Reference Intervals of Several Renal and Hepatic Function Parameters for Apparently Healthy Adults From Eastern China

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Background: Biochemical substances relating to renal and hepatic function are influenced not only by individual factors such as gender, lifestyle, and age but also by ecological factors, such as altitude, climate, and ethnic background. The purpose of the present study was to establish reference intervals for 16 biochemical substances relating to renal and hepatic function in healthy Chinese adults. **Methods:** A total of 2,405 apparently healthy individuals (18–77 years of age) were chosen as reference individuals in the present study. The 16 biochemical substances relating to renal and hepatic function were analyzed using a HITACHI RL7600 analyzer. The reference in-

tervals were established using nonparametric methods to estimate the 2.5 and 97.5 percentiles of the distribution as the lower and the upper limits, respectively. **Results:** Age- and gender-appropriate reference intervals were established for some biochemical substances relating to renal and hepatic function in healthy Chinese adults. **Conclusion:** The reference intervals established in this study can provide a useful clinical tool for the assessment of the kidney and liver damage. In addition, the established reference intervals can be adopted in other clinical laboratories after further validation. *J. Clin. Lab. Anal.* 29:235–241, 2015. ©

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Key words: Reference values; Kidney function tests; Liver function tests

INTRODUCTION

Reference intervals are used by the clinician as an important tool to differentiate between healthy and diseased populations (1,2). Kidney and liver function tests can be useful to determine whether the kidney and liver are performing their tasks adequately. Therefore, these tests are important items in occupational health examinations. Many clinical laboratories are either using the manufacturers' reference intervals or those published in textbooks without further validation. As we know, many reference intervals provided by manufacturers are derived from laboratory data on foreign populations. However, biochemical substances relating to renal and hepatic function are influenced not only by individual factors such as gender, lifestyle, and age but also by ecological factors, such as altitude, climate, and ethnic background (3–5). In addition, differences can be found between repeated tests on the same person due to different experimental methods, different experimental analyzers, different laboratory conditions, and intra-individual variations (6–8). Appropriate reference intervals for adults will help clinical physicians make an early diagnosis and decide

whether medical intervention is required. Therefore, it is essential to establish reference intervals using the native population. Reference intervals for populations from the Western countries are widely published in the textbooks (9,10) and scientific literature (11). However, there is a general lack of published reference intervals for populations living in China. Although a study by Chan et al. (12) reported the reference intervals of common serum analytes for adults living in Hong Kong, reference intervals for adults living in Shanghai City are rarely reported.

In this study, we report reference intervals for 16 biochemical substances related to renal and hepatic function using 2,405 apparently healthy Chinese adults according to the recommendations of the International Federation

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of Clinical Chemistry (IFCC) (1, 8, 13–15) and Clinical Laboratory Standards Institute (CLSI) (3).

MATERIALS AND METHODS

Study Population

The reference sample group started with 3,615 adult individuals (18–77 years of age) from eastern China, covering cities and villages. The population studied didn't consist of migrant workers. Informed written consent was obtained from all of the subjects. Information regarding age, gender, environment, dietary habits, smoking or alcoholic habit, drugs, physical activity, ethnic background, disease if any, anthropometric parameters, and career were obtained in respect to each subject. To select apparently healthy persons, the physical health status was assessed by a physical examination and some clinical laboratory assays, including those for glucose, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV), human immunodeficiency virus (HIV). The exclusion criteria were as follows: (a) exposure to HIV or hepatitis; (b) hypertension, chronic renal failure, diabetes mellitus, or other diseases that could affect the values of the biochemical substances measured in this study; (c) had taken drugs within 2 weeks, undergone surgery during the past 6 months; (d) pregnant women; (e) excessive smoking (12 cigarettes/day) and drinking (30 g/day); (f) obesity (body mass index ≥ 30); (g) excessive exercise; (h) fasting glucose ≥ 7.0 mmol/L; (i) the results of glucose, bilirubin, and protein in urine were abnormal. A total of 1,201 individuals were excluded and finally 2,414 apparently healthy adults were included in this study. The median body mass was 23.12 kg/m² in male and 22.03 kg/m² in female. The present study was approved by the Ethics Committee at Shanghai Jiao Tong University Affiliated Sixth People's Hospital.

