European Journal of Therapeutics https://doi.org/10.58600/eurjther.20232902-1343.y

Original Research

Determination of Reference Intervals of Biochemistry Parameters in Healthy Individuals in Gaziantep Province

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ABSTRACT

Objective: Reference values have gained universal acceptance as the most powerful material that helps the decision-making-implementation process of the clinical laboratory. These values may be affected by the geographical location, dietary habits and other lifestyle changes of individuals applying to the clinical laboratory. The aim of our study to determine the reference ranges for the biochemistry test panel, thyroid function tests, and insulin hormone levels, which are frequently needed by clinicians for the province of Gaziantep, with samples obtained from healthy individuals.

Methods: In the study phase, the selection of reference individuals was carried out using the direct method a priori. For the study group, healthy individuals (224 men, 243 women) between the ages of 18-45 were selected. Reference intervals (95% limit) were calculated according to the non-parametric method.

Results: When the reference intervals obtained in our study were compared with the reference intervals of the manufacturer, there were differences (> 10% lower or higher) in the upper and lower limits in urea (female and male), creatinine (male), HDL (female), AST (female and male), ALT (female), GGT (female), ALP (common), Lipase (common), CK (male), iron (male), TSH (female and male) markers. Male and female reference intervals for HDL, AST, ALT, and TSH were significantly different. Manufacturer reference ranges for these parameters were common to both sexes.

Conclusion: As a result, differences were determined between most of the the reference intervals obtained in our study and the reference intervals we routinely use. We think that the difference in the reference intervals is due to the differences in dietary habits and environmental factors.

Keywords: Reference range, biochemistry tests, direct method, regional differences

INTRODUCTION

Reference values have gained universal acceptance as the most powerful material that helps the decision-making-implementation process of the clinical laboratory [1]. Clinical laboratories provide services to clinicians and patients in order to evaluate health status, diagnosis of disease, degree of disease, drug dose, and sometimes surgical intervention with the tests they measure [2]. Reference values and ranges form the basis for the interpretation of laboratory test results and help the clinician to distinguish between healthy and sick individuals [3]. For this reason, each clinical laboratory should determine the reference values and reference interval of its own population or prove the suitability of the current values to the population.

The importance of reference intervals (RIs) has been recognized in the laws of the United States, and the Clinical Laboratory Improvement Amendments requests laboratories that offer, modify, or develop their own measurements of the FDA-approved test system, and manufacturers, to verify that the RIs are compatible for their own patient population [2]. Article 5.5.5 of the ISO 15189 Special Conditions for Quality and Competence Standard, which is a clinical laboratory accreditation standard, is related to RIs. Accordingly, before the analysis and after each update in the analysis procedures, the RIs are reviewed, and the necessary changes are provided by the laboratory specialists [4]. Having sufficient data is extremely important when determining the reference range. Statistical methods used in the reference range determination are highly dependent on the distribution type of the reference population and the number of data [5]. According to C28-A3 standards, at least 120 data will be sufficient for the statistical evaluation of data in reference range analysis. This number is also valid for main subgroups such as age and gender [6,7].

In this study, we determined the reference ranges for the most frequently studied biochemistry test panel, thyroid function tests, and insulin hormone levels in our central laboratory for Gaziantep province in accordance with the C28-A3 standards.

How to cite: Örkmez M and Tarakcioglu M (2023) Determination of Reference Intervals of Biochemistry Parameters in Healthy Individuals in Gaziantep Province. Eur J Ther. 29(2):173-178. https://doi.org/10.58600/eurjther.20232902-1343.y

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Received: 10.05.2023 • **Accepted:** 26.05.2023 • **Published Online:** 27.05.2023

pISSN: 2564-7784
eISSN: 2564-7040

METHODS

Subjects

The reference range determination study was planned as the 'Prior' choice of the 'Direct' method proposed by the International Federation of Clinical Chemistry, among many methods related to the subject [6]. Questionnaires with added questions were applied to individuals aged 18-45 in Gaziantep, selected as the study group, according to the US National Clinical Laboratory Standards Committee C28-A3 standards and by evaluating preanalytical factors. Exclusion criteria were: BMI ≥ 30, alcohol consumption ≥ 70 g/day, smoking >20 cigarettes/day, Hb (female) <12.5, Hb (male) <13.5, chronic systemic disease (CRP> 5 mg/L), having an acute disease within the last 14 days, currently known carrier state for HBV, HBC or HIV, pregnancy, and being in the postpartum first year. According to the preliminary evaluations of these questionnaires, taking into account the exclusion criteria, 224 male and 243 female healthy individuals.

