Clinica Chimica Acta 490 (2019) 142–146

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Pediatric reference intervals of liver and renal function tests from birth to adolescence in Chinese children as performed on the Olympus AU5400

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ARTICLE INFO

Keywords:
Reference intervals
Pediatric
Liver function tests
Kidney function tests

ABSTRACT

Background: The growth and development of children and adolescents influence values of liver and renal function tests. The purpose of this study was to determine age- and gender-specific reference intervals for liver and renal function tests in apparently healthy Chinese children and adolescents.

Methods: A total of 63,086 apparently healthy children and adolescents (0–15 y) were chosen as reference individuals in this study. The 15 biochemical analytes relating to liver and renal function were measured using an Olympus AU5400 analyzer. Reference intervals were partitioned according to age and/or gender subgroups using the Harris and Boyd's method and established using non-parametric methods.

Results: Our results showed that all analytes except for cholinesterase (ChE) and α1-microglobulin (α1-MG) required partitioning by age. Gender partitions were also required for alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), creatinine (Cre), and uric acid (UA). Age- and gender-appropriate reference intervals for liver and renal function tests were established for apparently healthy Chinese children and adolescents.

Conclusions: When establishing pediatric reference intervals, partitioning by age and/or gender is essential. Those reference intervals can be adopted in other clinical laboratories after appropriate validation.

1. Introduction

Clinicians rely on the availability of reliable and suitable reference intervals to decide whether patients require further testing and examination. Liver and renal function tests can be useful to determine whether the liver and kidney are performing their tasks adequately. Therefore, these tests are an important part of routine health checks.

Many of clinical laboratories adopt the reference intervals reported by the medical literature or the diagnostic test manufacturer [1]. As we know, reference intervals provided by manufacturers were established mainly based on American and European populations. Given that laboratory test results could be influenced by differences in dietary, genetic, environmental, and social factors, using reference intervals derived from foreign population may lead to a wrong interpretation, which might influence the outcome. In addition, the clinical interpretation of these results in pediatrics is executed in the context of age- and gender-specific dynamics because physiological development leads to changes in many analytes measured, particularly during puberty and in the first ys of life [2]. Clinical laboratories should define reference intervals based on the local population.

However, establishing pediatric reference intervals is challenging [2–4]. On the one hand, obtaining sufficient samples from healthy children is challenging. On the other hand, reference interval value can be different due to gender and age. Appropriate pediatric reference intervals are often inadequate or even unavailable.

2. Methods

2.1. Study population

This study was approved by the institutional ethics committee of the Third Affiliated Hospital of Zhengzhou University. According to the Clinical and Laboratory Standards Institute (CLSI) EP28-A3c guidelines [5], we enrolled a total of 65,735 apparently healthy children and adolescents (0–15 y) between January 2016 and June 2018 from our hospital. The exclusion criteria were as follows: 1. diabetes mellitus,

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https://doi.org/10.1016/j.cca.2019.01.001
Received 17 September 2018; Received in revised form 1 January 2019; Accepted 2 January 2019
Available online 03 January 2019
0009-8981/

anemia, renal disease, hepatic disease or other diseases that may affect analytes measured in the study, 2. use of prescription drugs over the previous 14 days, 3. surgery experiences within 6 months, 4. obesity, 5. history of heart attack and 6. positive for hepatitis B virus (HBV), human immunodeficiency virus (HIV), and hepatitis C virus (HCV). A total of 2649 individuals were excluded and finally 63,086 apparently healthy children and adolescents were included in this study. For each subject, information regarding ethnicity, age, gender, weight, height, diet, disease if any, was obtained. Overweight and obesity were defined according to the World Health Organization (WHO) standards [6,7]. The data of children (0–5 y) with overweight [BMI-for-age values > + 2 SDs] and obesity (BMI-for-age values > + 3SDs) were excluded from this study. The data of children (5–15 y) with overweight(BMI-for-age values > + 1 SD)and obesity (BMI-for-age values > + 2SDs) were excluded from this study.