Specimen Collection and Handling

All blood specimens were collected in untreated vacuum tubes after an overnight fast. All blood specimens were left at room temperature (25°C) for 30 min to clot. Next, the blood in the vacuum tubes was centrifuged for 10 min at $1,200 \times g$. The values of the following analytes in serum collected were measured with the HITACHI RL7600 analyzer using the manufacturer's reagents: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bile acid (TBA), cholinesterase (ChE), gamma-glutamyl transpeptidase (GGT), total protein (TP), albumin (Alb), prealbumin (PA), total bilirubin (TBIL), direct bilirubin (DBIL), urea nitrogen (BUN), creatinine (Cre), uric acid (UA), retinol-binding protein (RBP), and cystatin-C (Cys-C). All ana-

lytes were measured within 2 h. Table 1 summarizes the test methods for each analyte. Inter- and intraassay coefficients of variation for each analyte obtained from the manufacturer also summarized in Table 1. A calibration procedure was carried out using multicalibrator serum to ensure accuracy and precision. Commercial quality control materials at concentrations within and outside the reference intervals were measured at least once every day. All the daily quality control runs were within ± 2 SD from their target values.

Statistical Analysis

All statistical analyses were performed with SPSS (Version 17.0, SPSS Inc. Chicago, Illinois, USA). Prior to the reference interval calculations, the results from all of the subjects were divided into gender for male and female, and age ranging from 18 to 27, 28 to 37, 38 to 47, 48 to 57, 58 to 67, and 68 to 78 years old. Outliers were removed from each group when the observed value of D was over one-third of the R according to Dixon's test (17), where D is the absolute difference between the largest (or smallest) value and the next largest (or smallest) value, and R is the range of all observations. After the exclusion of obvious observed outliers, we used the normal deviate test to decide whether the separate reference intervals will be essential for the defined subgroups of subjects according to the Harris and Boyd method (16).

The following formula was used to calculate the Z -values

$$Z = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\left(\frac{s_1^2}{n_1}\right) + \left(\frac{s_2^2}{n_2}\right)}}$$

and then the calculated statistic Z -values were compared with the critical values calculated as,

$$Z^* = 3\sqrt{\left(\frac{n_1 + n_2}{240}\right)}s_i$$

where $\bar{x}_i$, s_i , and n_i are the mean, standard deviation, and the sample size of subgroup i , respectively. If the calculated Z value exceeds the critical value, then separate reference intervals should be established for each subgroup. Reference intervals were established using a nonparametric method to estimate 2.5 and 97.5 percentiles of distribution as the lower and the upper limits, respectively. Differences in medians were assessed by the Mann–Whitney U test. The Spearman correlation was used to test the relationship between the body mass index and each analyte. A value of $P < 0.05$ was considered significantly different.

TABLE 1. Summary of Test Methods and the Inter- and Intra-Assay Imprecision for Each Analyte

Analyte	Method	Intraassay imprecision (CV)	Interassay imprecision (CV)
ALT	IFCC standard method (without pyridoxal phosphate activation)	4.15–6.78%	4.56–7.01%
AST	IFCC standard method (without pyridoxal phosphate activation)	3.85–5.15%	3.96–5.48%
ALP	IFCC recommended method (colorimetric assay)	1.35–4.56%	2.35–5.04%
GGT	Enzymatic colorimetric assay	2.56–5.17%	3.01–6.27%
ChE	Dithiobisnitrobenzoic acid photometric	2.87–5.64%	3.12–6.56%
TBA	3- α -hydroxysteroid dehydrogenase	1.67–3.85%	2.01–4.39%
TP	Colorimetric, biuret reagent	1.78–3.12%	1.96–3.65%
Alb	Colorimetric, bromocresol	1.15–4.24%	1.85–5.01%
PA	Immunoturbidimetric analysis	0.97–1.75%	1.08–1.87%
TBIL	Colorimetric assay (diazonium ion)	5.12–8.84%	6.12–8.96%
DBIL	Colorimetric assay (Jendrassik-Grof method)	4.13–6.95%	5.13–7.01%
Cre	Compensated Jaffé	2.27–4.56%	3.27–5.13%
UA	Enzymatic colorimetric assay	1.85–3.06%	2.64–3.76%
BUN	Enzymatic, urease	2.16–6.51%	3.16–7.15%
RBP	Immunoturbidimetric analysis	1.35–3.23%	1.68–3.41%
Cys-C	Automated particle-enhanced immunoturbidimetric method	0.69–1.13%	0.95–1.69%

CV, coefficients of variation.

