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Determination of Reference Intervals of Healthy Adults Aged Between 20-50 Years in Izmir

[İzmir İlinde 20-50 Yaş Arası Sağlıklı Bireylerde Referans Aralıklarının Saptanması]

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ABSTRACT

Objective: For clinical diagnosis and therapeutic management, clinicians have to be sure of the reference limits of the laboratory parameters. International guidelines recommend every laboratory to establish their own reference intervals for healthy individuals belonging to a group of homogenous healthy population. Considering varied dietary habits and geographical differences in Turkey, there is a need for a specific reference interval for Turkish population. In this context, this study sought to answer the question whether referance ranges should be calculated seperately for different subpopulations in Turkey.

Methods: The reference intervals for clinical chemistry and hormone tests were estimated for the local Turkish population living in Izmir, according to the International Federation of Clinical Chemistry (IFCC) recommendations. The study included 274 healthy adults (133 women, 141 men) aged between 20-50 years. The health status was confirmed by history, physical examination and a questionnaire prepared according to the Clinical and Laboratory Standards Institute (CLSI/NCCLS) recommendations. The central 95% reference intervals were determined non-parametrically by direct method. The reference intervals were compared with similar (by age and health status) reference interval studies estimated in Turkey.

Results: Differences were observed between reference intervals given by the manufacturer and the intervals established by reference interval studies from other regions of Turkey.

Conclusion: Because of the difficulties of establishing reference ranges from healthy subjects, determining common reference ranges will identify the regional differences and will provide important contributions to the clinicians.

Key words: Direct method, nonparametric, reference limits, Turkish population

ÖZET

Amaç: Klinik tanı ve tedavi kararı aşamasında, klinisyenlerin laboratuvar testlerinin referans aralıklarından emin olmaları önemlidir. Uluslararası kılavuzlar, her laboratuvarın sağlıklı bireylerden oluşan homojen bir sağlıklı popülasyondan kendi referans aralıklarını belirlemelerini önermektedir. Türkiye'nin diyet çeşitliliği ve coğrafi farklılıklarını düşünürsek, Türk popülasyonuna özel referans aralıklarının belirlenmesi gerekliliği ortaya çıkar. Bu bağlamda, bu çalışma ile Türkiye'deki farklı popülasyonlar için ayrı ayrı referans aralığı mı hesaplanmalı sorusuna cevap arandı.

Yöntemler: Çalışmamızda İzmir'de yaşayan sağlıklı popülasyondan klinik biyokimya ve hormon testleri için referans aralıkları, Uluslararası Klinik Kimya ve Laboratuvar Tıbbı Federasyonu'nun (IFCC) belirlediği kriterlere göre hesaplandı. Çalışmaya, yaşları 20-50 arasında olan 274 sağlıklı erişkin (133 kadın, 141 erkek) alındı. Sağlık durumları anamnez, fiziksel muayene ile Klinik ve Laboratuar Standartları Enstitüsü (CLSI/NCCLS) önerilerine göre hazırlanan anket formuyla değerlendirildi. %95 merkezi alan temel alınarak direkt metodla nonparametrik olarak referans aralık sınırları hesaplandı. Sonuçlar Türkiye'de yapılmış olan benzer (yaş ve sağlık bakımından) referans aralık çalışmaları ile karşılaştırıldı.

Bulgular: Hesapladığımız referans aralıkları ile kit prospektüsünde önerilen referans aralıkları ve Türkiye'nin diğer bölgelerde yapılan referans çalışmalarındaki değerler arasında farklılıklar gözlendi.

Sonuç: Her laboratuvarın sağlıklı bireylerden referans aralığını belirlemesinin zorluğu nedeniyle ortak referans aralık belirleme çalışmaları yapılarak bölgeler arası farklılıkların belirlenmesinin klinisyenlere önemli katkılar sağlayacağını düşünüyoruz.

Anahtar kelimeler: Direkt metod, nonparametrik metod, referans aralıkları, Türk popülasyonu

Introduction

The reference intervals help clinicians to evaluate laboratory results, to consider the risk of some disease, and to diagnose for some disease. Lying between two reference limits, reference intervals are descriptive of a defined population determined from apparently healthy individuals. In clinical laboratories apart from the qualitative tests, most of the biochemistry tests are quantitative, so reference intervals are needed for all tests.

It is difficult principally for small laboratories to find suitable healthy volunteers and establish the reference limits especially for costly parameters such as hormones and vitamins, tumor markers. Besides it is hard to maintain reference intervals for all tests with ever changing methodologies and instrumentation.