2.2. Sample collection and handing

Serum samples were collected from all subjects after obtaining parental permission. Blood was taken between 8:00 and 11:00. Subjects fasted for > 8 h before blood samples were collected into vacuum tubes in the morning. All samples were left at 25 °C for 30 min to clot. Next, samples were centrifuged at 1200 ×*g* for 10 min. We have evaluated the influence of interference factors such as hemolysis, jaundice, lipidemia, and rheumatoid factor on the results of biochemical substances measured in this study. The results were not significantly affected by hemolysis (hemoglobin 5.0 g/l), lipidemia (triglyceride 8 mmol/l), jaundice (bilirubin 600 μmol/l) and rheumatoid factor (450 IU/ml) according to the manufacturer's instructions. Hemolytic samples were collected again. There was no obvious interference from hemolysis, jaundice, lipidemia, and rheumatoid factor. All qualified samples were analyzed within 2 h after separation. The serum samples were stored at −70 °C until further analysis.

2.3. Instruments, reagents and methods

The activities and concentrations of the following analytes were measured with the Olympus AU5400 automatic analyzer (Olympus Ltd.): alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), cholinesterase (ChE), total protein (TP), albumin (Alb), direct bilirubin (DBIL), total bilirubin (TBIL), urea nitrogen (BUN), uric acid (UA), creatinine (Cre), β2-microglobulin (β2-MG), α1-microglobulin (α1-MG), and cystatin-C (Cys-C). All assays were performed according to the manufacturer's instructions. Performed tests, methods, and analytical details were listed in Table 1. ALT, AST, ALP, GGT, ChE, TP, Alb, TBIL, DBIL, UA, Cre, BUN, β2-MG were measured using reagents from Beijing Wantai Drd Co, Ltd. α1-MG was measured using reagents from InTec Products Inc. Cys-C was measured using reagents from AusBio Laboratories Co., Ltd. Abnormal and normal controls were run daily and used to calculate the precision. Accuracy was calculated from external quality assessment (EQA) schemes organized by the Chinese National Center for Clinical Laboratories. If controls were out of range, no analysis was performed.

2.4. Statistical analysis

All statistical analyses were performed with SPSS 17.0 software and in accordance with CLSI C28-A3 document [5]. Briefly, the data were graphed using histograms and scatter plots to identify outliers; Outliers were excluded with Dixon's rule [5,8]. Gender and age partitions were first determined by visual inspection of the scatter plots and distribution for overall trends. And then, it was justified with Harris and Boyd's method currently recommended by the CLSI [9]. According to Harris and Boyd's method [10], z values were calculated and then compared with the critical values calculated using the formula:

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

where s_i, χ_i, and n_i are the SD, mean, and the sample size of subgroup*i*, respectively. If Z value was > Z^{*} value, partitioning reference intervals according to age or gender is necessary. The lower (2.5th percentiles) and upper (97.5th percentiles) reference intervals were calculated using non-parametric rank methods. The 90% confidence intervals (*CI*) of the reference intervals were also calculated.

3. Results

The reference population started with 65,735 apparently healthy children and adolescents (0–15 y) from our hospital. After applying these exclusion criteria, our study included 63,086 subjects. Subsequently, one outlier was detected in ChE and Cre. Two outliers were detected in BUN. Three outliers were detected for ALP, TP, Alb, TBIL, α1-MG, and Cys -C. Four outliers were detected in ALT, GGT, Cre, and UA. Five outliers were detected in AST, DBIL. These outliers were excluded from further analysis. Pediatric reference intervals for selected liver and renal function parameters are summarized in Table 2, partitioned by age and/or gender, as per the Harris and Boyd's method. And 90% confidence intervals of lower and upper reference limits were also calculated. ALT activities increased gradually, reached its peak between 2 and 7 months, and then decreased gradually. AST activities increased

Table 1
Performed tests, abbreviations, methods, and analytical details.

