Hospital Based Reference Values for 18 Clinical Chemistry Analytes Using the Data of the Kartal Dr. Lütfi Kirdar Training and Research Hospital Biochemistry Laboratory Patient Data

Kartal Lütfi Kırdar Eğitim ve Araştırma Hastanesi Biyokimya Laboratuvarı Hasta Verileri Kullanılarak 18 Klinik Kimya Analiti İçin Referans Değerlerinin Belirlenmesi

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ABSTRACT Objective: Our aim was to estimate hospital based "health related" reference values for the most frequently used 18 clinical chemistry analytes using the hospital patient database. **Material and Methods:** We used a posterior and partially selected method to determine reference values. A total of 15.716 outpatients (58.58% women, 41.52% men), (ages 13-73) non-smoking, and without alcohol consumption and chronic disease were enrolled in the study. For all analytes 2.5% and 97.5% of the reference values were calculated for 4 different age groups (13-24, 25-44, 45-64, >65) in both men and women. **Results:** Most of the analytes showed age-specific values (except for high-density lipoprotein-cholesterol (HDL-c) in females and protein total and bilirubin total in males). Age matched men and women had different values for most analytes, except for glucose, cholesterol, protein total, albumin and lactate dehydrogenase (LDH). Only protein total, glucose and alkaline phosphatase (ALP) reference values were similar to those of manufacturers we used in the laboratory. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), g-glutamyl transpeptidase (GGT), creatine kinase (CK), LD, creatinine, and urea had lower; albumin and uric acid had higher reference limits. **Conclusion:** These age- and sex-specific reference values, different from those of the manufacturers in many respects, reflect our hospital population more reliably.

Key Words: Reference values; database

ÖZET Amaç: Bu çalışmada amacımız, laboratuvarımızın hasta veri tabanından yararlanarak en sık kullanılan 18 klinik kimya analitinin 'sağlıklı kişiler' için referans değerlerini belirlemek amaçlandı. **Gereç ve Yöntemler:** Referans değerlerini belirlemede posterior ve kısmi yöntemler kullanıldı ve hasta seçimi belirlenmiş verilere göre yapıldı. Çalışmaya sigara ve alkol kullanmayan ve herhangi bir kronik hastalığı bulunmayan toplam 15.716 poliklinik hastası (%58,58 kadın, %41,52 erkek, yaş aralığı 13-73) dahil edildi. Hem kadınlarda hem de erkeklerde dört farklı yaş grubu için (13-24, 25-44, 45-64, > 65) referans değerlerinin %2,5 ve %97,5 çeyrek değerleri hesaplandı. **Bulgular:** Analitlerin çoğunluğu [kadınlarda yüksek yoğunluklu lipoprotein kolesterol, (HDL)-c, erkeklerde total protein ve total bilirubin hariç] yaşa göre değişen değerler gösterdi. Glikoz, kolesterol, total protein, albumin ve laktat dehidrogenaz (LD) hariç analitlerin çoğunda hem kadınlarda hem de erkeklerde yaş gruplarına göre farklı değerler elde edildi. Yalnızca total protein ve alkalen fosfataz (ALP) referans değerleri laboratuvarımızda kullanılan cihaz ve kit üreticisinin sunduğu değerlerle benzerdi. Buna karşılık aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), γ-glutamil transferaz (GGT), kreatin kinaz (KK), LD, kreatinin ve üre referans değerleri üretici firmanınkinden daha düşük, albümin ve ürik asidin değerleri ise daha yüksek bulundu. **Sonuç:** Yaşa ve cinsiyete özgül bu referans değerlerinin birçok açıdan üretici firmanınkinden farklı olduğu ve bizim hastane popülasyonumuzu daha iyi temsil ettiği sonucuna vardık.