This study was carried out in accordance with the Helsinki Declaration Decisions, Patient Rights Regulation and Ethics Committee Rules, and approval was obtained from the Gaziantep Ethics Committee (on May 5, 2011, with Decision No. 05/2011-55). Volunteers gave written informed consent to participate in the study and were briefed on the results upon request.

Biochemical Analysis

The subjects fasted prior to sample collection and the time of sampling was set at 7 to 10 am. Within 20 to 30 minutes of selection, the samples were centrifuged at 1200 x g for 10 min at room temperature. Blood samples were centrifuged within 20–30 minutes of withdrawal from each volunteer. One aliquot of 1 ml was prepared and stored at -80 ±2 oC for up to six months until analysis. The frozen serum samples were transferred to a refrigerator (+4–6 oC) for about 2–3 hours for thawing before examination and then transferred to the analyzer within 6 hours of thawing. Glucose, urea, creatinine, uric acid, total protein, albumin, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, calcium, total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), amylase, creatine kinase (CK), creatine kinase MB (CK-MB), iron, magnesium, sodium, potassium, chlorine, lipase and unsaturated iron binding capacity (UIBC) measurements were performed on the Abbott Architect C16000 autoanalyzer (Abbott Inc., Wiesbaden, Germany). Free T3 (fT3), free T4 (fT4), thyroid stimulating hormone (TSH) and insulin hormone measurements were made on the Abbott Architect I2000 (Abbott Inc., Wiesbaden, Germany). Hemoglobin (Hb) values measured in order to identify healthy individuals were measured on the Becman Coulter LH780 (Becman Coulter Inc, California, USA) device, and CRP and HbsAg values were measured using the Abbott Architect I2000 (Abbott Inc., Wiesbaden, Germany) device. Original kits from the company representative were used for each device.

Statistical Analysis

To calculate RIs, data were transferred into SPSS version 20.0 (SPSS Inc., Chicago, USA) and MedCalc version 14.12.0 (MedCalc Software, Mariakerke, Belgium). Reference limits describe the central 95% of the reference population. Non-parametric statistics were used for the determination of RIs. Non-parametric methods typically include the central 95th percentile of reference values and use the 2.5th and 97.5th percentile as the lower and upper reference limit, respectively. Dixon's range test, recommended by the IFCC for statistical analysis in reference interval studies, was used to detect and eliminate extreme values as outliers. Confidence intervals of 90% (90% CI) of reference limits were determinated following IFCC recommendations [8,9].

RESULTS

Five hundred forty-five samples were collected from candidate reference individuals between the ages of 18-45 (mean age 30.7±7.8 years) living in Gaziantep province. A total of 78 samples were excluded from the study because of Hb levels less than 12.5 g/dL in women (n=35) and less than 13.5 g/dL in men (n=13), CRP levels above five mg/L (female n=8, male n=5) and HbsAg (+) (female n=6, male n=11). After exclusions, the study group was formed with the remaining 467 samples (243 females, 224 males).

Table 1 includes the demographic and characteristic data for the male and female genders of the reference individuals in the study group. The degree of influence of the factors (alcohol, smoking, exercise) that might affect the reference ranges on the data was analyzed with the "Mann-Whitney U test". No statistically significant difference was found in terms of their effects (p> 0.05). Table 2 shows the RIs of 33 analytes determined by the direct method in males (n:224) and females (n:243) participants and 90% CIs of reference limits. It also includes a comparison of the reference ranges determined with the reference ranges from the literature and the manufacturer. When the reference intervals obtained in our study were compared with the reference intervals of the manufacturer, there were differences (>10% lower or higher) in the upper limits of urea (female and male), creatinine (female and male), HDL (female), TBIL (common), AST (female and male), ALT (female), GGT (female), ALP (common), Lipase (common), CK (male), iron (female and male), TSH (female and male) markers. There were differences (>10% lower or higher) in the lower limits of urea (female and male), creatinine (male), HDL (male and female), TBIL (common), AST (female and male), GGT (female and male), ALP (common), Mg (common), iron (male), TSH (female and male), fT3, fT4 markers. Male and female reference intervals for HDL, AST, ALT, and TSH were significantly different. Manufacturer reference ranges for these parameters were common to both sexes.