Test (abbreviation)	Method	Reagent	Control	Calibrator	Calibration method	Bias(%)	CV(%)
Alanine aminotransferase (ALT)	Alanine substrate method	Wantai	Randox	Randox	Single-point calibration	0.89	2.1
Aspartate aminotransferase (AST)	Aspartate substrate method	Wantai	Randox	Randox	Single-point calibration	−1.25	1.8
Alkaline phosphatase (ALP)	NPP substrate-AMP buffer method	Wantai	Randox	Randox	Single-point calibration	3.17	2.8
Albumin (Alb)	Bromocresol green method	Wantai	Randox	Randox	Single-point calibration	−1.42	1.2
α1-microglobulin (α1-MG)	Immunoturbidimetric method	Intec	InTec	InTec	Six-point calibration	1.89	3.8
Urea nitrogen (BUN)	Urease-glutamate dehydrogenase method	Wantai	Randox	Randox	Single-point calibration	−2.93	2.4
β2-microglobulin (β2-MG)	Particle-enhanced immunoturbidimetric method	Wantai	Randox	Randox	Six-point calibration	1.78	2.8
Cholinesterase (ChE)	Butyrylthio choline substrate method	Wantai	Randox	Randox	Single-point calibration	1.37	3.4
Creatinine (Cre)	Sarcosine oxidase method	Wantai	Randox	Randox	Single-point calibration	0.84	1.3
Cystatin-C (Cys-C)	Latex enhanced immunoturbidimetric assay	AusBio	AusBio	AusBio	Five-point calibration	0.68	0.9
Direct bilirubin (DBIL)	Vanadate oxidase method	Wantai	Randox	Randox	Single-point calibration	1.03	3.8
Gamma-glutamyl transpeptidase (GGT)	GCANA substrate method	Wantai	Randox	Randox	Single-point calibration	−3.14	1.8
Total protein (TP)	Biuret method	Wantai	Randox	Randox	Single-point calibration	0.41	1.3
Total bilirubin (TBIL)	Vanadate oxidation method	Wantai	Randox	Randox	Single-point calibration	1.24	3.3
Uric acid (UA)	Uricase method	Wantai	Randox	Randox	Single-point calibration	−2.22	1.5

The total analytical imprecision for the experimental method used to calculate the reference intervals is given for each assay as an average coefficient of variation (CV %) of two concentrations of internal controls through 1 year.

Table 2
Pediatric reference intervals for selected liver and renal function parameters.