Anahtar Kelimeler: Referans değerleri; veri tabanı

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Reference value of an analyte is a clinical decision point. It is very important in clinical practice because the test result itself is directly compared with it. When a new test is put in use or a new method is introduced instead of a traditional one, setting up of the reference values should immediately and precisely be performed since it is directly related with the diagnostic performance of that test. The validation of a test should at least include the analysis of some samples from healthy individuals if the available resources are not sufficient.[1] International Federation of Clinical Chemistry (IFCC) and National Committee of Clinical Laboratory Standards (NCCLS) have established the selection criteria of reference individuals.[2,3] In the direct method reference individuals are selected according to well-defined criteria. This may be done in two ways; prospectively (a priori) and retrospectively using the patient data (a posteriori). In the indirect method, patient data is used without any selection; in a cumulative set of data, the portion that shows Gaussian distribution is formulated. Indirect methods are not recommended by IFCC since selection of individuals is not sufficiently characterized.

On the other hand, indirect methods are encouraged by some authors because of their economical and physical advantages.[4,5] The major opinion is that any person in any section of his/her regular life is not an optimal reference for anyone who attends hospital demanding medical care. Therefore, a hospital population is a good reference for laboratory test results, if any selection can be made out of hospital patient data. In this study, we selected data of patients who did not smoke, did not use alcohol and had chronic disease and excluded in-patients of all clinics and outpatients from emergency, hemodialysis, transplantation, diabetes and pregnancy departments. This is a posteriori, newly called a partially selected method, since it is a combination of direct and indirect methods and is more economic, less cumbersome and more reliable than the conventional other populations' reference values we used.

MATERIAL AND METHODS

DATA SELECTION

This was a retrospective study run between June-September 2005, Venipuncture staff was informed to note smoking, alcohol abuse and chronic disease states of all patients. Then laboratory data were investigated. In order to eliminate results related with pathologic conditions and repeated measurements, hospitalized patients and outpatients from emergency, hemodialysis, transplantation, diabetes and pregnancy departments were excluded. Finally the data of 15.716 outpatients (58.58% female, 41.52% male), (age 13-73 years) were included in the study. Final number of patients used in calculation was 2249 men, 4066 women for albumin; 2060 men, 3050 women for bilirubin total; 1543 men, 2277 women for calcium; 2324 men, 3980 women for cholesterol; 4137 men, 5015 women for creatinine; 2072 men, 4099 women for high-density lipoprotein cholesterol (HDL-c); 1497 men, 3164 women for iron; 4960 men, 7748 women for glucose; 2010 men, 3692 women for protein total; 2300 men, 4145 women for triglycerides; 4574 men, 6598 women for urea; 2062 men, 4117 women for uric acid; 5082 men, 6548 women for aspartate aminotransferase (AST): 5091 men, 6542 women for alanine aminotransferase (ALT); 3622 men, 4073 women for alkaline phosphatase (ALP); 1423 men, 3271 women for creatine kinase (CK); 2931 men, 4865 women for γ-glutamyl transpeptidase (GGT); and 2147 men, 3051 women for lactic dehydrogenase (LD) parameters.

ANALYTIC METHODS

Blood samples were collected into evacuated collection tubes (Becton Dickinson Vacutainer Tubes, no 367896) without any additives in the morning (07.00-10.00 a.m.) after 8-12 hours of fasting. After 15 minutes of centrifugation at 1500 g, sera were collected. Analyses were carried out by Roche Diagnostics Modular System analyzers (Germany) with original Roche Diagnostics reagents. Method used for each analyte was given in Table 1.

Two levels of control sera (Roche Diagnostics) were used for internal quality for all analytes except for HDL-c.

TABLE 1: Summary of test methods.

Albumin	Bromcresol gren
Bilirubin	Diazonium dye
Calcium	0-cresolphytalein
Cholesterol	Cholesterol oxidase
Creatinine	Jaffe
HDL cholesterol	PEG modified cholesterol oxidase
Iron	Ferrozine dye
Glucose	Glucose oxidase
Total protein	Biuret
Triglycerides	Glycerophosphate oxidase
Urea	Urease
Uric acid	Uricase
AST	Aspartate to oxaloacetate
ALT	Alanine to pyruvate
ALP	p-nitrophenyl phosphate
CK	Creatine phosphate to creatine, HK, G-6-PD
GGT	γ-glutamyl-3-carboxy-4-nitroanilide
LDH	Pyruvate to lactate

HDL: High density cholestrol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, CK: Creatine kinase, GGT: Gama glutamyl transpeptidase, LDH: Lactate de hydrogenase.