According to guidelines [1], it is recommended that if a laboratory fails to establish their own reference intervals, they can collect samples from just 20 reference individuals to verify the manufacturer's reference intervals. If no more than 2 of 20 samples fall outside the provided reference intervals, the laboratory can safely adopt the interval. If 3 or more samples fall outside the given reference interval, they may have a problem and have to increase the sample size.

The intervals of clinical chemistry and hormone tests currently used in our laboratory are either from diagnostic package inserts without giving details of the original source of the data or from the textbooks.

In this study, reference intervals for routine biochemistry parameters and hormone tests were established according to the recommendations of IFCC in 90% confidence intervals for the 2.5th and 95th percentile by nonparametric method [1-8].

Materials and Methods

Subjects

Reference individuals were selected from two factories' apparently healthy employees native to Izmir. The potential reference individuals categorized according to the questionnaire and health investigations. The questionnaire was prepared according to the CLSI/NCCLS recommendations [9]. Obesity or underweight, pregnancy, habits of excessive drinking or smoking (smoking > 20 pieces/day), acute infection or recent recovery from illness or surgery were our other exclusion criterias. The selected individuals were aged between 20-50 years (141 male, 133 female). The mean ages of male and female were 34.31±5.62, 32.34±6.99 years respectively. The local Ethic Review Board approved the study (No: 4005), which was conducted according to the Declaration of Helsinki.

Analytical procedures

Venous blood samples were drawn from each individual at resting conditions in the morning after an overnight fasting using evacuated blood collection gel tubes (Vacutainer, Becton Dickinson, Plymouth, UK). The specimens were allowed to clot and centrifuged at 3000 g for 10 minutes. Serum samples were analyzed within 4 hours of blood collection. The analyzed tests were as follows: glucose, calcium, sodium (Na^+), potassium (K^+), chloride (Cl^-), blood urea nitrogen (BUN), creatinine, uric acid, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), triglycerides (TG), thyroid stimulating hormone (TSH), free triiodothyronin (fT3), free thyroxine (fT4), alpha feto protein (AFP), total prostate specific antigen (tPSA), folate and vitamin B_{12}. Analytes, methods and analyzers were listed in the Table 1.

The following instruments were used: Architect (Abbott, Wiesbaden, Germany) and Advia Centaur (Siemens Diagnostics, USA). For each analyzer, reagents and calibrators were from the same manufacturer. Bio-Rad controls level 1 and level 2 (Bio-Rad Laboratories, Milano, Italy) were used for internal quality check in Abbott Architect autoanalyzer. Ligand plus 1,3 (Bayer HealthCare) and Seronorm™ Immunoassay Lyo L-1, Lyo L-3 controls were used in Advia Centaur (Table 2). External quality control products of Bio-Rad laboratories were used (Bio-Rad Laboratories, Milano, Italy).

Statistical analysis

Computation of reference intervals:

The nonparametric method was preferred and applied according to the IFCC recommendations [1-7]. The numbers of the subgroups were enough for the nonparametric method [10].

First of all the individuals were categorized according to their gender before statistical analysis.

Partitioning: Lahti method was used for partitioning. Lahti *et al* [11] developed a new method for partitioning gaussian or log-gaussian distributed subgroups. *Distance criteria* of the Lahti method is expressed in distances between the reference limits of the subgroup distributions.

As laboratory data are often fundamentally log-gaussian, especially when stripped of values associated with disease and partitioned into well defined subgroups, the *distance criteria* was preferred for partitioning. Natural logarithmic transformations were performed to obtain Gaussian distributions of the parameters in order to apply the *distance criteria*. (Table 3). (Of course it is possible to use Lahti method for non-Gaussian distributed subgroups for partitioning without logarithmic transformation [12]).

The *distance criteria* are as follows:

- If the ratio (*R*) of the standard deviations (larger one divided by the smaller one) of the subgroups exceeds 1.5, partitioning is recommended.

Table 1. Analytical methods and analyzer

Analyte	Method	Analyzer
AFP, IU/mL	Luminescence	Advia Centaur
ALP, U/L@ 37°C	PNP, AMP	Architect
ALT, U/L@ 37°C	UV without P5P	Architect
AST, U/L@ 37°C	UV without P5P	Architect
BUN, mmol/L	Urease, kinetic	Architect
Calcium, mmol/L	Arsenazo	Architect
Chloride, mmol/L	ISE indirect	Architect
T.Cholesterol, mmol/L	Enzymatic colorimetric(CHOD-PAP)	Architect
Creatinine, (mmol/L)	Alkaline picrate	Architect
Folate, nmol/L	Luminescence	Advia Centaur
Free T3, pmol/L	Luminescence	Advia Centaur
Free T4, pmol/L	Luminescence	Advia Centaur
Glucose, mmol/L	Hexokinase	Architect
HDL-C, mmol/L	Direct, non-immunological	Architect
Potassium, mmol/L	ISE indirect	Architect
Sodium, mmol/L	ISE indirect	Architect
Total PSA, mg/L	Luminescence	Advia Centaur
Triglycerides, mmol/L	Enzymatic colorimetric(GK/GPO)	Architect
TSH, mIU/L	Luminescence	Advia Centaur
Uric acid, mmol/L	Uricase/peroxidase colorimetric	Architect
Vitamin B12, pmol/L	Luminescence	Advia Centaur