RESULTS

Seven outliers were detected in ALT, AST, and GGT, two outliers in ALP, and all the results for these subjects were excluded from this study. Finally, 2,405 subjects (1,256 men and 1,149 women) were selected as reference individuals. The calculated reference intervals for 16 biochemical substances are summarized in Table 2, partitioned by age and/or gender, as per the Harris and Boyd method. Table 2 shows the established reference intervals for 16 biochemical substances and their comparison with the reference intervals currently used in our laboratory. The results for ALT, GGT, Alb, PA, UA, Cre, and RBP appear generally higher in males than in females ($P < 0.05$). Necessity to partition reference intervals into gender-specific subgroups was demonstrated statistically in ALT, GGT, Alb, PA, UA, Cre, and RBP ($Z > Z^*$). In addition, the groups of individuals over 68 years old had lower values of ALT, ChE, and Alb compared with the groups of individuals aged 18 to 67 years old ($P < 0.05$). The groups of individuals over 28 years old had higher activities of AST and ALP compared with the groups of individuals aged 18 to 27 years old ($P < 0.05$). In contrast, TBA concentration was higher at the age of 68 to 77 years old ($P < 0.05$). The groups of individuals aged 18 to 57 years old had lower concentrations of TBIL and DBIL compared with the groups of individuals aged 58 to 77 years old ($P < 0.05$). In contrast, GGT and RBP values were higher at the age of 18 to 57 years old ($P < 0.05$). PA demonstrated a decreased concentration with increasing age ($P < 0.05$). However, the concentration of other analytes such as TP, UA, Cre, BUN, and Cys-C appeared to have no differences between age groups. Necessity to partition reference intervals into gender-specific subgroups was demonstrated statistically in ALT, AST,

ChE, GGT, ALP, TBA, Alb, TBIL, DBIL, PA, and RBP ($Z > Z^*$). Compared with the values currently used in our laboratory, both the upper and lower limits of the study reference intervals displayed an increase in the ALT, AST, ALP, GGT, TBA, TBIL, DBIL, BUN, and Cys-C values. However, PA, Cre, and RBP showed a decrease in both the lower and the upper limits. Cys-C showed the maximum percentage of the study population (41.61% of the males and 36.10% of the females) outside the existing reference intervals, followed by UA (19.24% of the males and 24.13% of the females).

Reference intervals for healthy adults that have been reported in other studies are listed in Table 3. The reference intervals established in this study differ to some extent from those published in the literature.

The correlations between body mass index and each of the tested serum analytes are summarized in Table 4. The serum ALT, GGT, and UA values were significantly correlated with the body mass index in males and females.

DISCUSSION

The interpretation of laboratory testing results depends, to a significant extent, on the use of reference intervals. However, many clinical laboratories adopt the manufacturers' reference intervals without further validation. As we know, many reference intervals provided by manufacturers are derived from laboratory data on foreign populations. The use of inappropriate reference intervals will directly affect the clinical doctors making medical diagnoses and management decisions.

As with other clinical laboratories in China, our laboratory has used the reference intervals provided by manufacturers or published in textbooks. However, the details

TABLE 2. Study Reference Intervals, Existing Reference Intervals, and the Percent of Individuals Outside the Existing Reference Intervals