STATISTICAL EVALUATION AND CALCULATION OF REFERENCE VALUES

Data were classified into 4 subgroups (13-24, 25-44, 45-64, >65 years/old) according to ages. Each subgroup was again divided according to gender and independent samples t-test was used to evaluate sex related differences in each age subgroup. Distribution of each sub-subgroup was evaluated by using the Kolmogorov Smirnov test, Kruskal Wallis nonparametric ANOVA was used to evaluate age related differences in each sex group.

Frequency data were transferred into GraphROC package program (designed by V Kairisto, University of Turku, Department of Clinical Chemistry, Turku, Finland). Calculations were done with "indirect method for ordinary limits" method. Outliers were removed by ± 4 SD method and nonparametric limits for 2.5% and 97.5% percentiles were defined.

Descriptive statistics and comparisons among groups were performed with SPSS 13.0 statistical package program. $p< 0.05$ was considered statistically significant.

RESULTS

Between-day reproducibility for 2 levels of control sera with normal and abnormal values was represented in Table 2. Distributions of all parameters

TABLE 2: Between-day precisions of two levels of control sera for each analyte.

	Level 1			Level 2		
	n	mean	CV(%)	n	mean	CV %
Albumin (g/dL)	27	45.3	2.64	27	30.3	3.62
Bilirubin (µmol/L)	25	16.6	4.04	25	83.6	3.33
Calcium (mmol/L)	22	2.10	2.4	22	3.38	2.87
Cholesterol (mmol/L)	27	2.33	3.26	27	4,50	2.85
Creatinine (µmol/L)	20	107	6.15	20	4154	6.46
HDL-c (mmol/L)				25	0.69	4.39
Iron (µmol/L)	20	20.75	1.63	20	30.30	2.06
Glucose (mmol/L)	26	5.41	3.83	26	14.20	3.56
Total protein (g/L)	20	67.9	1.94	20	50.20	2.28
Triglycerides (mmol/L)	26	1.33	2.55	26	2.35	2.69
Urea (mmol/L)	26	6.91	3.90	26	24.50	2.06
Uric acid (µmol/L)	22	277.2	2.,61	22	648	2.28
AST (U/L)	21	43.38	2.36	21	139.62	2.35
ALT (U/L)	27	45.7	2.56	27	111.67	2.80
ALP (U/L)	20	82.53	2.39	20	219.35	2.98
CK (U/L)	27	155.78	1.99	27	512.33	1.88
GGT (U/L)	20	40.12	2.63	20	191.76	2.31
LD (U/L)	22	319.73	2.26	22	514.45	2.31

(n= Number of days, CV= Coefficient of Variation), HDL: High density cholestrol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, CK: Creatine kinase, GGT: Gama glutamyl transpeptidase, LDH: Lactate de hydrogenase.

TABLE 3: Distribution of subgroups by Kolmogorov Smirnov analysis (KS) and effect of age on 18 biochemical analytes (Kruskal Wallis nonparametric ANOVA).

	Women			Men		
Analytes	**n**	**KSp's**	**ANOVA p's**	**n**	**KSp's**	**ANOVA p's**
Albumin	2066	0.00	0.00	1572	0.00	0.00
Bilirubin	1564	0.00	0.00	2102	0.00	0.57
Calcium	2288	0.00	0.00	1842	0.00	0.00
Cholesterol	2263	0.00	0.00	1798	0.00	0.00
Creatinine	3822	0.00	0.00	1999	0.00	0.00
HDL-c	2100	0.00	0.855	1825	0.00	0.00
Iron	1022	0.02	0.35	1555	0.00	0.00
Glucose	3833	0.00	0.00	1802	0.00	0.00
Total protein	1902	0.00	0.00	2217	0.00	0.25
Triglycerides	2180	0.00	0.00	1791	0.00	0.00
Urea	3810	0.00	0.012	1970	0.00	0.00
Uric acid	1992	0.00	0.00	2045	0.00	0.00
AST	3897	0.00	0.00	1847	0.00	0.00
ALT	3873	0.00	0.01	2100	0.00	0.00
ALP	2713	0.00	0.00	1877	0.00	0.00
CK	695	0.00	0.035	1660	0.00	0.00
GGT	2483	0.00	0.00	1791	0.00	0.00
LD	1865	0.00	0.00	1709	0.00	0.00