Table 2. Analytical performance (internal quality control results)

		Manufacturer	Lab. results							
ASSAY	Level	Mean	Intraassay				Interassay			
			n	Mean	SD	%CV	n	Mean	SD	%CV
AFP (IU/mL)	1	6.8	6	6.86	0.44	6.42	14	6.80	0.43	6.34
	3	94	6	121.8	3.84	3.16	13	122.14	4.84	3.96
ALP (U/L)	1	93	6	93.83	0.98	1.04	22	107.55	6.39	5.94
	2	414.5	7	449.57	7.41	1.65	22	437.64	14.40	3.29
ALT (U/L)	1	28.5-45.5	7	30.29	0.49	1.61	20	30.85	1.50	4.85
	2	84.3-135	7	103.14	1.22	1.18	26	94.66	3.84	4.05
AST (U/L)	1	37.3	7	34.43	0.54	1.55	23	35.92	1.49	4.15
	2	186	7	185.14	2.34	1.26	26	178.35	4.36	2.45
BUN (mmol/L)	1	5.36	7	5.36	0.0	0.0	23	5.92	0.39	6.52
	2	16.83	7	16.93	0.19	1.13	27	16.48	0.39	2.38
Ca (mmol/L)	1	2.19	7	2.25	0.0	0.0	26	2.15	0.06	2.57
	2	2.90	7	3.02	0.03	1.06	26	2.86	0.17	5.84
Cl (mmol/L)	1	97.3	8	98.7	0.70	0.72	29	99.6	3.27	3.28
	2	85.3	8	85.25	1.04	1.21	27	84.7	1.33	1.56
Cholesterol (mmol/L)	1	6.84	8	6.08	0.02	0.27	22	6.83	0.24	3.57
	2	2.59	8	2.43	0.02	0.95	22	2.60	0.05	1.74
Creatinine (mmol/L)	1	181.2	7	176.8	0.00	0.00	20	170.7	11.5	6.96
	2	539.2	7	545.4	3.5	0.79	20	539.2	14.1	2.69
Folate (nmol/L)	1	25.80	5	26.82	2.38	8.86	18	28.11	2.54	9.1
	3	7.32	6	6.82	1.36	9.20	17	6.98	0.68	9.8
FT3 (pmol/L)	1	2.25	6	2.36	0.08	3.26	18	2.34	0.12	5.31
	3	8.79	6	8.39	0.37	4.40	19	8.84	0.48	5.34
FT4 (pmol/L)	1	9.55	6	10.06	0.9	8.9	21	9.8	0.65	6.6
	3	35.7	6	35.2	2.7	7.7	22	34.7	2.06	6.0
Glucose (mmol/L)	1	5.0	7	4.80	0.03	0.62	25	5.0	0.08	1.61
	2	16.5	7	16.90	0.20	1.21	25	16.03	0.37	2.33
HDL-C (mmol/L)	1	1.89	12	1.90	0.02	0.98	20	1.71	0.05	2.74
	2	0.81	12	0.93	0.02	2.21	20	0.79	0.04	4.46
K (mmol/L)	1	3.86	6	3.70	0.00	0.00	40	3.86	0.07	1.76
	2	5.86	6	5.97	0.05	0.87	34	5.61	0.21	3.74
Na (mmol/L)	1	147	8	144.4	1.06	0.73	31	144.5	4.77	3.30
	2	128	8	128.6	1.69	1.31	27	127.1	2.69	2.12
Triglycerides (mmol/L)	1	2.07	6	2.16	0.01	0.40	21	2.09	0.07	3.32
	2	0.95	6	1.00	0.01	0.59	22	0.93	0.03	2.67
TPSA(mg/L)	1	1.97	5	1.68	0.02	1.37	19	1.74	0.09	4.89
	3	14.7	5	12.27	0.44	3.58	19	12.86	0.54	4.20
TSH(mIU/L)	1	0.39	5	0.36	0.02	5.70	20	0.376	.018	4.73
	3	18.3	6	17.90	0.71	3.96	23	17.49	1.20	6.86
Uric acid (mmol/L)	1	0.29	7	0.29	0.00	0.76	19	0.29	0.00	1.59
	2	0.54	7	0.56	0.00	1.05	22	0.55	0.00	1.55
Vitamin B12 (mmol/L)	1	834	6	819	48.9	5.97	13	808	46.1	5.71
	3	210	6	209	13	6.19	12	211	17	8.07