DISCUSSION

In this study, it was aimed to determine the RIs for the most frequently studied biochemistry tests in our central laboratory. At the beginning of this study, it was started with the question of why RIs are so important. Today, due to the developing technology and increasing test diversity, clinicians make more laboratory requests and decide on clinical diagnosis, patient follow-up, and surgical intervention with the interpretation of the results.

Table 1. Demographic and characteristic data of the reference population

	Female	Male
n	243	224
Age (years)	29.7±7.8	31.7±7.7
BMI (kg/m2)	24.6±4.9	25.8±3.1
Smoking (Yes)	19	24
Alcohol consumption (Yes)	5	12
Exercise (Yes)	7	20

Table 2. Reference intervals estimated with direct method using non-parametric calculation

Test Name	Unıt	Method	Gender	RIs			Manufac-turer	Literature (14)
				LL-CI	LL-UL	UL-CI		
Glucose	mg/dL	Hexokinase	C	68-71	70-103	101-106	70-105	74-100
Urea	mg/dL	Urease	F	11-12	11-34 (•,▪)	32-39	19-42	13-43*
			M	16-18	17-39 (•,▪)	38-44	15-55	
Creatinine	mg/dL	Jaffe/picrate	F	0.51-0,56	0.52-0.85 (▪)	0.81-0.92	0.57-1.1	0.45-0.75
			M	0.53-0.67	0.63-1.05 (•,▪)	1.01-1.09	0.72-1.25	0.62-1.1
Urıc Acıd	mg/dL	Urokinase	F	1.9-2.3	2.1-6 (•)	5.5-7.1	2.6-6	2.3-6.6
			M	2.8-3.5	3.1-7.9 (•)	7.3-8.1	3.5-7.2	4.4-7.3
Total Proteın	g/dL	Biuret	C	5.7-6.1	6-8.1	8-8.2	6.4-8.3	6.4-8.3
Albumın	g/dL	Bromocresol green	C	3.38-3.62	3.46-4.9	4.81-4.95	3.5-5	3.5-5.2
Total Cholesterol	mg/dL	Enzymatic	C	108-121	114-259	234-258	<200**	<200**
Trıglyceride	mg/dL	Enzymatic	F	30-46	40-220	189-225	<170**	<150**
			M	36-51	43-305	285-364		
LDL	mg/dL	Liquid se-lective de-tergent	C	48-58	54-159	156-174	<130**	<100**
HDL	mg/dL	Accelerator selective detergent	F	25-34	33-68 (•,▪)	66-75	40-60	40-60
			M	25-29	27-55 (•)	53-59		
Total Bilirubin	mg/dL	Colorimetric	C	0.2-0.25	0.23-1.32 (•,▪)	1.25-1.39	0.2-1.2	0-2
Direct Bilirubin	mg/dL	Colorimetric	C	0.08-0.1	0.09-0.51	0.48-0.64	0-0.5	0-0.2
AST	U/L	Enzymatic	F	8-10	9-27 (•,▪)	24-28	5-34	<31
			M	9-10	9-38 (•,▪)	34-43		<35
ALT	U/L	Enzymatic	F	4-5	4-32 (▪)	30-39	0-55	<34
			M	5-6	5-53	48-61		<45