HDL: High density cholestrol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, CK: Creatine kinase, GGT: Gama glutamyl transpeptidase, LDH: Lactate de hydrogenase.

for males and females were non-Gaussian (Table 3). For men all parameters except for protein total and bilirubin total and for women all parameters except for HDL-c showed age specific differences (Table 3). Age matched men and women had different values for many analytes, except for glucose, cholesterol, protein total, albumin and LD (Table 4).

Striking properties of the population were as follows: 25-44 years/old men had the highest iron levels among all age groups, while women at the same age group had the lowest (menses). Women had higher HDL-c levels than age-matched men did. Cholesterol levels gradually increased with age in both sexes. Men had higher creatinine levels than age-matched women. Among men 13-24 year-olds had higher ALP levels than others did. ALP activities of males were higher than the values of females until 45 years old; the values were similar between 45-64 years but women had higher ALP levels over 65.

DISCUSSION

Subject based references provide the best information about an individual patient's situation, but since this is often impossible to obtain, population based references are used widely.[4,5] Though we knew that selected high-quality reference individuals (direct method) was the preferred way, we used our laboratory data for obtaining true reference values associated with our hospital population. Using the data of large hospitals has been recently recommended by many authors.[6,7] In order to interpret laboratory test results obtained in a clinical setting, a clinical laboratory specialist needs to use reference values comparable to the patients' setting in many respects. Furthermore some authors suggested that reference data from several populations-that is for both control and diseased persons-should be used in the interpretation of a single test.[8,9] This need spontaneously grew as our laboratory database gradually got larger. We realized that our cumulative statistics gave rather different values than the manufacturer's references we used.

What advantages did we get? We used an easier and economic way to obtain reference individuals by using a posteriori indirect method. Selection of reference individuals is the most im-

TABLE 4: Calculated sex and age specific reference values of 18 biochemistry analytes and statistical significance of difference between the values of men and women of the same age group.