Table 3. Partitioning criteria of analytes

		Lahti model		
	R	D(s) (0.25–0.75 s)	Decision for one end	Decision on partitioning
AFP	1.01			
Lowerlimit		0	No partitioning	No partitioning
Upper limit		0.245	No partitioning	
ALP	1.03			
Lower limit		0.620	Marginal	Marginal No partitioning #
Upper limit		0.271	Marginal	
ALT	1.21			
Lower limit		1.252	Partitioning	Partitioning
Upper limit		0.163	No partitioning	
AST	1.07			
Lower limit		0.906	Partitioning	Partitioning
Upper limit		0.512	Marginal	
B12	1.01			
Lower limit		0.178	No partitioning	No partitioning
Upper limit		0.099	No partitioning	
BUN	1.57*			
Lower limit				Partitioning
Upper limit				
Ca	1.10			
Lower limit		1.259	Partitioning	Partitioning
Upper limit		0.750	Marginal	
Chol	1.07			
Lower limit		0.240	No partitioning	No partitioning
Upper limit		0.240	No partitioning	
Cl	1.17			
Lower limit		0.538	Marginal	Marginal ÞNo partitioning #
Upper limit		0.538	Marginal	
Crea	1.29			
Lower limit		4.89	Partitioning	Partitioning
Upper limit		1.06	Partitioning	
Folate	1.03			
Lower limit		0.279	Marginal	Marginal No partitioning #
Upper limit		0	No partitioning	
FT3	1.02			
Lower limit		0.503	Marginal	Marginal No partitioning #
Upper limit		0	No partitioning	
FT4	0.99			
Lower limit		0.461	Marginal	Marginal No partitioning #
Upper limit		0.131	No partitioning	
Glucose	1.19			
Lower limit		0.215	No partitioning	Marginal No partitioning #
Upper limit		0.320	Marginal	
HDL	1.46			
Lower limit		0.618	Marginal	Partitioning
Upper limit		0.830	Partitioning	
K	1.25			
Lower limit		0.617	Marginal	Partitioning
Upper limit		1.358	Partitioning	
LDL	1.05			
Lower limit		0.180	No partitioning	Marginal No partitioning #
Upper limit		0.360	Marginal	
Na	1			
Lower limit		0	No partitioning	Marginal No partitioning #
Upper limit		0.709	Marginal	
TG	1.17			
Lower limit		0.611	Marginal	Partitioning
Upper limit		4.50	Partitioning	
TSH	1.13			
Lower limit		0.215	No partitioning	Marginal ÞNo partitioning #
Upper limit		0.324	Marginal	
UA	1.32			
Lower limit		5.47	Partitioning	Partitioning
Upper limit		3.79	Partitioning	

Analyses were performed with using logarithmically transformed test values.

R: ratio of the subgroups standard deviations

D(s): distance as a scale unit, D(s) (0.25–0.75 s): critical distance in this criteria

* The ratio (R)of the subgroups standard deviations (larger by smaller) was larger than 1.5, therefore partitioning is recommended.

Decision with nonstatistical considerations

Table 4. Reference intervals (defined by the 2.5 and 97.5 percentiles) and manufacturer suggestions