Analyte (Unit)	Gender	Age (Years)	N	Median	Study RI	Existing RI	OOR (M; F)
ALT(U/l)#	M	18–67	1,045	21.00	8.00–76.80		
	M	68–77	211	16.00	7.00–44.00		
	F		1,149	13.00	6.00–55.00		
AST(U/l)	M&F		2,405	18.00	7.00–72.00	0.00–65.00	4.44; 1.59
	F	18–27	189	16.00	11.00–34.25		
	F	28–77	960	18.00	12.00–46.00		
ALP(U/l)	M&F		2,405	19.00	12.00–47.00	8.00–37.00	6.17; 4.29
	F	18–27	189	61.50	36.62–113.25		
	F	28–77	960	67.00	37.00–125.82		
GGT(U/l)#	M&F		2,405	69.00	39.00–122.82	15.00–112.00	5.34; 2.86
	M	18–57	838	30.00	12.00–90.00		
	M	58–77	418	25.00	11.00–77.75		
ChE(U/l)	F		1,149	18.00	9.00–67.00		
	M&F		2,405	24.00	10.00–84.82	0.00–50.00	18.42; 4.76
TBA(μmol/l)	F	18–67	950	327.00	202.20–511.40		
	F	68–77	199	303.00	178.80–458.20		
	M&F		2,405	333.50	184.35–510.82	203.00–460.00	11.59; 8.73
TP(g/l)	M	18–67	1,045	3.60	1.10–12.70		
	M	68–77	211	4.00	1.27–14.65		
	M&F		2,405	3.50	1.00–12.70	0.00–10.00	6.08; 4.76
Alb(g/l)#	M&F		2,405	68.00	57.00–81.00	60.00–80.00	7.56; 9.05
	M	18–67	1,045	45.00	38.00–53.00		
	M	68–77	211	41.00	32.25–48.00		
PA(mg/l)#	F	18–67	950	44.00	38.00–52.80		
	F	68–77	199	42.00	33.60–49.00		
	M&F		2,405	45.00	36.00–53.00	35.00–55.00	1.56; 1.27
TBIL(μmol/l)	M	18–57	838	272.00	163.40–400.30		
	M	58–67	207	239.50	114.25–378.55		
	M	68–77	211	213.00	116.00–345.25		
DBIL(μmol/l)	F		1,149	223.00	126.10–326.80		
	M&F		2,405	249.00	134.00–382.47	200.00–400.00	14.31; 16.84
	F	18–57	760	10.10	4.02–20.70		
UA(μmol/l)#	F	58–77	389	12.00	5.41–28.08		
	M&F		2,405	11.30	5.00–26.28	0.00–18.00	13.90; 12.22
	F	18–57	760	3.50	1.35–7.14		
Cre(μmol/l)#	F	58–77	389	3.95	1.70–9.18		
	M&F		2,405	4.00	1.60–9.28	0.00–6.00	17.36; 12.86
	M		1,256	340.00	174.00–519.57		
BUN(mmol/l)	F		1,149	251.00	129.87–398.00		
	M&F		2,405	308.00	156.17–505.00	210.00–430.00	19.24; 24.13
	M		1,256	72.00	51.42–97.00		
RBP(mg/l)#	F		1,149	55.00	38.77–77.00		
	M&F		2,405	66.00	43.00–94.00	53.00–115.00	3.37; 4.79
	M&F		2,405	4.80	2.80–8.28	2.50–6.40	12.83; 17.78
Cys-C(mg/l)	M	18–57	838	47.00	28.00–68.00		
	M	58–77	418	41.00	21.00–62.25		
	F		1,149	39.00	22.00–63.22		
Cys-C(mg/l)	M&F		2,405	44.00	24.00–66.00	30.00–70.00	5.92; 15.87
	M&F		2,405	1.00	0.70–1.50	0.00–1.00	41.61; 36.10

N, number of subjects; RI, reference interval; OOR, out of the range (the percent of individuals outside the existing reference intervals); M, Male; F, Female.

$P < 0.05$ and $Z > Z^*$.

of the procedures used to establish the reference intervals are not mentioned. These details include the studied population, experimental method, specimen collection, etc. In addition, many reference intervals currently used are not divided into subgroups by gender or age. Our results

showed that ALT, GGT, Alb, PA, UA, Cre, and RBP values appear generally higher in males than in females ($P < 0.05$). The higher ALT and Cre values in men than in women may be explained by the greater muscle mass in men than in women (23). The higher UA in men than in

TABLE 3. Published Reference Intervals of These Measured Analytes for the Adult Population

