14)
TC	0	0.53 (0.44, 0.60)	0.09 (0.07, 0.10)	0.09 (0.07, 0.11)
TG	0.35	0.40 (0.29, 0.51)	0.14 (0.14, 0.12)	0.13 (0.09, 0.16)
HDL-C	0.67	0.07 (0.00, 0.10)	0.13 (0.16, 0.11)	0.17 (0.16, 0.17)
LDL-C	0	0.50 (0.39, 0.60)	0.12 (0.13, 0.11)	0.17 (0.18, 0.15)
Na	0.14	0.28 (0.00, 0.39)	0.07 (0.12, 0.00)	0.06 (0.05, 0.06)
K	0	0.21 (0.21, 0.22)	0.04 (0.00, 0.07)	0.10 (0.13, 0.06)
Cl	0.19	0.06 (0.09, 0.00)	0.20 (0.19, 0.21)	0.21 (0.24, 0.19)
Ca	0.21	0.19 (0.17, 0.22)	0.10 (0.13, 0.06)	0.06 (0.08, 0.02)
IP	0.24	0.22 (0.25, 0.18)	0.13 (0.06, 0.18)	0.10 (0.04, 0.14)
Mg	0.2	0.12 (0.16, 0.06)	0.13 (0.15, 0.09)	0.10 (0.13, 0.06)
AST	0.45	0.17 (0.17, 0.16)	0.11 (0.07, 0.14)	0.11 (0.11, 0.11)
ALT	0.61	0.24 (0.29, 0.16)	0.11 (0.11, 0.12)	0.15 (0.15, 0.15)
LDH	0	0.27 (0.10, 0.36)	0.06 (0.07, 0.05)	0.15 (0.13, 0.17)
ALP	0.22	0.38 (0.11, 0.51)	0.10 (0.09, 0.11)	0.12 (0.07, 0.16)
GGT	0.76	0.29 (0.22, 0.37)	0.14 (0.14, 0.15)	0.11 (0.12, 0.09)
CK	0.62	0.18 (0.25, 0.00)	0.12 (0.07, 0.16)	0.06 (0.00, 0.10)
AMY	0.1	0.10 (0.14, 0.00)	0.10 (0.12, 0.08)	0.05 (0.09, 0.00)

SDR represents standard deviation ratio, the ratio of the standard deviation for a given factor to that for a net between-individual variation. By use of 3-level nested ANOVA, the magnitudes (SD) of between-sex, -age, -birth place (or –geographical region representing place of residence) variation were computed relative to the net between-individual SD as SDR. SDR-sex, SDR-age, and SDR-birth place (or SDR-region) denote SDR for between-sex, between-age, and between-birth place (or between-region) differences, respectively. The SDRs in parentheses represent those computed after partitioning data to males (M) and females (F) by use of 2-level nested ANOVA, setting age and birth place (or region) as the target factors. The light and dark gray backgrounds indicate 0.3<SDR≤0.5 and 0.5<SDR, respectively.

MRA results by inclusion of dummy variables for geographical regions (defined as place of residence by setting Marmara as the reference region), age, BMI, levels of alcohol consumption, cigarette smoking and physical exercise as explanatory variables are shown in Table 3 for males and females. The degree of association of each explanatory variable with a target variable was expressed as a standard partial regression coefficient, which takes a value between –1.0 and 1.0, and corresponds to a partial correlation coefficient (r_p). The absence of apparent regional differences was confirmed with almost all $|r_p|$ well below the cut-off level of 0.2. Significant BMI-related changes were observed for UA, GLU, TG, ALT, and GGT in males, and for UA, TG, HDL-C, and GGT in females. Significant age-related changes were observed for ALB, UN, GLU, TC, LDL-C, K, Ca, and ALT in males, and for ALB, UN, CRE, GLU, TC, LDL-C, Na, and ALP in females. Test results for all 25 analytes were not significantly associated with the levels of alcohol consumption, cigarette smoking or physical exercise in either males or females (Table 3).

RIs were derived both parametrically and non-parametrically after applying secondary exclusion based on the LAVE method in a mode allowing a single abnormal result in the analytes chosen for exclusion (UA, TG, HDL-C, LDL-C, AST, ALT, LDH, GGT and CK). RIs and 90% CIs of the LRLs and URLs are shown for males and females in Table 4. RIs determined by the parametric and non-parametric methods were generally identical for TP, ALB, UN, CRE, TBil, DBil, Na, K, Cl, Ca, Mg, and AMY. For these analytes, there were no changes in RIs with the application of secondary exclusion methods, although the 90% CIs for the URLs were generally narrower with the parametric method (Table 4). In contrast, the ranges of RIs for GLU, TC, TG, HDL-C, LDL-C ALT and GGT were wider by the non-parametric method than by parametric methods as reported by Ichihara [20]. In addition, their RIs showed a clear tendency toward lowering of the URLs when stricter criteria for secondary exclusion were applied. Therefore, as shown in Table 4, for those analytes a set of two RIs were derived from two ways of applying the LAVE method, one allowing one abnormal result (regular method) and the other not allowing any abnormal results in other analytes as well as not allowing those with BMI >28 kg/m^2 or those receiving regular medication. The URLs decreased significantly with the stricter criteria, and the difference between the parametric and non-parametric methods tended to get narrower even though the data size was greatly reduced except for HDL-C, which showed the opposite direction of change. Age-related RIs are shown in Table 5 for ALB, UN and ALP, TC, LDL-C, TG and GLU, for which significant age-related differences (SDR>0.3) were observed by nested ANOVA (Table 2). The high SDR-age for TG and ALP originated from females. Although the SDR-age was <0.3 for UA, CRE, Na, LDH and GGT for the combined data, age-related differences (SDR>0.3) were observed in females (Table 2).