Age	Gender	n	Mean	SD	SD left	SD right	Intervals	Effect of gender
ALBUMIN (g/L)								
13-24	Men	290	43	6.0	3.8	3.8	35-51	p= 0.710
	Women	519	43	5.3	3.9	3.9	35-52	
25-44	Men	598	42	6.5	4.0	4.0	34-50	p= 0.802
	Women	1235	43	4.0	3.9	3.9	34-50	
45-64	Men	956	39	7.0	5.0	5.0	33-52	p= 0.03
	Women	1591	42	5.0	3.0	3.0	38-50	
> 65	Men	405	37	6.0	6.0	6.0	29-50	p< 0.001
	Women	721	39	6.0	3.6	3.6	35-49	
BILIRUBIN Total (µmol/L)								
13-24	Men	280	10.9	10.4	4.27	4.27	2.39-19.5	p< 0,001
	Women	370	10.3	8.55	3.76	3.76	2.56-16.4	
25-44	Men	504	11.8	6.49	4.78	4.78	2.56-18.8	p< 0,001
	Women	863	10.3	10.3	3.07	3.07	2.22-13.5	
45-64	Men	897	16.6	8.89	4.27	4.27	1.71-17.4	p< 0,001
	Women	1143	10.1	7.86	3.2	3.2	1.71-13.7	
> 65	Men	379	11.8	6.15	4.95	4.95	3.08-17.8	p= 0,03
	Women	674	11.3	5.81	4.95	4.95	2.74-16.6	
CALCIUM Total (mmol/L)								
13-24	Men	97	2.52	0.15	0.12	0.12	2.15-2.72	p= 0.7
	Women	139	2.47	0.12	0.08	0.08	2.27-2.67	
25-44	Men	347	2.45	0.12	0.11	0.11	2.12-2.67	p= 0.05
	Women	650	2.45	0.15	0.10	0.10	2.30-2.70	
45-64	Men	562	2.42	0.17	0.14	0.14	2.02-2.70	p= 0.35
	Women	962	2.45	0.15	0.10	0.10	2.07-2.70	
> 65	Men	537	2.37	0.17	0.15	0.15	2.15-2.72	p= 0.37
	Women	526	2.42	0.15	0.13	0.13	2.20-2.72	
CHOLESTEROL Total (mmol/L)								
13-24	Men	228	4.20	0.93	0.84	0.84	2.34-5.43	p= 0.07
	Women	498	4.39	1.04	0.88	0.88	2.18-5.59	
25-44	Men	522	4.55	1.04	0.98	0.98	2.44-6.26	p= 0.93
	Women	1349	4.73	1.06	0.95	0.95	2.47-6.16	
45-64	Men	865	4.71	1.24	1.03	1.03	3.27-7.35	p= 0.98
	Women	1804	5.40	1.15	1.01	1.01	3.45-7.43	
> 65	Men	709	4.73	1.27	0.96	0.96	2.80-6.63	p< 0.001
	Women	827	5.20	1.22	1.22	1.22	2.28-7.12	
CREATININE (µmol/L)								
13-24	Men	327	86.6	103.4	14.1	14.1	43.3-99.0	p< 0.001
	Women	411	71.6	96.3	10.6	10.6	42.4-76.9	
25-44	Men	545	75.1	19.4	11.4	11.4	53.0-99.8	p< 0.001
	Women	992	63.6	18.5	11.4	11.4	35.3-75.1	
45-64	Men	2186	76.0	18.5	12.3	12.3	49.5-106.0	p< 0.001
	Women	2241	61.8	20.3	9.72	9.72	38.0-77.7	
> 65	Men	1079	94.5	29.1	20.3	20.3	44.2-114.9	p< 0.001
	Women	1371	74.2	22.9	14.1	14.1	38.8-93.7	
HDL-c (mmol/L)								
13-24	Men	195	1.15	0.33	0.26	0.26	0.67-1.71	p= 0.04
	Women	473	1.43	0.36	0.31	0.31	0.85-2.13	
25-44	Men	471	1.12	0.30	0.28	0.28	0.54-1.69	p< 0.001
	Women	1267	1.38	0.34	0.33	0.33	0.59-1.89	
45-64	Men	750	1.15	0.31	0.28	0.28	0.46-1.61	p= 0.01
	Women	1699	1.38	0.35	0.30	0.30	0.70-1.89	
> 65	Men	656	1.17	0.36	0.29	0.29	0.46-1.61	p< 0.001
	Women	660	1.32	0.39	0.29	0.29	0.67-1.89	

TABLE 4: Calculated sex and age specific reference values of 18 biochemistry analytes and statistical significance of difference between the values of men and women of the same age group (*continued).*