Analyte (Unit)	Gender	N	Age range (years)	Region	Method	Reference interval	Reference
ALT (U/l)	M	401	20–79	Beijing, China	Enzymatic rate	8.1–55.9	(17)
		140	19–48	Southern Tanzania	Not stated	9.1–55.3	(19)
		207	18–50	Kenya		9–42	(20)
	F	487	20–79	Beijing, China	Enzymatic rate	6.2–44.4	(17)
		133	19–48	Southern Tanzania	Not stated	6.7–44.9	(19)
		153	18–50	Kenya		6–27	(20)
AST (U/l)	M&F	887	20–79	Beijing, China	Enzymatic rate	12.0–38.0	(17)
	M	137	19–48	Southern Tanzania	Not stated	15.2–53.4	(19)
		209	18–50	Kenya		16–47	(20)
	F	132	19–48	Southern Tanzania	Not stated	13.5–35.2	(19)
		152	18–50	Kenya		12–33	(20)
	M&F	236	19–48	Southern Tanzania	Not stated	45.6–158.4	(19)
ALP (U/l)	M	401	20–79	Beijing, China	Colorimetric assay	45.1–113.0	(17)
		200	18–50	Kenya		45–147	(20)
	F	487	20–79	Beijing, China	Colorimetric assay	30.2–108.6	(17)
		148	18–50	Kenya		43–126	(20)
	M	401	20–79	Beijing, China	Enzymatic colorimetric assay	9.0–63.8	(17)
		136	19–48	Southern Tanzania	Not stated	9.3–120.8	(19)
GGT (U/l)	F	204	18–50	Kenya		6–69	(20)
		487	20–79	Beijing, China	Enzymatic colorimetric assay	7.0–44.0	(17)
	M	130	19–48	Southern Tanzania	Not stated	7.3–51.8	(19)
		149	18–50	Kenya		7–49	(20)
	M&F	888	20–79	Beijing, China	Colorimetric assay	67.9–85.9	(17)
		267	19–48	Southern Tanzania	Not stated	66.3–85.1	(19)
TP (g/l)	M	154	18–50	Kenya		67–93	(20)
		207	18–50	Kenya		66–89	(20)
	F	888	20–79	Beijing, China	Bromocresol green method	43.3–54.5	(17)
		137	19–48	Southern Tanzania	Not stated	37.06–50.72	(19)
	M	156	18–50	Kenya		38–51	(20)
		131	19–48	Southern Tanzania	Not stated	35.57–49.27	(19)
TBIL (μmol/l)	F	208	18–50	Kenya		38–48	(20)
		401	20–79	Beijing, China	Colorimetric assay	5.5–28.7	(17)
	M	129	19–48	Southern Tanzania	Not stated	6.0–42.0	(19)
		201	18–50	Kenya		4.9–29.7	(20)
	F	486	20–79	Beijing, China	Colorimetric assay	4.6–22.8	(17)
		126	19–48	Southern Tanzania	Not stated	5.2–41.0	(19)
DBIL (μmol/l)	M	156	18–50	Kenya		3.4–27.0	(20)
		887	20–79	Beijing, China	Colorimetric assay	1.5–8.6	(17)
	M	116	19–48	Southern Tanzania	Not stated	0.93–8.43	(19)
		201	18–50	Kenya	Not stated	0.5–4.9	(20)
	F	117	19–48	Southern Tanzania	Not stated	0.70–5.83	(19)
		146	18–50	Kenya	Not stated	0.2–3.7	(20)
UA (μmol/l)	M	401	20–79	Beijing, China	Kinetic UV assay	203.4–521.0	(17)
		204	18–50	Kenyans	Not stated	191–461	(18)
	F	137	19–48	Southern Tanzania	Not stated	196–459	(19)
		487	20–79	Beijing, China	Kinetic UV assay	155.0–397.6	(17)
	M	153	18–50	Kenyans	Not stated	142–362	(18)
		133	19–48	Southern Tanzania	Not stated	148–360	(19)
Cre (μmol/l)	F	400	20–79	Beijing, China	Enzymatic colorimetric assay	55.0–99.9	(17)
		206	18–50	Kenyans	Not stated	56–99	(18)
	M	138	19–48	Southern Tanzania	Not stated	48–96	(19)
		486	20–79	Beijing, China	Enzymatic colorimetric assay	42.0–76.8	(17)
	F	154	18–50	Kenyans	Not stated	48–85	(18)
		132	19–48	Southern Tanzania	Not stated	40–81	(19)
BUN (mmol/l)	M	401	20–79	Beijing, China	Kinetic UA assay	3.1–8.1	(17)
		366	18–50	Kenyans	Not stated	1.9–5.9	(18)
	F	135	19–48	Southern Tanzania	Not stated	1.57–5.01	(19)
		487	20–79	Beijing, China	Kinetic UA assay	2.4–7.1	(17)
	M	130	19–48	Southern Tanzania	Not stated	1.47–4.61	(19)