Analye (Unit)	Age	Gender	*N*	Outliers	Lower limit (*CI*)	Upper limit (*CI*)
ALT(U/l)	0 to < 15d	F+ M	3318	0	3.0 (3.0–4.0)	39.0 (36.0–43.0)
	≥15d to < 1 m	F+ M	1345	0	4.0 (3.0–5.0)	45.0 (43.0–48.0)
	≥1 m to < 2 m	F+ M	1835	0	7.0 (6.0–8.0)	50.0 (48.0–51.0)
	≥2 m to < 7 m	F+ M	12,883	1	11.0 (11.0–11.0)	56.0 (55.0–56.0)
	≥7 m to < 1 y	F+ M	10,562	1	9.0 (9.0–9.0)	52.0 (52.0–53.0)
	≥1 y to < 2 y	F+ M	10,926	1	8.0 (8.0–8.0)	45.0 (44.0–46.0)
	≥2 y to < 3 y	F+ M	5604	0	7.0 (7.0–7.0)	38.0 (36.0–40.0)
	≥3 y to < 8 y	F+ M	11,634	1	6.0 (6.0–7.0)	36.0 (35.0–37.0)
	≥8 y to < 16 y	M	2683	0	7.0 (6.0–7.0)	46.0 (44.0–48.0)
		F	2292	0	6.0 (6.0–6.0)	40.0 (38.0–41.0)
AST(U/l)	0 to < 8d	F+ M	2216	0	12.0 (10.0–13.0)	58.0 (52.0–67.0)
	≥8d to < 1 m	F+ M	2446	1	13.0 (12.0–13.0)	62.0 (57.0–68.0)
	≥1 m to < 1 y	F+ M	25,280	2	21.0 (20.0–21.0)	67.0 (67.0–68.0)
	≥1 y to < 2 y	F+ M	10,926	1	21.0 (21.0–21.0)	60.0 (59.0–61.0)
	≥2 y to < 3 y	F+ M	5604	0	19.0 (19.0–20.0)	53.0 (52.0–55.0)
	≥3 y to < 6 y	F+ M	8748	1	18.0 (18.0–18.0)	49.0 (48.0–50.0)
	≥6 y to < 13 y	F+ M	6945	0	16.0 (15.0–16.0)	43.0 (42.0–45.0)
	≥13 y to < 16 y	M	494	0	13.0 (12.0–14.0)	38.0 (35.0–43.0)
		F	422	0	12.0 (11.0–13.0)	32.0 (29.0–34.0)
GGT(U/l)	0 to < 3 m	F+ M	9424	1	17.0 (16.0–17.0)	64.0 (64.0–64.0)
	≥3 m to < 4 m	F+ M	2205	0	13.0 (13.0–13.0)	60.0 (59.0–61.0)
	≥4 m to < 5 m	F+ M	2634	0	11.0 (11.0–11.0)	53.0 (51.0–54.0)
	≥5 m to < 8 m	F+ M	7770	0	9.0 (9.0–9.0)	45.0 (44.0–46.0)
	≥8 m to < 1 y	F+ M	7910	1	8.0 (8.0–8.0)	40.0 (39.0–41.0)
	≥1 y to < 7 y	M	14,473	1	8.0 (8.0–8.0)	35.0 (34.0–36.0)
		F	12,369	1	7.0 (7.0–7.0)	31.0 (30.0–31.0)
	≥7 y to < 14 y	M	3115	0	9.0 (9.0–9.0)	35.0 (33.0–37.0)
		F	2661	0	8.0 (8.0–8.0)	32.0 (30.0–37.0)
	≥14 y to < 16y	M	281	0	9.0 (8.0–10.0)	44.0 (41.0–53.0)
		F	240	0	8.0 (6.0–9.0)	35.0 (27.0–37.0)
ALP(U/l)	0 to < 8d	F+ M	2216	0	43.0 (40.0–50.0)	344.0 (324.0–353.0)
	≥8d to < 15d	F+ M	1102	0	51.0 (47.0–60.0)	361.0 (342.0–378.0)
	≥15d to < 1 m	F+ M	1345	0	59.0 (53.0–65.0)	406.0 (375.0–423.0)
	≥1 m to < 5 m	F+ M	9601	0	102.0 (99.0–107.0)	482.0 (479.0–485.0)
	≥5 m to < 7 m	F+ M	5118	0	67.0 (64.0–73.0)	422.0 (417.0–429.0)
	≥7 m to < 1 y	F+ M	10,562	1	49.0 (47.0–51.0)	358.0 (355.0–366.0)
	≥1 y to < 9 y	F+ M	29,255	2	66.0 (62.0–66.0)	339.0 (336.0–341.0)
	≥9 y to < 15 y	F+ M	3610	0	74,0 (71.0–79.0)	388.0 (388.0–408.0)
	≥15y to < 16y	M	145	0	85.0 (83.0–87.0)	407.0 (391.0–413.0)
		F	129	0	44.0 (43.0–46.0)	306.0 (299.0–311.0)
ChE(U/l)	0 to < 1 m	F+ M	4663	0	1.5 (1.3–1.6)	8.2 (7.9–8.5)
	≥1 m to < 16 y	F+ M	58,422	1	4.2 (4.0–4.2)	13.6 (13.4–13.8)
TP(g/l)	0 to < 4 m	F+ M	11,629	1	42.2 (41.8–42.5)	65.7 (65.4–66.0)
	≥4 m to < 1 y	F+ M	18,314	1	50.0 (49.7–50.2)	71.8 (71.7–72.0)
	≥1 y to < 10 y	F+ M	30,222	1	55.1 (54.9–55.2)	76.1 (76.0–76.3)
	≥10 y to < 16 y	F+ M	2918	0	59.4 (559.0–60.3)	80.4 (79.9–80.8)
Alb(g/l)	0 to < 1 m	F+ M	4663	0	21.6 (20.1–22.5)	46.3 (46.0–46.6)
	≥1 m to < 3 m	F+ M	4762	0	28,1 (27.9–28.8)	48.5 (45.6–49.8)
	≥3 m to < 1 y	F+ M	20,518	2	33.5 (33.3–33.7)	51.1 (51.0–51.3)
	≥1 y to < 16 y	F+ M	33,140	1	35.0 (34.9–35.2)	52.2 (52.1–52.3)
TBIL(μmol/l)	0 to < 3d	F+ M	1075	0	5.3 (3.7–6.7)	39.1 (34.7–39.8)
	≥3d to < 2 m	F+ M	5423	0	3.5 (3.4–3.8)	35.6 (34.9–36.1)
	≥2 m to < 3 m	F+ M	2927	0	3.5 (3.4–3.7)	30.5 (29.7–31.3)
	≥3 m to < 4 m	F+ M	2205	0	2.8 (2.7–2.9)	20.8 (19.9–22.0)
	≥4 m to < 1 y	F+ M	18,314	1	2.7 (2.7–2.7)	14.6 (14.4–14.8)
	≥1 y to < 2 y	F+ M	10,926	1	3.1(3.0–3.1)	13.6 (13.4–13.8)
	≥2 y to < 9 y	F+ M	18,329	1	37 (3.7–3.8)	16.6 (16.4–16.8)
	≥9 y to < 12 y	F+ M	2412	0	4.7 (4.5–4.9)	21.2 (19.8–21.8)
	≥12 y to < 16 y	F+ M	1472	0	5.0 (4.7–5.3)	24.9 (23.7–26.1)
DBIL(μmol/l)	0 to < 2 m	F+ M	6497	1	1.1 (1.0–1.2)	10.0 (9.8–10.2)
	≥3 m to < 1 y	F+ M	20,519	1	0.6 (0.6–0.6)	4.5 (4.4–4.6)
	≥1 y to < 3 y	F+ M	16,529	2	0.6 (0.6–0.6)	3.7 (3.7–3.8)
	≥3 y to < 12 y	F+ M	15,137	1	0.8 (0.8–0.9)	4.9 (4.8–5.0)
	≥12 y to < 16y	F+ M	1472	0	1.1 (1.0–1.2)	7.4 (7.0–8.0)
BUN(mmol/l)	0 to < 1 y	F+ M	29,944	1	0.8 (0.8–0.8)	5.1 (5.1–5.2)
	≥1 y to < 7 y	F+ M	26,843	1	1.3 (1.2–1.3)	5.9 (5.7–6.0)
	≥7 y to < 16 y	F+ M	7297	0	1.6 (1.6–1.6)	6.6 (6.6–6.7)