Analyte, Method		Non- parametric Method			M. RI	
		n	RI	90% Confidence Interval		
AFP (IU/mL)	M& F	272	<**8.1**	6.6-27	**<6.7**	
ALP (U/L) 37ºC	M& F	274	**44-134**	39-48 127-140	**40-150**	
ALT (U/L) 37ºC	M	141	**6-44**	5-7 42-68		
	F	133	**3-40**	1-4 28-76	**<55**	
AST (U/L) 37ºC	M	141	**12-32**	11-13 28-55	**5-34**	
	F	133	**10-28**	4-11 24-39		
BUN (mmol/L)	M	141	**2.86-6.78**	2.14-3.21 6.07-8.93	**3.21-7.50**	
	F	133	**2.14-6.07**	0.36-2.50 5.71-8.21		**2.50-7.14**
T.Calcium (mmol/L)	M	141	**2.28-2.64**	2.15-2.30 2.60-2.80		**2.10-2.55**
	F	133	**2.16-2.57**	2.13-2.18		2.53-2.75
Chloride (mmol/L)	M&F	274	**103-111**	102-104 110-112		**98-107**
T. Cholesterol (mmol/L)	M&F	274	**3.13-6.97** <5.18*	2.85-3.39 6.73-7.62		**3.63-5.18**
Creatinine (mmol/L)	M	140	**71-101**	67.2-72.5 99-108.7	**62-115**	
	F	133	**56-92**	47.7-61 87.5-102.5		**53-97**
Folate (nmol/L)	M& F	264	**12.7-45.3**	10.7-13.34 45.3-45.3		**7.0-45.3**
Free T3 (pmol/L) Luminescence	M& F	274	**4.57-8.02**	3.02-4.77 7.82-9.29	**3.5-6.5**	
Free T4 (pmol/L)	M& F	274	**13.2-25.0**	12.5-13.8 23.6-28.0		**11.5-22.7**
Glucose (mmol/L)	M& F	274	**4.00-5.83**	3.89-4.11 5.77-6.05	**3.89-5.83**	
HDL-C (mmol/L)	M	141	**0.80-1.71** >1.04*	0.78-0.88 1.66-1.92	**0.78-1.68**	
	F	133	**0.91-2.02** >1.30*	0.83-0.96 1.87-2.12	**0.91-1.94**	
LDL-C (mmol/L)	M& F	273	**1.55-4.77**	1.19-1.71 4.35-5.03	**<3.37**	
Sodium (mmol/L)	M& F	274	**139-147**	138-139 147-148	**136-145**	
Potassium (mmol/L)	M	141	**3.70-5.70**	3.60-3.80 5.20-6.30	**3.5-5.1**	
	F	133	**3.60-5.07**	3.30-3.70 4.80-5.20		
Total PSA (µg/L)	M	141	**<2**	1.89-2.11	**<2**	
Triglycerides (mmol/L)	M	140	**0.57-3.54** <1.70*	0.41-0.70 3.00-4.23	**0.45-1.70**	
	F	132	**0.44-2.25** <1.70*	0.38-0.50 1.58-2.90		
TSH (mIU/L)	M& F	272	**0.60-6.25**	0.05-0.69 5.05-7.69	**0.35-5.50**	
Uric acid (mmol/L)	M	141	**0.22-0.48**	0.20-0.24 0.44-0.48	**0.21-0.43**	
	F	133	**0.13-0.38**	0.11-0.15 0.35-0.47	**0.15-0.35**	
Vitamin B12 (pmol/ L)	M&F	272	**142-953**	129-156 856-1144	**156-672**	

M: male, F: female, RI: reference interval, Confidence Interval (lower-upper limit), MRI: reference intervals suggested by manufacturers
* Universal discriminatory values.

- If $R \leq 1.5$, the distances between the upper and lower limit pairs of the subgroups are divided by the narrower standard deviation. (Critical distance is 0.25*s*-0.75*s*). (*s*): scale unit.
- If both distance lower (D_L) and distance upper(D_U) are <0.25 *s*, partitioning is not recommended.
- If either D_L or D_U or both lie in the interval (0.25 *s,* 0.75 *s*) and neither one is ≥0.75 *s,* then the decision is made by *nonstatistical considerations*: according to the clinical practice, and medical literature.
- If either D_L or D_U or both are ≥ 0.75*s*, partitioning is recommended.

Outliers: The frequency histograms of the two subgroups (male and female) were prepared and examined visually in order to decide on outliers. Dixon-Reed method was applied to detect outliers [13,14] according to the following steps:

The smallest (or largest) value in a distribution may be an outlier if the difference between the two smallest (or largest) value is greater than one third of the difference between the maximum and minimum values of the distribution.

If there are more than one outlier, it is recommended that 1/3 rule should be applied to the least extreme outlier as it is the weakest part of that rule.

Non-parametric estimation: After deletion of outliers, the reference limits were calculated by nonparametric method recommended by IFCC. The estimation of the intervals was performed by using the 2.5 and 97.5 centile of the distribution by non-parametric method:

Non-parametric method is based on sorting the reference values in assending order of magnitude. The limits of the conventional 95% reference interval have rank numbers equal to: lower limit: 0.025 (*n*+1), upper limit: 0.975 (*n*+1). The reference limits are the corresponding rank numbers. If the corresponding rank number is not an integer, the limit has to be calculated by interpolation between two values.

The 90% confidence intervals for lower and upper 95% reference limits were determined by using rank number defining tables from IFCC [7].

The effect of smoking status was evaluated with Mann-Whitney U test. In our study group, 76 male and 51 female were smoking. There was no statistically significant difference between smoking and non-smoking status for the tests.