TABLE 3. Continued

Analyte (Unit)	Gender	N	Age range (years)	Region	Method	Reference interval	Reference
Cys-C (mg/l)	M&F	270	20–65	Denmark	Not stated	0.54–1.21	(21)
	M	100	18–56	Mexico	Particle-enhanced nephelometric immunoassay	0.71–1.02	(22)
	F	100	18–56	Mexico	Particle-enhanced nephelometric immunoassay	0.58–0.98	(22)

M, Male; F, Female

TABLE 4. Spearman Correlation Between the Serum Analytes and Body Mass Index

Analyte	Male correlation	Female correlation
ALT	0.319*	0.327*
AST	0.031	0.143*
ALP	−0.031	0.261*
GGT	0.135*	0.213*
ChE	0.051	−0.054
TBA	0.061	0.045
TP	0.053	0.034
Alb	−0.015	−0.164*
PA	−0.021	−0.174*
TBIL	−0.131	−0.039
DBIL	−0.071	−0.137*
Cre	−0.081	−0.061
UA	0.227*	0.256*
BUN	0.093	0.054
RBP	0.047	0.031
Cys-C	0.067	0.058

* $P < 0.05$.

women may be due to the higher clearance rate in women than in men (24). The gender difference in GGT may be due to extra production from the prostate gland in men as compared to women who have no prostate gland. Lower Alb, PA, and RBP concentrations in women compared to men indicate that the synthesis of these analytes is gender dependent. In addition, age differences in the reference intervals established were observed in ALT, AST, ALP, GGT, ChE, TBA, Alb, PA, TBIL, DBIL, and RBP in this study. Age differences were also observed for ALT, AST, ALP, Alb, GGT, TBIL, and DBIL in other studies (17,18,20). However, age differences for PA and RBP were rarely reported in the literature. Our concern is whether the separate reference intervals will be essential for the defined subgroups of subjects. According to the Harris and Boyd's criteria, if the calculated Z value exceeds the critical value, then partitioning the reference intervals into age- or gender-specific was necessary.

In establishing reference intervals, completely "healthy" adults-based values are optimal. Nevertheless, "health" is

a relative concept lacking a universal definition (25). In this study, each of the subjects underwent an overall physical examination with complete histories. In sampling, we also applied strict exclusion criteria. Last, a total of 2,405 apparently healthy adults aged 18 to 77 years (1,256 men and 1149 women) were selected randomly from the Sixth People's Hospital Affiliated to Shanghai Jiao Tong University. With this carefully selected reference sample group, we reported reference intervals for 16 biochemical substances for apparently healthy adults from eastern China. The reference intervals established in this study differ, to some extent, from those published in the literature (17–22). The differences can be explained by the differences in the experimental methods, laboratory conditions, geographical locations, sample sizes, races, etc.

We acknowledge that there is at least one limitation in the present work. The mean values of the 16 biochemical substances in healthy adults are not compared with those in children.

In summary, we reported reference intervals for biochemical substances related to renal and hepatic function for apparently healthy adults from eastern China. The reference intervals established in this study can serve as a useful clinical tool for the assessment of kidney and liver damage. In addition, the reference intervals can be adopted in other clinical laboratories after local validation.