(*continued on next page*)

Table 2 (*continued*)

Analye (Unit)	Age	Gender	*N*	Outliers	Lower limit (*CI*)	Upper limit (*CI*)
Cre(μmol/l)	0 to < 3d	F+ M	1075	0	15.6 (15.2–17.2)	61.1 (47.7–73.7)
	≥3d to < 8d	F+ M	1141	0	12.5 (10.9–15.0)	53.1 (47.1–74.8)
	≥8d to < 15d	F+ M	1102	0	12.8 (10.6–13.6)	47.7 (46.4–53.4)
	≥15d to < 3 m	F+ M	6106	1	12.4 (12.2–12.6)	35.6 (34.4–36.7)
	≥3 m to < 7 m	F+ M	9956	1	12.2 (12.1–12.4)	28.0 (27.7–28.5)
	≥7 m to < 2 y	F+ M	21,489	1	13.1 (12.9–13.2)	30.1 (29.8–30.4)
	≥2 y to < 3 y	F+ M	5604	0	15.3 (15.0–15.6)	34.4 (33.5–35.4)
	≥3 y to < 6 y	F+ M	8748	1	17.4 (17.2–17.8)	38.9 (38.5–39.9)
	≥6 y to < 8 y	F+ M	2886	0	20.1 (19.7–20.4)	44.9 (44.4–45.8)
	≥8 y to < 11 y	F+ M	2820	0	23.8 (23.1–24.5)	51.0 (50.0–52.2)
	≥11 y to 15y	M	986	0	27.9 (26.6–30.1)	64.2 (62.8–67.0)
		F	895	0	23.9 (19.6–27.1)	55.8 (54.5–56.9)
	≥15 y to 16 y	M	145	0	32.9 (32.9–34.0)	92.5 (90.0–92.5)
		F	129	0	30.3 (29.8–31.4)	76.0 (73.3–77.0)
UA(μmol/l)	0 to < 1 m	F+ M	4663	0	56.3 (51.9–60.1)	287.4 (269.0–352.1)
	≥1 m to < 4 m	F+ M	6967	0	82.6 (80.7–84.1)	312.5 (307.4–317.1)
	≥4 m to < 1 y	F+ M	18,313	2	87.0 (85.2–88.5)	361.4 (357.6–365.9)
	≥1 y to < 10 y	F+ M	30,221	2	97.1 (95.7–98.5)	405.6 (401.4–410.0)
	≥10 y to 13 y	F+ M	2002	0	120.8 (111.6–126.4)	436.7 (422.7–453.1)
	≥13 y to < 16 y	M	499	0	147.0 (128.7–164.6)	518.6 (480.1–551.4)
		F	417	0	72.7 (64.6–102.4)	424.1 (395.8–449.3)
β2- MG (mg/l)	0 to < 3 m	F+ M	9425	0	1.6 (1.5–1.7)	5.3 (5.2–5.4)
	≥3 m to < 1 y	F+ M	20,520	0	1.4 (1.4–1.5)	4.2 (4.2–4.3)
	≥1 y to < 15 y	F+ M	33,140	1	1.1(1.1–1.1)	3.3 (3.3–3.4)
	≥15 y to 16 y	F+ M	274	0	1.2 (1.0–1.2)	3.0 (2.9–3.6)
α1-MG (mg/l)	0 to < 16 y	F+ M	63,083	3	10.2 (10.1–10.2)	28.7 (28.5–28.9)
Cys –C(mg/l)	0 to < 4 m	F+ M	11,629	1	0.8 (0.8–0.8)	2.1 (2.0–2.1)
	≥4 m to < 1 y	F+ M	18,314	1	0.7 (0.7–0.7)	1.7 (1.7–1.8)
	≥1 y to < 2 y	F+ M	10,926	0	0.6 (0.6–0.6)	1.4 (1.3–1.4)
	≥3 y to < 16 y	F+ M	16,609	1	0.5 (0.5–0.5)	1.1 (1.1–1.1)