IRON (µmol/L)								
Age	**Gender**	**n**	**Mean**	**SD**	**SD left**	**SD right**	**Intervals**	**Effect of gender**
13-24	Men	544	15.0	8.73	7.08	6.94	3.6-28.1	p= 0.04
	Women	596	14.1	8.01	6.67	6.67	3.6-26.8	
25-44	Men	211	15.6	7.51	7.94	7.94	4.8-30.4	p< 0.001
	Women	516	10.8	6.80	5.90	5.90	1.8-16.6	
45-64	Men	435	15.9	8.12	6.44	6.44	3.2-28.4	p< 0.001
	Women	1335	13.0	7.08	6.71	6.71	2.8-26.3	
> 65	Men	307	15.3	8.05	7.16	7.16	4.1-29.1	p< 0.001
	Women	717	12.9	6.06	6.15	6.15	3.9-26.3	
GLUCOSE (mmol/L)								
Age	**Gender**	**n**	**Mean**	**SD**	**SD left**	**SD right**	**Intervals**	**Effect of gender**
13-24	Men	621	5.09	0.77	0.52	0.52	3.79-5.88	p= 0.07
	Women	998	4.80	0.66	0.53	0.53	3.57-5.83	
25-44	Men	1085	5.06	0.88	0.61	0.61	3.52-5.94	p= 0.7
	Women	2198	5.00	0.71	0.59	0.59	3.63-5.88	
45-64	Men	1644	5.99	0.99	0.60	0.60	3.85-6.21	p= 0.17
	Women	2831	5.94	0.82	0.60	0.60	3.79-6.32	
> 65	Men	1610	5.26	0.98	0.94	0.94	3.57-6.32	p= 0.64
	Women	1721	5.33	0.89	0.75	0.75	3.52-6.49	
PROTEIN Total (g/L)								
Age	**Gender**	**n**	**Mean**	**SD**	**SD left**	**SD right**	**Intervals**	**Effect of gender**
13-24	Men	281	74	8.0	6.0	6.0	54-89	p= 0.87
	Women	498	75	8.3	5.5	5.5	66-87	
25-44	Men	580	74	8.0	5.6	5.6	64-86	p= 0.50
	Women	1139	75	7.0	4.8	4.8	68-87	
45-64	Men	860	73	9.0	6.6	6.6	63-88	p= 0.05
	Women	1427	76	7.0	5.0	5.0	66-87	
> 65	Men	289	73	8.9	6.0	6.0	62-87	p< 0.001
	Women	628	74	7.8	6.0	6.0	66-88	
TRIGLYCERIDES (mmol/L)								
Age	**Gender**	**n**	**Mean**	**SD**	**SD left**	**SD right**	**Intervals**	**Effect of gender**
13-24	Men	209	1.56	1.29	0.92	0.92	0.45-2.69	p< 0.001
	Women	477	1.25	0.84	0.47	0.47	0.20-1.74	
25-44	Men	477	1.65	0.90	0.83	0.83	0.20-2.57	p< 0.001
	Women	1285	1.44	0.93	0.62	0.62	0.12-2.02	
45-64	Men	802	1.98	1.27	0.70	0.70	0.45-2.79	p= 0.07
	Women	1735	1.84	1.20	0.70	0.70	0.39-2.78	
> 65	Men	812	1.37	0.61	0.62	0.62	0.35-2.10	p< 0.001
	Women	648	1.55	0.61	0.72	0.72	0.57-2.41	
UREA (mmol/L)								
Age	**Gender**	**n**	**Mean**	**SD**	**SD left**	**SD right**	**Intervals**	**Effect of gender**
13-24	Men	179	4.78	2.32	1.72	1.72	1.99-6.80	p< 0.001
	Women	895	4.33	3.15	1.01	1.01	1.49-5.31	
25-44	Men	1221	6.14	4.98	1.39	1.39	2.15-7.63	p< 0.001
	Women	2139	4.54	2.98	1.14	1.14	1.49-5.97	
45-64	Men	1971	7.12	5.39	1.62	1.62	1.82-8.13	p< 0.001
	Women	2798	6.25	2.98	1.46	1.46	1.82-7.30	
> 65	Men	1203	8.10	2.58	2.04	2.04	1.99-9.96	p< 0.001
	Women	766	6.64	2.75	1.99	1.99	2.15-8.96	
URIC ACID (µmol/L)								
Age	**Gender**	**n**	**Mean**	**SD**	**SD left**	**SD right**	**Intervals**	**Effect of gender**
13-24	Men	228	297	95.2	77.3	77.3	119-437	p< 0.001
	Women	537	261	71.4	59.5	59.5	119-345	
25-44	Men	530	315	107	71.4	71.4	154-446	p< 0.001
	Women	1319	255	71.4	59.5	59.5	107-351	
45-64	Men	908	327	95.2	77.3	77.3	130-434	p< 0.001
	Women	1659	285	77.3	71.4	71.4	119-392	
> 65	Men	396	351	119	101	101	154-559	p< 0.001
	Women	602	315	101	95.2	95.2	142-481	

TABLE 4: Calculated sex and age specific reference values of 18 biochemistry analytes and statistical significance of difference between the values of men and women of the same age group (*continued*).