The RIs for AMY, Na and K were narrower than the RIs given in the manufacturers' inserts (Table 6), while the RIs for LDH, UN, GLU, Ca, IP and Cl were similar to the manufacturers' suggestions. The RIs for TP, CRE, TBil, GGT, CK, ALP, AST (females), ALT (females) and UA (males) showed moderate to marked differences for the upper limits. Mg, TP, ALB, AST and ALT showed similar differences for the lower limits. TC, TG and LDL-C had wider RIs because the upper limits given in the manufacturers' inserts correspond to clinical decision limits (CDLs). As SDR-age for UN, TC, TG, LDL-C and ALP were found to be very high in females (Table 2), RIs for these analytes in females were partitioned by ages <50 and ≥50 and were found to be higher in the older age group (Table 6).

Discussion

These data show that RIs for the 25 commonly tested analytes are similar for the seven major geographical regions of Turkey. Reference values for UA, UN, TBil, CRE, TG, HDL-C, ALT, AST, CK, and GGT are different for males and females, and the values for ALB, UN, ALP, TC, LDL-C, TG and GLU vary with age. BMI levels are clearly associated with test results for UA, GLU, TG, HDL-C, LDL-C, ALT and GGT either in males or females or both. In fact, for these analytes, the RIs derived, especially by the non-parametric method, were wider compared with the RIs derived by applying the stricter criteria limiting individuals with BMI <28 kg/m^2. The levels of alcohol drinking, cigarette smoking and physical exercise had no effect on RIs for any of the 25 analytes examined.

RIs established by a multicenter study were expected to become wider than those established by a single laboratory due to the inclusion of between-laboratory variation [23]. Yamamoto et al. [23] recently established common RIs for standardized clinical laboratory tests in Japan by nationwide collaboration of more than 100 accuracy-certified core laboratories. Although the reported RIs were close to those reported by Ichihara et al. [24], it was necessary to adjust the dataset for unbalanced age and male/female ratios and to delete results from some laboratories which showed biases exceeding the allowable

Table 3 MRA results (r_p) for sources of variation of reference values in males and females.

Analytes	N	Regions (place of residence)						Age	BMI	Exercise level	Alcohol level	Smoking level
		A	EA	SEA	CA	MED	BS					
Males												
TP	1188	0.03	0.08	0.07	0.02	0.02	0.04	0.15	0.06	0.00	−0.05	−0.18
ALB	1188	−0.03	0.03	−0.02	−0.01	−0.02	−0.01	−0.38	0.05	0.04	−0.01	−0.09
UN	1188	−0.04	0.10	0.03	0.05	0.00	0.08	0.22	0.07	0.01	−0.01	−0.03
CRE	1187	−0.01	0.12	0.06	0.13	0.11	0.20	0.07	0.07	0.01	0.01	−0.03
UA	1188	0.00	0.13	0.04	0.11	0.07	0.02	0.05	0.29	0.01	0.08	−0.09
TBil	1188	−0.08	−0.01	−0.10	0.04	−0.03	−0.04	0.05	0.04	0.01	−0.01	−0.12
DBil	1185	−0.06	0.04	−0.04	0.03	0.00	−0.02	0.09	0.07	0.01	−0.01	−0.14
GLU	1188	0.01	−0.05	0.04	0.01	0.05	0.03	0.23	0.21	0.00	0.00	−0.10
TG	1188	−0.11	0.03	0.01	−0.03	−0.03	−0.06	0.08	0.26	−0.01	0.01	0.09
TC	1188	−0.11	−0.04	−0.06	−0.05	−0.07	−0.07	0.31	0.11	−0.02	0.04	0.05
HDL-C	1188	−0.09	−0.22	−0.10	−0.07	−0.09	−0.07	0.12	−0.17	0.01	0.07	−0.14
LDL-C	1188	−0.09	−0.02	−0.09	−0.03	−0.06	−0.05	0.31	0.11	−0.02	0.02	0.08
Na	1188	0.04	0.06	0.05	0.05	0.07	0.06	0.01	0.02	0.05	−0.02	0.02
K	1188	0.12	0.15	0.14	0.04	0.13	0.18	0.20	0.03	−0.02	0.00	0.08
Cl	1188	0.05	0.10	0.10	0.01	−0.08	−0.02	0.13	0.01	0.03	−0.01	0.03
Ca	1187	0.00	0.11	0.10	0.01	−0.01	0.02	−0.21	0.07	0.05	−0.03	−0.02
IP	1188	0.00	−0.07	0.00	0.06	−0.04	−0.02	−0.18	0.08	0.07	0.08	0.12
Mg	1188	0.11	0.09	0.05	0.12	0.06	0.05	0.08	0.05	0.00	0.00	0.02
AST	1188	−0.02	0.03	0.05	0.01	0.00	0.06	0.08	0.18	−0.02	0.01	−0.05
ALT	1188	−0.05	0.10	0.08	0.04	0.02	0.03	−0.24	0.31	0.00	0.06	−0.01
LDH	1188	0.01	−0.04	−0.04	0.00	−0.01	−0.08	0.07	0.19	0.02	−0.05	0.01
ALP	1188	−0.06	0.04	0.02	−0.03	−0.02	−0.01	0.02	0.02	0.00	−0.02	0.04
GGT	1188	0.00	0.13	0.03	0.01	0.01	0.02	0.03	0.24	−0.01	0.08	0.05
CK	1188	0.03	0.14	0.07	0.05	0.03	0.06	−0.18	0.09	0.06	−0.05	0.01
AMY	1188	−0.01	0.08	0.03	0.02	0.02	−0.03	0.16	0.14	−0.01	0.00	−0.09
Females												
TP	1225	−0.01	−0.07	0.06	−0.02	−0.04	−0.07	0.13	0.06	−0.01	0.05	−0.19
ALB	1225	0.01	−0.07	0.04	0.03	0.05	−0.01	−0.23	0.14	0.03	0.01	−0.03
UN	1225	−0.01	−0.03	−0.07	0.00	−0.07	−0.01	0.43	0.09	0.02	0.05	−0.04
CRE	1224	−0.01	−0.01	−0.09	0.08	0.00	0.03	0.22	0.06	−0.04	0.03	−0.01
UA	1225	−0.02	0.00	−0.05	0.01	−0.08	−0.11	0.06	0.26	0.01	0.04	−0.02
TBil	1225	−0.04	−0.01	−0.19	−0.01	−0.03	−0.02	0.01	0.14	−0.03	0.00	−0.05
DBil	1225	−0.06	0.07	−0.05	0.00	−0.01	0.00	0.04	−0.18	−0.03	0.02	−0.11
GLU	1225	0.02	−0.08	−0.02	−0.04	0.05	0.03	0.21	0.17	0.00	0.01	−0.02
TG	1225	0.01	0.05	0.08	−0.01	−0.02	0.01	0.22	0.26	−0.01	0.04	0.06
TC	1225	−0.05	−0.06	−0.05	−0.05	−0.02	−0.03	0.39	0.09	0.02	0.08	0.05
HDL-C	1225	−0.08	−0.14	−0.14	−0.05	−0.03	−0.05	0.07	−0.26	0.01	0.07	−0.07
LDL-C	1225	−0.03	−0.02	−0.01	−0.03	0.00	0.00	0.34	0.19	0.01	0.05	0.06
Na	1225	0.05	0.03	0.03	0.02	0.10	0.10	0.27	0.01	0.00	0.02	−0.06
K	1225	0.09	0.11	0.07	0.04	0.07	0.11	0.13	0.13	−0.02	0.02	0.00
Cl	1225	0.18	0.25	0.24	0.16	0.05	0.20	0.01	0.02	0.03	−0.03	0.00
Ca	1224	0.00	−0.01	0.06	0.03	0.01	0.01	0.00	0.06	0.05	0.05	−0.09
IP	1225	0.02	−0.03	−0.06	0.06	0.03	0.10	0.04	−0.19	0.05	0.04	0.06
Mg	1225	0.10	0.00	0.07	0.06	0.06	0.09	0.02	0.05	0.02	0.01	0.03
AST	1225	−0.04	−0.07	0.03	0.06	−0.03	0.01	0.11	0.01	0.03	0.02	−0.01
ALT	1225	−0.10	−0.10	−0.05	−0.01	−0.01	−0.07	0.00	0.14	0.03	0.05	0.05
LDH	1225	0.03	0.00	0.05	0.05	0.01	−0.02	0.19	0.18	0.05	−0.01	−0.05
ALP	1225	0.01	0.12	0.11	0.05	0.02	0.03	0.28	0.19	−0.03	0.00	−0.05
GGT	1225	−0.02	0.03	0.00	0.06	−0.01	0.00	0.15	0.23	−0.04	0.05	0.03
CK	1225	0.01	0.03	0.10	0.04	0.05	0.07	0.06	0.12	0.06	0.02	0.00
AMY	1225	0.02	0.03	0.08	0.05	0.06	0.04	0.17	−0.19	0.01	−0.01	−0.10