ALT alanine a minotransferase, *AST* aspartate a minotransferase, *GGT* gamma-glutamyl transpeptidase, *ALP* alkaline phosphatase, *ChE* cholinesterase, *TP* total protein, *Alb* albumin, *DBIL* direct bilirubin, *TBIL* total bilirubin, *BUN* urea nitrogen, *UA* uric acid, *Cre* creatinine, *β2- MG* β2- microglobulin, *α1- MG* α1- microglobulin, *C ys-C* cystatin-C.

gradually, reached its peak between 1 and 12 months, and then decreased gradually. They required 8–9 age partitions. Gender partitions were also required for ALT and AST in the 8- to 15-y and 13- to 15-y partitions, respectively. GGT activities decreased gradually with increasing ages. GGT and ALP required 8–9 age partitions. Gender partitions were also required for GGT and ALP in the 1- to 15-y and 15-y partitions, respectively. ChE required 1 age partition. α1-MG required no age and gender partitioning. TP and Alb levels increased gradually with increasing ages. They required 4 age partitions. No gender differences were observed for both analytes. Reference intervals for TBIL and DBIL were higher during the first y of life. Following the first y, the values decreased to their lowest concentrations and then gradually increased with age. Furthermore, no gender differences were observed. The levels of BUN and UA increased gradually with increasing ages. Cre levels gradually decreased to their lowest concentrations between 3 and 7 months and then gradually increased with age. They required 3–11 age partitions. Gender partitions were also required for Cre and UA in the 11- to 15-y and 13- to 15-y partitions, respectively. However, BUN required no gender partitioning. β2-MG and Cys-C concentrations decreased gradually with increasing ages. They required 4 age partitions. However, they required no gender partitioning.

4. Discussion

Liver and kidney function tests are commonly applied in routine clinical evaluation, diagnosis, treatment, and prognosis. ALT, BUN, and Cre levels in children and adolescents are quite different from those of healthy adults [11]. In addition, reference intervals of children and adolescents are in a non-fixed range because their eating habits and physiological development may have an impact on the reference intervals [12]. Therefore, it is necessary to establish suitable reference intervals for liver and renal function tests in apparently healthy Chinese children and adolescents.

Determining reference intervals through recruitment of completely healthy individuals is time-consuming and costly. Because of the dynamic changes occurring with child growth and physiological development, the recruitment of pediatric reference individuals is particularly challenging. This often leads to the need for gender- and age-specific partitioning of reference intervals which requires a large sample size. Given these challenges, the idea of determining reference intervals from hospital patient data is very attractive to clinical laboratories. This concept introduced by Hoffmann [13] has been proved useful by other studies [14,15]. In sampling, we tried to ensure that there were at least 120 samples for each subgroup.

Our results showed that all analytes except for α1-MG required partitioning by age. Gender partitions were also required for ALT, AST, GGT, ALP, Cre, and UA. The rest of the analytes required no gender partitioning. Age-related changes in analytes levels were observed more commonly than gender-related differences. Analytes measured in this study showed multiple separated age-related reference intervals. The reference intervals determined in this study reflected pediatric growth and development changes during childhood.

Although reference intervals need to be subdivided based on statistical analysis, it is not clear whether these statistical differences are medical relevant. Reference interval update required every few ys because the reference population change with nutrition and environment factors. In addition, the diversity of detection system can result in differences of test results. Reference intervals established in this study reflect growth and development throughout childhood.

It is acknowledged that the most important limitation of this study consists of the skewed distribution of individual age. The 90% confidence intervals of the lower and upper limits of reference intervals for teenagers may be wide due to the relatively small number of subjects.

In summary, reference intervals established in this study reflect growth and development throughout childhood. These reference intervals can be used in any other laboratory after further validation and

transfer as recommended by CLSI C28-A3 [5].

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