The age-related changes in lipid concentrations of the subjects were evaluated. First of all the variables of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides were tested statistically for normality by *One-Sample Kolmogorov-Smirnov* Test. For the Gaussian distributed data (T. Cholesterol, and LDL cholesterol) A one-way analysis of variance (ANOVA) was applied. For non-Gaussian distributed data (triglycerides, HDL cholesterol), non parametric *Kruskal-Wallis* Test was used (Table 5).

Alcohol consumption was an exclusion criteria, so 5 individuals drinking alcohol were excluded from the study.

Analysis was carried out using SPSS 15 (SPSS Inc., Chicago, IL, USA).

Table 5. Age-related changes in lipid profile according to mean and median values

		T.Cholesterol (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	Triglycerides (mmol/L)
Group 1 (age 20-30 y)	N	94	95	98	95
	Mean	4.64	2.85		
	Median	4.65	2.85	1.24	0.90
	SD	0.74	0.62	0.31	0.44
	Min	2.90	1.48	0.77	0.39
	Max	6.19	4.35	2.08	2.23
Group 2 (age 31-40 y)	N	132	130	134	126
	Mean	4.94	3.06		
	Median	4.87	3.0	1.17	1.06
	SD	0.86	0.68	0.27	0.49
	Min	3.16	1.66	0.77	0.42
	Max	7.43	4.84	2.12	2.74
Group 3 (age 41-50 y)	N	37	37	37	33
	Mean	5.12	3.12		
	Median	5.05	3.13	1.18	1.11
	SD	0.89	0.72	0.22	0.57
	Min	2.85	1.45	0.81	0.41
	Max	6.79	4.33	1.59	2.48
p value		0.003*	0.03*	0.3	0.01*

Statistically significant at *p<0.05 level.
For non-Gaussian distributed tests, only median values were given.

Results

Table 4 shows the analytical methods, reference limits, lower and upper limits calculated for each test, based on the nonparametric procedures.

The ratio of the standart deviations of the subgroups (R) was 1.57 for BUN, so it was partitioned for both sex (Table 3).

After exclusion, the number of reference individuals decreased for some analytes from total subject number of 274 as seen in the Table 4.

The reference intervals for ALP, ALT, AST and creatinine (male) were narrower and B_{12}, uric acid (female) reference intervals were wider than the intervals provided by the manufacturer. The upper and lower limits for TSH, fT3, fT4, K^+ (male) and uric acid (male) were slightly higher than the manufacturer suggestions (Table 4). The upper limit for AFP was higher than the values given in the kit inserts. For folate, only the lower limit that we suggested was slightly higher than the limit provided by the manufacturer.

No partitioning was needed for cholesterol and LDL cholesterol. The upper limits were higher for cholesterol and LDL cholesterol for both sex. Triglyceride values of men were higher than women. Mean serum total cholesterol, LDL-C, and triglyceride levels were increased with age (Table 5). The reference intervals for glucose, BUN, calcium (female), creatinine (female), potassium (female) and sodium were similar to manufacturer suggestions. Both the upper limit and lower limit were higher for calcium (male) and uric acid (male). For chloride, the lower level was higher than the manufacturer. For tPSA, the upper limit was same as the kit insert.

Discussion

Establishing reference intervals for clinical laboratories is hard, and costly, especially the direct estimation from healthy adults. Most of the laboratories, particularly the small ones use the values provided by manufacturers, but especially there are no detailed information about the reference populations in the kit inserts. Because of many biological diversities of the community, it is imposible to find healthy group as mentioned in the studies on determination of reference ranges. However, while planning the study, preanalytical factors should be minimized. Ichihara et al [15,16], planned to avoid the possible bias attributable to differences in physical activity and/or climatic influences in their common reference interval study. They choosed the participants among the hospital staff.

In the current study the volunteers were choosen from the staff working indoors. One group was from a food factory, the other group was from the service staff of an automotive industry in Izmir. Medical doctor of the two workplaces was the same, and the participants were selected with his cooperation. The participants were free from the commercial toxic agents according to their periodic health screening reports.

In this study, the reference intervals of common serum analytes have been set up for the local Turkish population.

For ALP, Cl^-, folate, glucose, Na^+, LDL-C, fT3, fT4 and TSH, decisions on partitioning were made by nonstatistical considerations. Decisions were made by consulting to the literature [17] and to the package inserts of several different manufacturers.