r_p, standardized partial regression coefficient; $r_p \geq 0.20$ was considered significant. The light gray and dark gray background colors indicate $0.2 \leq r_p < 0.3$ and $0.3 \leq r_p$, respectively. A, Aegean; CA, Central Anatolia; SEA, Southeast Anatolia; EA, East Anatolia; MED, Mediterranean; BS, Black Sea. These six dummy variables represent regions (places of residence) with Marmara set as the reference region. BMI, body mass index. Levels of (regular) exercise, alcohol (daily consumption), and smoking (cigarette) were graded into 8, 5, and 3 categories, respectively, according to the criteria described in the text.

Table 4 RIs and CIs derived by parametric and non-parametric methods in males and females.

		Males															Females											
Analyte	Units	n	Parametric							Non-parametric							n	Parametric							Non-parametric				
			LL 90% CI			RI		UL 90%CI		LL 90% CI			RI		UL 90%CI			LL 90% CI			RI		UL 90%CI		LL 90% CI			RI		UL 90%CI	
			LL	UL	LL	Me	UL	LL	UL	LL	UL	LL	Me	UL	LL	UL		LL	UL	LL	Me	UL	LL	UL	LL	UL	LL	Me	UL	LL	UL
TP	g/L	1244	65.9	66.8	66	74	82	81.1	82.6	66.0	67.6	67	74	82	80	82.2	1202	65.1	66.1	66	73	82	81.2	82.4	65.6	67.0	66	73	82	80.1	82.1
ALB	g/L	1261	40.8	41.3	41	46	50	49.7	50.1	41.0	42.0	41	46	50	49.0	51.0	1220	39.3	40.0	40	44	49	48.7	49.3	40.0	41.0	40	45	49	48.0	49.2
UN	mmol/L	1241	2.9	3.08	2.97	4.5	7.26	7.08	7.29	2.72	3.05	2.87	4.57	7.41	6.92	7.45	1201	2.25	2.39	2.32	3.88	6.64	6.38	6.89	2.03	2.25	2.21	3.88	6.78	6.60	6.96
UA	µmol/L	1259	211	227	220	327	409	458	476	203	222	213	325	476	462	482	1217	144	155	148	244	357	369	360	144	155	148	244	363	352	372
CRE	µmol/L	1243	57.5	59.2	58.4	71.6	92	91.1	94.6	57.5	59.2	58.4	70.8	89.3	88.5	92	1191	48.6	50.5	49.5	58.4	70.8	69.9	71.7	50.5	51.5	51.4	58.4	69	67.3	69.9
TBil	µmol/L	1234	3.59	3.93	3.8	9.6	22.4	21.0	24.1	3.42	3.93	3.6	8.6	23.9	23.9	27.4	1201	2.56	2.9	2.7	7.0	15.9	15.3	17.9	3.42	3.59	3.4	6.8	17.1	17.1	20.1
DBil	µmol/L	1229	0.85	1.36	1.02	3.59	7.18	6.84	7.86	1.71	1.88	1.71	3.42	8.5	8.50	8.72	1204	0.51	1.53	1.36	2.73	6.84	5.81	9.57	1.71	1.88	1.71	3.42	6.84	6.84	7.01
GLU[a]	mmol/L	1241	3.79	4.01	3.96	4.89	6.32	6.16	6.38	3.74	3.96	3.96	4.89	6.87	6.27	7.26	1220	3.85	4.12	4.01	4.78	5.99	5.72	6.32	3.85	4.01	3.9	4.31	6.21	5.99	6.43
GLU[a,b]		527	3.79	4.01	3.96	4.78	5.83	5.66	5.88	3.74	3.96	3.96	4.78	5.94	5.72	6.21	531	3.79	3.96	3.85	4.73	5.55	5.51	5.61	3.85	4.00	3.85	4.3	5.61	5.44	5.83
TC[a]	mmol/L	1243	3.15	3.32	3.22	4.82	6.82	6.57	6.97	3.02	3.32	3.17	4.8	6.92	6.85	7.12	1198	3.12	3.35	3.25	4.72	5.46	6.77	7.17	3.11	3.32	3.27	3.25	6.95	6.80	7.27
TC[a,b]		471	3.15	3.32	3.20	4.60	6.42	6.3	6.72	3.02	3.32	3.15	4.65	6.17	6.15	6.65	456	3.15	3.32	3.22	4.45	5.92	5.77	6.05	3.10	3.27	3.25	4.42	5.92	5.80	5.97
TG[a]	mmol/L	1229	0.53	0.63	0.55	1.43	3.92	3.78	4.04	0.5	0.6	0.55	1.45	4.13	4.03	4.29	1220	0.46	0.51	0.47	1.04	2.99	2.94	3.25	0.46	0.5	0.47	1.04	3.14	3.01	3.26