Age	Gender	n	Mean	SD	SD left	SD right	Intervals	Effect of gender
AST (U/L)								
13-24	Men	312	23.5	14	14.2	5.9	11-30	P<0.001
	Women	435	22.1	11	3.8	3.8	10-25	
25-44	Men	775	18	5.1	4.2	4.8	10-32	P<0.001
	Women	1416	18	4.6	3.4	3.4	12-29	
45-64	Men	1899	20.8	7.4	3.4	3.4	10-32	P<0.001
	Women	2812	23.3	12.2	4.9	4.9	9-28	
>65	Men	2096	22.7	12.4	5.4	5.4	7-27	P=0.03
	Women	1885	24.2	14.4	2.9	2.9	12-23	
ALT (U/L)								
13-24	Men	309	23.7	16.9	8.8	8.8	7-31	P<0.001
	Women	439	21.6	15.8	7.4	7.4	3-27	
25-44	Men	781	16.6	6.4	4.3	3.3	7-34	P<0.001
	Women	1427	16.6	6.0	6.4	6.4	3-28	
45-64	Men	1925	20.7	11.1	9.3	9.3	2-31	P<0.001
	Women	2792	22.2	10.9	6.2	6.2	4-27	
>65	Men	2076	17.5	7.8	6.5	6.5	4-27	P=0.49
	Women	1884	19	7.1	7.1	7.1	3-27	
ALP (U/L)								
13-24	Men	194	272	105	109	109	112-407	P<0.001
	Women	251	222	116	46.3	46.3	103-278	
25-44	Men	832	154	53.9	54.9	54.9	89-282	P<0.001
	Women	1316	168	71	47.3	47.3	84-259	
45-64	Men	710	251	130	56	56	82-293	P=0.12
	Women	1235	221	97.3	53	53	86-294	
>65	Men	1886	146	76.0	103	103	9-224	P<0.001
	Women	1271	190	52.3	48.8	48.8	82-270	
CK (U/L)								
13-24	Men	566	153.8	174	60.2	60.2	24-205	P<0.001
	Women	717	75.5	30.8	24.6	24.6	38-115	
25-44	Men	191	107	88.6	69	69	25-177	P<0.001
	Women	314	54.4	31	27	27	18-99	
45-64	Men	345	110	71	54	54	26-184	P<0.001
	Women	1540	79	53	36	36	15-139	
>65	Men	321	227	70	48.5	48.5	15-156	P<0.001
	Women	700	55	60	12.5	12.5	12-80	
GGT (U/L)								
13-24	Men	414	25.4	20.5	7.6	8.4	7-31	P=0.03
	Women	573	22.5	14.7	10.3	10.3	6-32	
25-44	Men	660	26.3	18.6	13.9	14.4	6-44	P<0.001
	Women	1279	26	16.9	13	13	6-39	
45-64	Men	621	38	28.8	18	28	6-28	P<0.001
	Women	1128	27	22.0	10	10	4-22	
> 65	Men	1236	24.8	19.0	9.6	9.6	4-31	P<0.001
	Women	1885	26		8.3	8.3	6-37	
LDH (U/L)								
13-24	Men	213	416	233	53.9	53.9	197-405	P=0.40
	Women	454	377	174	55.2	55.2	214-429	
25-44	Men	435	358	99	59.4	59.4	210-442	P=0.60
	Women	520	364	187	61	61	214-451	
45-64	Men	867	433	298	91.3	91.3	129-486	P=0.05
	Women	1451	381	134	57.7	57.7	143-448	
> 65	Men	632	337	89	64	64	235-486	P=0.84
	Women	626	389	89	56	56	267-486	

(n:number, SD: standard deviation; Mean and SD: Mean and SD values of the original reference distribution; SD left, SD right: SD values of underlying distribution; Intervals: Suggested health related intervals. HDL: High density cholestrol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, CK: Creatine kinase, GGT: Gama glutamyl transpeptidase, LDH: Lactate de hydrogenase).