In the current study, the reference intervals obtained for thyroid hormones showed differences in lower and upper limits (Table 4). The upper and lower limits for TSH, fT3, and fT4 were slightly higher than the manufacturer suggestions. Comparing the reference intervals of thyroid profile established in Bursa [18] and Denizli [19], which were also determined from healthy adults, their reference intervals for thyroid hormones were different from ours (Table 6). We obtained our results with third generation TSH assay which quantifies thyrotropin to a lower reportable value of 0.01 mIU/L, useful for patients with subnormal TSH concentrations. A study from Poland [20] reported that ethnic features and iodine intake were the possible factors that might influence the TSH values. Another study reported by Quinn et al. [21] established thyroid hormone reference values specific for the Chinese population because of ethnic differences. In a letter to the editor from Germany [22] recommended to confirm common reference interval for TSH, because reference values were mostly influenced from iodine status of the population investigated. Besides thyroid ultrasonography, a sensitive thyroid autoantibody measurement should be known to define a representative TSH reference interval usable for therapeutic decisions especially in elderly patients. For those reasons we need new reference intervals according to the regional iodine status and thyroid ultrasonography of the reference individuals or a common reference interval study for thyroid hormones like other common reference interval studies for some other laboratory tests [23-25].

The upper reference limits determined in our study for serum total cholesterol, and triglycerides were higher

when compared with the “universal” discriminatory values of 5.18 mmol/L for serum cholesterol, 1.70 mmol/L for triglycerides (NCEP: National Cholesterol Education Program) [26]. According to the ATP III classifications, the lower cut-off limit for HDL-C has to be 1.04 mmol/L for male and 1.30 mmol/L for female, but in our study 14.3% female and 27.7% male had HDL-C levels below those cut-off limits.

For LDL-C, 33.1% male and 22.5% female had elevated LDL-C levels above 3.37 mmol/L (the upper cut off

limit of ATP III) in the current study.

For some analytes, reference intervals are replaced by decision limits (e.g., cholesterol, glycated hemoglobin,

Table 6. Non-parametric reference intervals determined at the same age group in western part of Turkey

Analyte	Izmir (our study)		Manufacturer		Denizli (19)*		Bursa (18)	
	Sex	R.I	Sex	R.I	Sex	R.I	Sex	R.I
AFP (IU/mL)	M& F	<8.1	M& F	<6.7	–	–	–	–
ALP(U/L), 37ºC	M& F	44-134	M& F	40-150	M	36-129	M	64-176
					F	31-120	F	51-141
ALT(U/L), 37ºC	M	6-44	M& F	<55	_	_	M	8-45
	F	3-40			_	_	F	6-26
AST(U/L), 37ºC	M	12-32	M& F	5-34	_	_	M	10-45
	F	10-28			_	_	F	9-32
BUN(mmol/L)	M	2.86-6.78	M	3.21-7.50	M	2.86-8.21	M	2.66–6.83
	F	2.14-6.07	F	2.50-7.14	F	2.14-6.78	F	2.00–6.17
T.Calcium (mmol/L)	M	2.28-2.64	M& F	2.10-2.55	_	_	M	2.20–2.60
	F	2.16-2.57			_	_	F	2.17–2.62
Chloride (mmol/L)	M&F	103-111	M& F	98-107	–	–	M&F	97-108
Total Cholesterol (mmol/L)	M&F	3.13-6.97	M& F	3.63-5.18	M&F	2.88-6.29	M&F	2.64-6.70
Creatinine (mmol/L)	M	71-101	M	62-115	M	79.6-141	M	53.0-110.5
	F	56-92	F	53-97	F	61.8-115	F	36.2-89.2
Folate (nmol/L)	M& F	12.7-45.3	M& F	7-45.3	–	–	M	6.5–33.2
							F	8.1–49.8
Free T3 (pmol/L)	M& F	4.57-8.02	M& F	3.5-6.5	M& F	2.00-6.78	M	4.25-6.37
							F	3.58-6.08
Free T4 (pmol/L)	M& F	13.2-25.0	M& F	11.5-22.7	M& F	10.3-24.5	M& F	11.1-21.4
Glucose (mmol/L)	M& F	4.00-5.83	M& F	3.89-5.83	M& F	3.89-6.99	M& F	3.55–5.60
HDL-C (mmol/L)	M	0.80-1.71	M	0.78-1.68	M	0.73-1.74	M	0.77–1.39
	F	0.91-2.02	F	0.91-1.94	F	0.91-2.15	F	0.80–1.68
Sodium (mmol/L)	M& F	139-147	M& F	136-145	–	–	M& F	133-151
Potassium (mmol/L)	M	3.70-5.70	M& F	3.50-5.10	_	_	M& F	3.4-5.0
	F	3.60-5.07			_	_		
Total PSA (mg/L)	M	<2	M	<2	_	_	_	_
Triglycerides (mmol/L)	M	0.57-3.54	M& F	0.45-1.70	M	0.40-3.38	M	0.39–3.37
	F	0.44-2.25			F	0.27-2.49	F	0.27–2.48
TSH(mIU/L)	M& F	0.60-6.25	M& F	0.35-5.50	M& F	0.30-4.17	M& F	0.51-3.51
Uric acid (mmol/L)	M	0.22-0.48	M	0.21-0.43	_	_	M	0.16–0.35
	F	0.13-0.38	F	0.15-0.35	_	_	F	0.06–0.24
Vitamin B12 (pmol/L)	M&F	142-953	M&F	156-672	–	–	M	158-1139
							F	235-1473