TG[a,b]		479	0.53	0.63	0.55	1.25	3.36	3.21	3.92	0.48	0.58	0.52	1.26	3.47	3.22	4.10	469	0.44	0.49	0.45	0.86	2.11	1.84	2.34	0.45	0.48	0.46	0.88	2.39	2.25	2.62
HDL-C[a]	mmol/L	1234	0.82	0.92	0.87	1.07	1.45	1.42	1.55	0.72	0.8	0.75	1.05	1.57	1.45	1.60	1199	0.85	0.95	0.87	1.27	1.92	1.9	2.02	0.82	0.92	0.87	1.27	1.92	1.85	2.01
HDL-C[a,b]		520	0.80	0.87	0.85	1.05	1.52	1.5	1.62	0.75	0.8	0.77	1.07	1.57	1.50	1.60	528	0.92	0.97	0.95	1.32	1.95	1.91	2.02	0.92	0.97	0.92	1.32	1.97	1.90	2.02
LDL-C[a]	mmol/L	1244	1.37	1.52	1.65	2.75	4.14	4.17	4.52	1.37	1.6	1.50	2.72	4.30	4.05	4.35	1203	1.25	1.45	1.40	2.55	4.22	3.9	4.37	1.32	1.42	1.37	2.57	4.20	4.10	4.47
LDL-C[a,b]		521	1.37	1.52	1.60	2.67	4.01	3.95	4.25	1.37	1.60	1.52	2.63	4.25	3.95	4.30	529	1.22	1.42	1.35	2.45	3.77	3.50	3.80	1.25	1.35	1.27	2.32	3.87	3.65	4.17
Na	mmol/L	1245	136	137	137	141	144	143	145	137	138	137	141	144	144	145	1199	136	137	136	140	144	143	144	137	138	137	140	144	143	145
K	mmol/L	1242	3.67	3.74	3.7	4.3	4.9	4.88	5.00	3.70	3.80	3.7	4.3	5.0	4.90	5.01	1200	3.70	3.76	3.7	4.2	4.9	4.88	5.00	3.70	3.80	3.7	4.2	5.0	4.92	5.04
Cl	mmol/L	1243	98	99	98	102	106	105	107	97	98	98	102	107	106	107	1203	98	99	99	103	107	106	108	99	100	100	103	107	107	108
Ca	mmol/L	1244	2.15	2.19	2.15	2.32	2.5	2.49	2.62	2.15	2.17	2.17	2.32	2.5	2.45	2.51	1203	2.11	2.13	2.12	2.27	2.47	2.45	2.48	2.11	2.15	2.12	2.27	2.47	2.45	2.50
IP	mmol/L	1244	0.73	0.77	0.76	1.05	1.40	1.38	1.43	0.73	0.8	0.76	1.05	1.40	1.39	1.47	1202	0.82	0.85	0.83	1.12	1.40	1.39	1.42	0.86	0.89	0.83	1.12	1.40	1.38	1.35
Mg	mmol/L	1243	0.80	0.83	0.82	0.98	1.10	1.09	1.12	0.78	0.83	0.82	0.98	1.10	1.06	1.14	1197	0.77	0.80	0.77	0.94	1.06	1.06	1.10	0.77	0.82	0.82	0.94	1.10	1.06	1.16
AST	U/L	1266	12.6	13.3	13	19	30	29.7	31.7	12.0	13.0	13	19	36	34.0	38.0	1218	10.5	11.1	11	16	25	24.3	26.5	10.0	11.0	11	16	28	26.1	29.2
ALT	U/L	1256	8.2	9.2	9	19	57	53.3	61.9	8.0	9.0	8	20	58	55.0	61.0	1219	6.8	7.2	7	13	28	26.0	29.2	6.0	7.0	7	13	33	29.0	35.1
ALT[b]		513	7.9	9.0	8	18	44	40.3	45.9	6.0	8.0	7	14	38	35.0	41.0	525	6.8	7.1	7	11	22	20.0	24.1	5.1	6.7	7	11	23	21.0	28.0
LDH	U/L	1252	125	132	130	162	221	210	225	124	130	126	167	231	227	238	1218	117	126	120	158	209	201	214	118	122	120	162	231	203	236
ALP	U/L	1236	40	46	43	72	116	113	120	41	44	42	72	120	116	123	1197	34	38	35	62	105	102	111	34	37	36	62	110	106	115
GGT	U/L	1257	10.1	11.9	11	24	70	68	77	10.0	11.0	11	24	78	69	82	1212	7.0	7.7	7	13	33	31	37	7.0	8.0	7	13	39	36	44
GGT[b]		517	10.0	11.5	11	21	57	48	62	10.0	11.0	11	22	58	49	68	512	7.0	7.7	7	12	24	20	26	7.0	8.0	7	12	27	26	32
CK	U/L	1249	45	54	48	97	227	221	248	43	49	47	95	252	239	266	1211	30	36	34	60	131	116	139	27	34	32	60	135	126	151
AMY	U/L	1238	32	37	34	64	117	112	122	27	36	31	64	121	116	132	1196	33	39	31	65	114	108	120	28	36	32	62	117	110	125