REFERENCES

1. Horn PS, Pesce AJ. Reference intervals: an update. *Clin Chim Acta* 2003;334:5–23.
2. Concordet D, Geffre A, Braun JP, Trumel C. A new approach for the determination of reference intervals from hospital based data. *Clin Chim Acta* 2009;405:43–45.
3. Horowitz GL, Altaie S, Boyd JC. Defining, establishing, and verifying reference intervals in the Clinical Laboratory: Approved Guideline, C28–3E. 3rd ed., Wayne: Clinical and Laboratory Standards Institute, 2008.
4. Schmucker DL. Liver function and phase I drug metabolism in the elderly. *Drug aging* 2001;18:837–851.
5. Millan-Calenti JC, Sanchez A, Lorenzo-Lopez L, Maseda A. Laboratory values in a Spanish population of older adults: A comparison with reference values from younger adults. *Maturitas* 2012;71:396–401.

6. Harris HE, Boyd JC. On dividing reference data into sub-groups to produce separate reference range. *Clin Chem* 1990;36:265–269.
7. Ross JW, Fraser MD, Moore TD. Analytic clinical laboratory precisions: state of the art for thirty-one analysis. *Am J Clin Pathol* 1980;74:521–529.
8. Solberg HE, Stamm D. IFCC recommendation: theory of reference values: Part 4. Control of analytical variation in the production, transfer and application of reference values. *Clin Chem Acta* 1991;202:S5–S10.
9. Kasper DL, Harrison TR. Principles of Internal Medicine, 16th ed., New York: McGraw-Hill, Medical Pub. Division, 2005.
10. Herold G. Innere Medizin. Eine Vorlesungsorientierte Darstellung, 2007 ed., Herold, Köln, 2006.
11. Kratz A, Ferraro M, Sluss PM, Lewandrowski KB. Case records of the Massachusetts General Hospital, weekly, 2004.
12. Chan AOK, Lee KC, Leung JNS, Shek CC. Reference intervals of common serum analytes of Hong Kong Chinese. *J Clin Pathol* 2008;61:632–636.
13. Dybkrer R, Solberg HE. Approved recommendation (1987) on the theory of reference values. part 6. Presentation of observed values related to reference values. *Clin Chim Acta* 1987;170: S36–S37.
14. Petitclerc C, Solberg HE. Approved recommendation (1987) on the theory of reference values. Part 2. Selection of individuals for the production of reference values. *Clin Chim Acta* 1987;170: S3–S12.
15. Solberg HE. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. *Clin Chim Acta* 1987;170:S17–S18.
16. Harris EK, Boyd JC. On dividing reference data into subgroups to produce separate reference ranges. *Clin Chem* 1990;36:265–259.
17. Yang S, Qiao R, Li Z, et al. Establishment of reference intervals of 24 chemistries in apparently healthy adult Han population of Northern China. *Clin Biochem* 2012;45:1213–1218.
18. Juma AA, Ngerawa JJ, Njaqi EN. Reference values for some renal function parameters for adult population in North-Rift Valley, Kenya. *Ind J Clin Biochem* 2012;27:40–45.
19. Saathoff E, Schneider P, Kleinfeldt V, et al. Laboratory reference values for healthy adults from southern Tanzania. *Trop Med Int Health* 2008;13:612–625.
20. Alice J, Eliud NM, Joseph JN. Estimation of reference values for liver function tests for adult population in North-Rift Valley, Kenya. *Asian J Med Sci* 2011;3:243–249.
21. Erlandsen EJ, Randers E, Kristensen JH. Reference intervals for serum cystatin c and serum creatinine. *Clin Chem Lab Med* 1998;36:393–397.
22. Croda-Todd M, Juarez E, Hernandez P, Flores G, Rivera G, Bocanegra-Garcia V. Reference intervals for serum cystatin in healthy Mexican adults. *Clin Chem Lab Med* 2007;45: 925–927.
23. Jannie W, Treuting JJ, Donald CC. Metabolic intermediates and inorganic ions, in Henry JB, ed: Clinical diagnosis and management, 16th ed., Philadelphia, London and Toronto: WB Saunders company. 1979. p 259–304.
24. Croda-Todd MT, Soto-Montano XJ, Hernandez-Cancino PA, Juarez-Aguilar E. Adult cystatin C reference intervals determined by nephelometric immunoassay. *Clin Biochem* 2007;4:1084–1087.
25. Walton RM. Establishing reference intervals: health as a relative concept. *Semin Avian Exot Pet Med* 2011;10:66–71.