* Reference (19) units were converted to SI units.

neonatal bilirubin) (NCEP Guidelines). Decision limit is a threshold above which or below which a specific medical action is recommended, therefore it is used for the diagnosis of the disease by discriminating non-diseased and diseased people for assessment of a risk factor. According to the NCEP guidelines, the typical cholesterol is not necessarily a healthy cholesterol, indeed, apparently healthy individuals whose cholesterols are above 5.18 mmol/L are at increased risk of coronary artery disease. Thus, the current reference interval, or better yet, the decision limit for cholesterol is 5.18 mmol/L. This is the value that most of the laboratories use as the "upper limit of the reference interval".

A Turkish heart study has provided the lipid and lipoprotein levels from six different regions of Turkey [27]. They reported that the Turks have abnormally low HDL-C like Turkish male and female living in Germany [28]. They surveyed approximately 9000 male and female

from six regions of Turkey with different dietary habits, however the HDL-C levels were uniformly low in all regions. The Turkish people were found to have low levels of HDL-C (mean values for all six regions: male: 0.88-0.98 mmol/L, female: 0.96-1.17 mmol/L), typically 0.26-0.39 mmol/L lower than in Europeans and North Americans. Similarly Turks living in Netherlands [29], and the United States [30] have low plasma HDL-C levels. They conclude that the abnormality may have genetic origin. In our study the mean values of HDL-C for male and female were found as 1.16 mmol/L and 1.38 mmol/L respectively. Several studies have implicated high triglyceride levels as a coronary artery disease risk factor especially in the context of low HDL-C levels. Turks have distinctively low levels of total and HDL-C, associated with high levels of hepatic lipase and fasting triglycerides [31]. Turkish heart study reported that triglyceride levels tended to be high in Turkish male

(~1.36-1.70 mol/L) as it is observed to those seen in Turks living in Germany [28]. Turkish female have lower triglyceride levels than male and these levels are similar to those of Turkish female living in Germany.

In our study, serum triglycerides were positively skewed; their median values were 1.28 and 0.87 mmol/L, male and female respectively. 10.7% of male had high triglyceride levels associated with low levels of HDL-C.

Comparing total cholesterol limits of our study (T.Chol reference limits for both sex: 3.13-6.97 mmol/L) with the same age group reference interval studies established in Bursa (T.Chol reference limits: 2.64-6.70 mmol/L) and Denizli (T.Chol reference limits: 2.88-6.29 mmol/L), their upper limits were as high as ours (Table 6). For triglycerides, the reference limits in the current study (male and female 0.57-3.54 mmol/L, and 0.44-2.25 mmol/L respectively) were as high as the intervals determined in Bursa (male and female 0.39-3.37 mmol/L, and 0.27-2.48 mmol/L respectively). LDL-C levels of our study (male and female together: 1.55-4.77 mmol/L) were similar to the levels determined in Denizli (male and female together 1.06-4.56 mmol/L).

For uric acid, our results were similar to the other two studies from Turkey [18,19], but comparing the reference intervals with the kit insert, especially for men, the upper limit of our study was higher.

Especially for male, our reference limits for calcium were similar to the study from Bursa [18], but higher than the kit insert.

For Vitamin B_{12}, our upper limits determined in Bursa were as high as ours (Table 6).

The calculated reference intervals for glucose, tPSA, BUN, AST, calcium (female), creatinine (female), potassium and sodium were found as similar as the manufacturer. But TSH, fT3, fT4, ALP, Vitamin B_{12}, calcium (male), creatinine (male), chloride, uric acid and folate levels showed differences from the kit inserts in the present study.

There are different geographic areas, different climates, various dietary habits in our country, but not much reference interval studies. Comparing the reference intervals of ours with Bursa and Denizli, the intervals were similar with small differences except for thyroid profile. In our opinion like Ichihara et al. [15,16], and Nordic Reference Interval Project [23,24] we need a common reference interval study comprising all seven regions of Turkey.

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