RI, reference interval; LL, lower limit of the RI; Me, median; UL, upper limit of the RI; CI, confidence interval (90%) of LLs and ULs were estimated by the bootstrap method. RIs were derived after applying the LAVE method in a mode allowing a single abnormal result in analytes chosen as exclusion criteria: GLU, UA, TG, AST, ALT, LDH, GGT and CK. An exception to this rule was adopted for the analytes marked by [a]applying the LAVE method in a strict mode which does not allow any abnormal result in the analytes chosen for exclusion and excludes those with BMI>28 kg/m^2. [b]These analytes have well-recognized CDLs. The RI results of GLU, TC, TG, HDL-C and LDL-C given in this table should not be confused with the CDLs for these analytes.

Table 5 Age-related RIs for ALB, UN, GLU, TC, TG, LDL-C and ALP derived by the parametric method in males+females, in males, and in females.

Analyte	Unit	Age years	Males+Females				Males				Females			
			n	LL	Me	UL	n	LL	Me	UL	n	LL	Me	UL
ALB	g/L	20–29	493	42	46	51	252	42	47	51	241	41	45	50
		30–39	590	41	45	50	309	42	46	50	280	40	44	49
		40–49	558	40	45	49	271	42	45	49	287	40	44	49
		50–59	387	41	44	48	196	41	45	49	191	40	44	48
		60–79	275	39	44	47	140	39	44	47	135	39	43	46
UN	mmol/L	20–29	478	2.26	3.96	6.19	251	2.88	4.28	6.9	230	2.12	3.52	5.76
		30–39	596	2.48	4.14	6.58	306	3.02	4.5	6.9	288	2.19	3.78	5.83
		40–49	555	2.62	4.24	7.16	264	3.06	4.6	7.7	290	2.44	3.88	6.15
		50–59	390	2.8	4.68	7.63	201	2.91	4.75	7.59	189	2.62	4.57	7.56
		60–79	274	3.45	5.22	9.1	142	3.42	5.5	9.25	135	3.02	5	8.53
GLU[a]	mmol/L	20–29	484	3.85	4.67	5.5	252	3.9	4.67	5.66	233	3.79	4.62	5.28
		30–39	598	3.9	4.78	5.66	310	3.96	4.78	5.77	286	3.9	4.73	5.55
		40–49	549	4.07	4.95	5.94	261	4.01	5	6.05	289	4.12	4.89	5.83
		50–59	383	4.23	5.06	6.65	193	4.34	5.11	6.93	189	4.07	5.06	6.27
		60–79	270	4.23	5.17	6.87	139	4.34	5.28	8.36	135	4.01	5.17	6.16
TC[a]	mmol/L	20–29	475	2.9	4.22	5.75	246	2.85	4.31	6	229	2.97	4.15	5.45
		30–39	579	3.47	4.62	6.32	302	3.3	4.7	6.52	279	3.52	4.55	6.05
		40–49	539	3.77	5	7.05	258	3.72	5.12	7.25	282	3.72	4.9	6.65
		50–59	376	3.62	5.27	7.22	192	2.99	5.2	6.92	185	3.82	5.37	7.55
		60–79	270	3.57	5.27	7.25	138	3.42	5.05	6.92	131	3.92	5.47	7.55
TG[a]	mmol/L	20–29	487	0.44	0.93	2.62	252	0.47	1.16	3.01	235	0.42	0.8	1.65
		30–39	593	0.51	1.14	3.35	308	0.69	1.46	4.07	284	0.45	0.93	2.26
		40–49	548	0.62	1.37	3.99	262	0.74	1.69	4.15	288	0.53	1.08	2.86
		50–59	387	0.61	1.45	3.56	195	0.63	1.57	4.05	192	0.62	1.37	3.3
		60–79	277	0.67	1.47	4.14	141	0.66	1.51	4.33	135	0.71	1.44	3.92
LDLC[a]	mmol/L	20–29	485	1.2	2.22	3.55	247	1.27	2.35	3.7	238	1.12	2.07	3.27
		30–39	595	1.57	2.55	4.02	306	1.62	2.67	4.2	290	1.5	2.42	3.72
		40–49	556	1.72	2.82	4.4	263	1.87	2.9	4.87	293	1.87	2.75	4.05
		50–59	384	1.34	3.05	4.52	197	1.62	3.05	4.45	188	1.85	3.05	5.02
		60–79	273	1.34	3.02	4.55	139	1.62	2.9	4.4	134	1.77	3.15	4.67
ALP	U/L	20–29	482	40	65	107	246	48	73	117	233	36	59	86
		30–39	595	39	66	111	307	46	73	118	287	32	59	89
		40–49	555	39	68	114	265	42	74	114	292	36	62	107
		50–59	390	45	75	128	198	42	72	115	191	47	78	132
		60–79	277	48	79	138	139	53	77	138	134	47	82	131