ALP	U/L	Enzymatic	C	32–38	35–107 (•,▪)	101–122	40–150	42–98 53–128
GGT	U/L	Enzymatic	F M	5–7 9–11	6–27 (•,▪) 10–59 (•)	26–32 53–67	9–36 12–64	2–35 1–24
LDH	U/L	From lactate to pyruvate	C	105–115	112–231(•)	221–239	125–243	125–220
Amylase	U/L	Enzymatic	C	29–35	32–113 (•)	106–119	25–125	28–100
Lipase	U/L	Colorimetric	C	8–11	9–54 (▪)	49–56	8–78	<38
CK	U/L	Enzymatic	F M	25–35 41–49	29–141(▪) 44–225 (•,▪)	125–162 201–259	29–168 30–200	46–171 34–145
CK–MB	U/L	Colorimetric	C	4–7	5–27	26–28	<24	
Iron	µg/dL	Colorimetric	F M	25–31 31–46	28–160 (▪) 42–179 (•,▪)	143–164 173–212	31–144 25–156	65–175 50–170
Iron Binding Capacity	µg/dL	Colorimetric	F M	16–123 84–144	107–446 130–344 (•)	411–500 324–377	110–370	250–425
Ca	mg/dL	Arsenazo III Complex	C	8–8.4	8.2–10.1	10–10.3	8.4–10.2	8.6–10.2
Mg	mg/dL	Arsenazo III Complex	C	1.78–1.84	1.82–2.8 (•)	2.75–2.83	1.6–2.6	1.6–2.6
Phosphorus	mg/dL	Phospho–molybdate	C	2.3–2.6	2.49–4.5	4.4–4.7	2.3–4.7	2.5–4.5
Na	mmol/L	ISE	C	131–132	131–145	143–146	136–145	136–145
K	mmol/L	ISE	C	3.53–3.7	3.62–5.03	4.84–5.14	3.5–5.1	3.5–5.1
Cl	mmol/L	ISE	C	98–100	99–110	109–111	98–107	98–107
TSH	µIU/mL	CMIA	F M	0.44–0.67 0.38–0.57	0.55–4.11 (•,▪) 0.47–3.53 (•,▪)	4.04–4.38 3.24–3.69	0.35–4.94	0.4–4.2
Ft3	pg/mL	CMIA	C	2.39–2.57	2.53–3.93(•)	3.86–4.03	1.71–3.71	2.1–4.4
Ft4	ng/dL	CMIA	C	0.84–0.87	0.86–1.32(•)	1.28–1.36	0.7–1.48	0.4–2.7
Insulin	µU/mL	CMIA	C	3–4	4–20	17–21	–	3–25

(*) Calculated by converting blood urea nitrogen to urea
(**) Optimal values expected in healthy individuals
(•) LL of obtained RIs different (> 10% lower or higher) from manufacturer RIs
(▪) UL of obtained RIs different (> 10% lower or higher) from manufacturer RIs
F: Female, M: Male, C: Common, RIs: Reference Intervals, LL: Lower Limit, UL: Upper Limit, CI: Confidence intervals, ISE: Ion Selective Electrode, CMIA: Chemiluminescent microparticle immunological assay

Clinical laboratories, due to their responsibilities in health care, should provide clinicians and patients with the essential guides for the correct interpretation of all the tests they offer. The primary source that clinicians use when interpreting test results is reference ranges [10,11]. These values not only affect the physician's decision but also cause negativities in the patient's life. For this reason, the RIs to be included in the guidelines should represent the population served by the clinical laboratory and there should be no room for doubt in these values [7,12].

The suitability of the RIs in the prospectuses of the manufacturer according to the results of non-parametric methods was

evaluated [13]. Our results showed that; The reference ranges determined for the lower and upper limits in twelve parameters differed from those used in the routine. There were differences >10% lower or higher in the upper and lower limits. These changes are notable considering that sometimes seemingly minor differences are of great importance in clinical decisions. Unlike other parameters, RIs are not given in the kit package insert for the insulin hormone, and it is recommended by the manufacturer that the responsible clinical laboratory should conduct a reference study. Male and female reference intervals for HDL, AST, ALT, and TSH were significantly different. Manufacturer reference ranges for these parameters were common to both sexes.

As a result of reference value studies, the most important question is that 'can the determined reference values be applied in practice?' In fact, this is the most critical result of reference interval studies. Therefore, RIs determined by a team of biochemists and clinicians should be evaluated one by one before the results can be put into practice. If the determined reference values are compatible with the reference interval data in the literature and the package insert, it can be said that the reference interval of your society is similar to the RIs of other societies. If different reference values have been obtained, then you have the chance to say that your society's reference range is diverse and you have the opportunity to interpret this different result.

In our study, the upper reference value for total cholesterol was found as 259 mg/dL. When the literature and kit prospectuses are examined and clinical practice is examined, it is seen that the desired value for total cholesterol in healthy individuals is below 200 mg/dL. The blood lipid values we obtained bring to mind the question, "Are we administering unnecessary drug treatment to healthy individuals? or will we conclude that we have high blood lipid values depending on the nutritional habits of the society and these values cannot be a reference for a healthy person?" At this stage, the discussion can be continued over the decision limits. Yes, the values determined as a result of this study are the reference values of our society, but in clinical practice, there is also a need for decision limit studies for analytes such as glucose, total cholesterol, LDL-cholesterol, and triglycerides. These approaches also show that the use of determined RIs in routine practice is a process that should be supported by new ideas and studies.