portant and cumbersome step of a reference values study. So many strict including and excluding criteria result with a small sample group. When subgrouping is necessary for sexes and ages, the numbers get even smaller. Another issue to consider is that how much the reference individual reflects the health situation of any patient who attends any clinics for demanding medical help.[10] Indirect sampling is not the recommended one because it uses uncharacterized and unselected individuals, ranges obtained by this method are larger and may intersect patient values, and finally each hospital population may differ from another according to specialization of the institute.[11] In this study we aimed to verify this indirect sampling to somewhat a "partially direct" sampling; we did not enroll patients who smoked, with alcohol abuse and with chronic disease in the study. Besides, we would exclude the extreme values for each subgroup, if there were any. In the recommendations of IFCC, different reference populations with well-characterized criteria are introduced as acceptable according to the intended use of reference values, Kouri used this approach in a more diagnosis-based study, thus nearly unaffected test results were selected. He also claimed that the best reference for a patient was another patient not suffering from that disease, but living under the same conditions. In this study, we supposed that our hospital's outpatient population living in the same area, under same surroundings with a health problem to be solved would also be considered an "under the same condition" population.

CONCLUSION

As a result, we got significantly different reference values for most frequently used 18 analytes than the traditional values we used (manufacturer's). We saw that ranges were sex- and age-specific, and thus each result should be reported with its corresponding interval. So on, interpretation of test results will be more accurate and safe. A priori, direct sampling methodology in calculation of reference values of greater populations is generally beyond the potential of a single laboratory and should be organized and sponsored by national authorities; a standard protocol should be carried on simultaneously in several laboratories so that the results could be used countrywide.

REFERENCES

1. Holmes EW, Kahn SE, Molnar PA, Bermes EW Jr. Verification of reference ranges by using a Monte Carlo sampling technique. Clin Chem 1994;40(12):2216-22.
2. National Committee for Clinical Laboratory Standards. How to define, determine, and utilize reference intervals in the clinical laboratory: approved guideline. NCCLS Document C28-A;Vol. 15, No. 4. Villanova, PA: NCCLS; 1995.
3. Solberg HE. International Federation of Clinical Chemistry (IFCC), Scientific Committee, Clinical Section, Expert Panel on Theory of Reference Values, and International Committee for Standardization in Haematology (ICSH), Standing Committee on Reference Values. Approved Recommendation (1986) on the theory of reference values. Part 1. The concept of reference values. J Clin Chem Clin Biochem 1987;25(5):337-42.
4. Kouri T, Kairisto V, Virtanen A, Uusipaikka E, Rajamäki A, Finneman H, et al. Reference intervals developed from data for hospitalized patients: computerized method based on combination of laboratory and diagnostic data. Clin Chem 1994;40(12):2209-15.
5. Kairisto V, Hänninen KP, Leino A, Pulkki K, Peltola O, Näntö V, et al. Generation of reference values for cardiac enzymes from hospital admission laboratory data. Eur J Clin Chem Clin Biochem 1994;32(10):789-96.
6. Harris EK. Effects of intra- and interindividual variation on the appropriate use of normal ranges. Clin Chem 1974;20(12):1535-42.
7. Winkel P. The use of the subject as his own referent. In: Gräsbeck R, Alström T, eds. Reference Values in the Laboratory Medicine. The Current State of the Art.1st ed. Chichester, UK: John Wiley& Sons, 1981.p.65-78.
8. Statland BE, Winkel P. Reference values: are they useful? Clin Lab Med 1984;4(1):61-70.
9. Sunderman FW Jr. Current concepts of "normal values," "reference values," and "discrimination values," in clinical chemistry. Clin Chem 1975;21(13):1873-7.
10. Solberg HE, Gräsbeck R. Reference values. Adv Clin Chem 1989;27:1-79.
11. Solberg HE. Using a hospitalized population to establish reference intervals: pros and cons. Clin Chem 1994;40(12):2205-6.