LL, lower limit of the RI; Me, median; UL, upper limit of the RI; yrs: years. [a]These analytes have well-recognized CDLs. The RI results of GLU, TC, TG, and LDL-C given in this table should not be confused with the CDLs for these analytes.

limits. Therefore, a multicenter study must be carefully organized for establishing common RIs so that they are minimally affected by between-laboratory analytical bias [25]. In the present study, analyses of all the samples were made by a single core laboratory, thus minimizing analytical variations. The trueness of the core laboratory results was ensured using certified reference materials.

It is known that Turks have a high prevalence of coronary heart disease [26], associated with some known risk factors [17, 27, 28]. Turks have distinctively low concentrations of HDL-C [26–30], associated with elevated hepatic lipase activity and fasting triglyceridemia [28]. Genetic and environmental factors are also important in modulating HDL-C concentrations in Turks [29, 31]. In the present study, the mean concentrations of HDL-C in men and women were 1.05 mmol/L and 1.32 mmol/L, respectively. The mean concentrations of TG and TC in men and women were 1.25 mmol/L and 0.86 mmol/L, and 4.6 mmol/L and 4.45 mmol/L, respectively. These values are comparable to those reported recently for men and women living in Bursa [15, 16]. However, the HDL-C concentrations in this study were lower than the values observed in several different regions in Asia [25]. Although there was no significant difference in any analyte by birth place or by area

Table 6 RIs derived by the parametric method in males+females, in males, and in females.

Analyte	Unit	Age	Males+Females			Males			Females			Suggested RIs by manufacturer or CDLs
			LL	Me	UL	LL	Me	UL	LL	Me	UL	
TP	g/L		66	73	82							60–78
ALB	g/L		41	45	49							35–52 (20–60 years)
												32–46 (60–90 years)
UN	mmol/L	<50				2.95	4.53	7.2	2.21	3.72	6.12	3.2–7.4 (M,<50 years)
												2.52–6.73 (F,<50 years)
		≥50							2.85	4.72	7.96	3.02–9.2 (M, >50 years)
												3.52–7.23 (F, >50 years)
UA	µmol/L					226	327	458	166	238	345	208–428 (M)
												154–357 (F)
CRE	µmol/L					59	72	92	50	58	71	63.72–110.62 (M)
												50.44–98.23 (F)
TBil	µmol/L					3.8	9.6	24.1	2.7	7.0	15.9	3.42–20.52
DBil	µmol/L		1.36	3.42	6.84							0.0–8.55
GLU[a]	mmol/L		3.96	4.84	5.88							<5.55[b]
TC[a]	mmol/L	<50	3.22	4.67	6.45	3.20	4.60	6.42	3.20	4.66	6.38	<5.00[b]
		≥50							3.93	5.56	7.92	
TG[a]	mmol/L	<50				0.53	1.25	3.39	0.46	0.93	2.52	<1.65[b]
		≥50							0.64	1.39	3.55	
HDL-C[a]	mmol/L					0.85	1.05	1.52	0.95	1.32	1.56	>1.50[b]
LDL-C[a]	mmol/L	<50	1.47	2.80	3.92	1.60	2.67	4.01	1.32	2.47	3.92	<2.5[b]
		≥50							1.78	3.20	4.91	
Na	mmol/L		137	140	144							136–145
K	mmol/L		3.7	4.2	4.9							3.5–5.1
Cl	mmol/L		99	103	107							98–107
Ca	mmol/L		2.15	2.30	2.47							2.10–2.55
IP	mmol/L		0.80	1.08	1.40							0.73–1.50
Mg	mmol/L		0.77	0.94	1.06							0.65–1.06
AST	U/L					13	19	30	11	16	25	5–34
ALT	U/L					9	19	57	7	19	28	0–55
LDH	U/L		126	164	220							125–220
ALP	U/L	<50	38	67	112	43	72	116	34	60	97	40–150
		≥50							47	79	133	
GGT	U/L					11	23	69	7	13	33	12–64 (M)
												9–36 (F)
CK	U/L					48	98	227	34	62	131	30–200 (M)
												28–168 (F)
AMY	U/L		34	63	119							25–125

LL, lower limit of the RI; Me, median; UL, upper limit of the RI. [a]These analytes have well-recognized CDLs. The RI results of GLU, TC, TG, HDL-C and LDL-C given in this table should not be confused with the CDLs for these analytes. [b]CDLs.

of residence, HDL-C was slightly lower (r_p=–[JC1]0.22) in participants from East Anatolia. This difference may be attributable to differences in dietary habits or other environmental or genetic factors.

The observed URLs of LDL-C, TC, TG, ALT, GGT and GLU are higher than the so-called CDLs. This suggests the presence of latent diseases, such as the metabolic syndrome or pre-diabetes. We applied stricter exclusion criteria for lipids (HDL-C, LDL-C, TC, and TG), ALT, GGT and GLU which are known to be associated with those latent diseases. The values for HDL, LDL-C, TC and TG are in good accordance with the values recently reported in a North Indian population [32]. However, the URLs derived for these parameters in serum are not meant to be used for clinical decision making, and we are aware that it is more appropriate to apply CDLs for these parameters in order to identify risk for certain diseases.