For HDL-cholesterol, the manufacturer's reference ranges are given as 40-60 mg/dL without discrimination between men and women. However, both in our study and in studies conducted in Bursa and Denizli, HDL cholesterol values in men and women were significantly different. For HDL reference values, we found 27-55 mg/dL for men and 33-71 mg/dL for women in our study. In the study conducted in Bursa, values of 30-54 mg/dL in men and 31-65 mg/dL in women were reported [15], while values of 28-67 mg/dL in men and 35-83 mg/dL in women were obtained in the study conducted with individuals living in Denizli [13]. These results show us once again how reference values differ between individuals living in different parts of the same society, as well as gender.

These data and reference range studies provide essential information about the habits of our own society. It allows us to have information about the relationship between tests and lifestyle factors, such as hyperlipidemia, which is seen as an essential factor in terms of cardiovascular diseases, which has been discussed for many years in our country, and hyperglycemia, which is the leading cause of diabetes with a prevalence of 16.5% today [16].

In the thyroid test panel, narrower RIs were obtained than the RIs of the manufacturer. In our study, 2.53-3.93 pg/mL for fT3, 0.86-1.32 ng/mL for fT4, and 0.55-4.11 µIU/mL for TSH in women and 0.47-3.53 µIU in men /mL results obtained. Endemic goiter and iodine deficiency are important public health problems in Turkey. In the survey study of Kologlu et al. [17], it was determined that there is a significant problem of goiter in many regions of Turkey, and it was stated that this was due to the insufficient iodine content of the waters and soil. The differences in these values obtained in the RIs of thyroid function tests can be explained by geographical and ethnic differences such as population, lifestyle, salt iodination and nutrition [18].

Reference values for liver enzymes AST and ALT were significantly different in men and women ($p<0.01$). The company gives RIs as single RIs for both genders. These results suggest that separate reference values should be given for men and women.

The results of this study once again showed that reference values may vary according to the population served by the clinical laboratory. Gender, geographical location, socio-economic level and related nutrition, smoking and alcohol use, exercise may be the reasons for these differences. Therefore, each clinical laboratory should perform reference range studies for its own population. As stated in the accreditation documents regulating clinical laboratory service conditions and standards, the reference values used should be validated at least [4].

To summarize, most laboratories in our country, as in the world, use literature or test kit manufacturer reference intervals. This may affect the clinical interpretation of physicians and lead to misdiagnosis and treatments [19]. According to the European Union's regulation on in vitro diagnostic medical devices, test kit manufacturers are responsible for providing appropriate reference ranges for use with their devices. Due to the difficulties of the direct method, most laboratories use these reference ranges. However, it is the task of laboratories to determine the suitability of these externally sourced reference intervals for use [20].

Our study is the first reference interval study of our region and includes many biochemical markers. The 18-45 age range was a good choice to reach healthy individuals but it was also the main limitation of our study. Our targets are new reference interval studies to be carried out up to age 65. Also, RIs are representative of the population and are, therefore, not a perfect fit for the individual. An improved solution may be to use the data of individuals to derive a personalized RI. [21].

As a result, differences were determined between the RIs obtained in our study and the RIs in the manufacturer and the literature. We think that the difference in the RIs is due to the differences in dietary habits and environmental factors. The different RIs obtained will be used in diagnostic laboratories after meetings with clinicians.

Peer-review: Externally peer-reviewed.

Conflict of interest: The authors declare no conflict of interest.

Funding: The authors declare no funding.

Author's Contributions: Conception; MO, MT Design; MO, MT, Supervision; MT, Fundings; MT, Materials; MO, MT, Data Collection and/or Processing; MO, Analysis and/or Interpretation; MO, MT, Literature; MO, MT, Review; MO, Writing;, Critical Review; MT.

Ethics Committee Approval: Ethics committee approval was obtained from the Gaziantep University Clinical Research Ethics Committee on May 5, 2011, with Decision No. 05/2011-55.

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