In the present study we found that the URLs of CRE were 92 µmol/L for males and 71 µmol/L for females. These values are comparable to the reported values of 97 µmol/L [24], and 93 µmol/L [33] for adult males and 70 µmol/L [24], and 69 µmol/L [33] for adult females, but are much lower than the previously reported values of 104 µmol/L [34], 105 µmol/L [35, 36] and 104 µmol/L [37] for males and 84 µmol/L [34], 92 µmol/L [35, 36] and 90 µmol/L [37] for

females. Those studies, however, used different methods and analyzers (Creatinine Plus, Roche Diagnostics and Roche Hitachi 717). The values in the present study were also lower than the values of 110 μmol/L for males and 98 μmol/L for females suggested by manufacturers (Table 6). As noted by Ceriotti et al. [38] in their assessment of available data for serum creatinine RIs, the results were obtained exclusively from white Europeans [35–37] and/or Australians [34]. The low values in the current study are probably related to the fact that regular practice of physical exercise is less common in Turkey, so that there is a relatively lower muscle mass compared with the Caucasian population (unpublished findings observed in the ongoing IFCC global study). An additional possible factor for the low CRE is low meat consumption across Turkey, compared with the Caucasian population, due to socioeconomic and cultural differences. Thus, the observed differences in the LRLs between our present study and the previous studies could be attributable to differences in study populations.

The observed RIs for GGT in both sexes and for AST and ALT in males are in good accordance with the values reported recently by an IFCC multicenter study including Milan, Bursa, Beijing and the Nordic countries [39]. The method in that study used P5P (the IFCC reference method), whereas in the current study P5P was not used, which may have given rise to the difference in data although we had recalibrated the test results based on the value assigned AST and ALT sera as shown in Table 2 of the Supplemental Data. However, our URLs for AST and ALT were lower especially in females, which appears to be caused by the application of LAVE method to exclude latent abnormal values. RIs observed in the present study, with the exception of a few analytes [GGT, AST (males), ALP (males), Cl, TC (females) and TG], were found to be similar to the values reported for Hong Kong, which were obtained on the Abbott Architect c16000 analyzer [40]. Taken together, these data re-emphasize that population-specific RIs for laboratory analytes should be determined for each population.

In conclusion, the lack of apparent regional differences in reference values for any of the analytes and nationwide standardization of the assays, the RIs reported in this manuscript can be used in common in all clinical chemistry laboratories in Turkey for the interpretation of laboratory results for the 25 analytes.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
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References

1. Horn PS, Pesce AJ. Reference intervals an update. Clin Chim Acta 2003;334:5–23.
2. Solberg HE. International Federation of Clinical Chemistry (IFCC). Approved recommendation on the theory of reference values. Part 1.The concepts of reference values. Clin Chim Acta 1987;167:111–8; J Clin Chem Clin Biochem 1987;25:337–42.
3. Petit Clerc C, Solberg HE. International Federation of Clinical Chemistry (IFCC). Approved recommendation on the theory of reference values. Part 2. Selection of individual for the production of reference value. Clin Chim Acta 1987;170:S1–12; J Clin Chem Clin Biochem 1987;25:337–42.
4. Solberg HE, Petit Clerc C. International Federation of Clinical Chemistry (IFCC). Approved recommendation on the theory of reference values. Part 3. Preparation of individuals and collection of the specimens for the production of reference values. Clin Chim Acta 1988;177:S3–11; J Clin Chem Clin Biochem 1988;26:593–8.
5. Solberg HE, Stamm D. International Federation of Clinical Chemistry (IFCC). Approved recommendation on the theory of reference values. Part 4. Control of analytical variation in the production, transfer and application of reference values. Clin Chim Acta 1991;202:S5–11; J Clin Chem Clin Biochem 1991;29:531–5.
6. Solberg HE. International Federation of Clinical Chemistry (IFCC). Approved recommendation on the theory of reference values. Part 5. Statistical treatment of collected reference values. Clin Chim Acta 1987;170:S13–32; J Clin Chem Clin Biochem 1987;25:645–56.
7. Dybkaer R, Solberg HE. International Federation of Clinical Chemistry (IFCC). Approved recommendation on the theory of reference values. Part 6. Presentation of observed values related to reference values. Clin Chim Acta 1987;170:S33–42; J Clin Chem Clin Biochem 1987;25:657–62.
8. CLSI and IFCC, C28-A3 document; Defining, establishing and verifying reference intervals in the clinical laboratory: approved guideline, 3rd ed., 2008;28:1–76.

9. Ceriotti F, Hinzmann R, Panteghini M. Reference intervals: the way forward. Ann Clin Biochem 2009;46:8–17.
10. Directive 98/79/EC of European Parliamant and the Council of 27 October 1998 ON invitro diagnostic medical devices. Offical J Eur Commun 7 December 1998; L331/1–L331/37.
11. International Organization for Standardization. ISO 15189:2012 Medical laboratories – Requirements for quality and competence, 3rd ed., 2012.
12. Ceriotti F. Common reference intervals the IFCC position. Clin Biochem 2009;42:297.
13. Ozarda Y, Ichihara K, Barth JH, Klee G, and on behalf of the CRIDL on Reference intervals and Decision Limits, IFCC. Protocol and standard operating procedures for common use in worldwide multicenter study on reference values. Clin Chem Lab Med 2013;51:1027–40.
14. Ichihara K, Ozarda Y, Klee G, Straseski J, Barth JH, Baumann N, et al. Utility of panel of sera for alignment of test results in the worldwide multicenter study on reference values. Clin Chem Lab Med 2013;51:1007–20.
15. Ozarda Ilcol Y, Aslan D. Determining reference value of blood chemistry profile in healthy subjects in Bursa. Turk J Biochem 2004;29:183–92.
16. Ozarda Ilcol Y, Aslan D. Use of patient data for indirect estimation of reference intervals for clinical chemical anaytes in Turkey. Clin Chem Lab Med 2006;44:867–76.
17. Ozarda Y, Sucu DK, Hizli B, Aslan D. Rate of T alleles and TT genotype at MTHFR 677C->T locus or C alleles and CC genotype at MTHFR 1298A->C locus among healthy subjects in Turkey: impact on homocysteine and folic acid status and reference intervals. Cell Biochem Func 2009;27:568–77.
18. Inal TC, Serteser M, Coskun A, Ozpinar A, Unsal I. Indirect reference intervals estimated from hospitalized population for throtropin annd free thyroxine. Croat Med J 2010;51:124–30.
19. Koseoglu M, Isleten F, Dursun S, Cuhadar S. Determination of reference intervals of healthy adults aged between 20–50 years in Izmir. Turk J Biochem 2004;35:215–24.
20. Ichihara K. Statistical considerations for harmonization of the global multicenter study on reference values. Clin Chim Acta 2014;432:108–18.
21. Ricos C, Alverez V, Cava F, Garcia-Lario CV, Hernandez A, Jimenez CV, et al. Current data bases on biyologic variation: pros, cons and progress. Scand J Clin Lab Invest 1999;59:491–500.
22. Ichihara K, Boyd J. An appraisal of statistical procedures used in derivation of reference intervals. Clin Chem Lab Med 2010;48:1537–51.
23. Yamamato Y, Hosogaya S, Osawa S, Ichıhara K, Onuma T, Saito A, et al. Nationwide multicenter study aimed at the establishment of common reference intervals for standardized clinical laboratory tests in Japan. Clin Chem Lab Med 2013;51:1–10.
24. Ichihara K, Ceriotti F, Tam TH, Sueyoshi S, Poon PM, Thong ML, et al. The Asian project for collaborative derivation of reference intervals: (1) strategy and major results of standardized analytes. Clin Chem Lab Med 2013;48:1593–601.
25. Ceriotti F. Prerequisites for use of common reference intervals. Clin Biochem Rev 2007;28:115–21.
26. Mahley RW, Palaoglu EK, Atak Z, Dawson-Pepin J, Langlois AM, Cheung V, et al. Turkish Heart Study: lipids, lipoproteins, and apolipoproteins. J Lipid Res 1995;36:839–59.
27. Onat A. Risk factors and cardiovascular disease in Turkey. Atherosclerosis 2001;156:1–10.
28. Bersot TP, Vega GL, Grundy SM, Paloglu EK, Atagündüz P, Ozbayrakçi S, et al. Elevated hepatic lipase activity and low levels of high density lipoprotein in a normotriglyceridemic, nonobese Turkish population. J Lipid Res 1999;40:432–8.
29. Mahley RW, Pepin J, Paloglu EK, Malloy MJ, Kane JP, Bersot TP. Low levels of high density lipoproteins in Turks, a population with elevated hepatic lipase activity: high density lipoprotein characterization and gender specific effects of apoprotein E genotype. J Lipid Res 2000;41:1290–301.
30. Onat A, Can G, Hergenç G, Uğur M, Yüksel H. Coronary disease risk prediction algorithm warranting incorporation of C-reactive protein in Turkish adults, manifesting sex difference. Nutr Metab Cardiovasc Dis 2012;22:643–50.
31. Mahley RW, Can S, Ozbayrakci S, Bersot TP, Tanir S, Palaoglu KE, et al. Modulation of high-density lipoproteins in a population in Istanbul, Turkey with low levels of high-density lipoproteins. Am J Cardiol 2005;96:547–55.
32. Yadav D, Gupta M, Mishra S, Sharma P. Reference interval for lipid profile in North Indian population from Rajasthan according to various partitioning criteria. Clin Chim Acta 2013;426:145–51.
33. Wang XJ, Xu GB, Li HX, Liu Y, Wang F. Reference intervals for serum creatinine with enzymatic assay and evaluation of four equations to estimate glomerular filtration rate in a healthy Chinese adult population. Clin Chim Acta 2011;412:1793–7.
34. Mazzachi BC, Peake MJ, Ehrhardt V. Reference range and method comparison studies for enzymatic and Jaffe creatinine assays in plasma and serum and early morning urine. Clin Lab 2000;46:53–5.
35. Rustad P, Felding P, Franzson I, Kairisto V, Lahti A, Mårtensson A, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. Scand J Clin Lab Invest 2004;64:271–84.
36. Mårtensson A, Rustad P, Lund H, Ossowicki H. Creatininium reference intervals for corrected methods. Scand J Clin Lab Invest 2004;64:439–42.
37. Junge W, Wilke B, Halabi A, Klein G. Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffe method. Clin Chim Acta 2004;344:137–48.
38. Ceriotti F, Boyd JC, Klein G, Henny J, Queraltó J, Kairisto V, et al.; IFCC Committee on Reference Intervals and Decision Limits (C-RIDL). Reference intervals for serum creatinine concentrations: assessment of available data for global application. Clin Chem 2008;54:1261.
39. Ceriotti F, Henny J, Queraltó J, Ziyu S, Ozarda Y, Chen B, et al. IFCC Committee on Reference Intervals and Decision Limits (C-RIDL); Committee on Reference Systems for Enzymes (C-RSE). Common reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) in serum: results from an IFCC multicenter study. Clin Chem Lab Med 2010;48:1593–601.
40. Lo YC, Armbruster DA. Reference intervals of common clinical chemistry analytes for adults in Hong Kong. eJIFCC, 2012. Available from: http://www.ifcc.org/ifcc- communications-publications-division. Accessed on 13 June, 2013.