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Age-Specific Reference Intervals for Routine Laboratory Biochemical Tests in The Iranian Adolescent Girls

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ABSTRACT

Introduction: For interpretation of the most common biochemical markers reference intervals (RIs) are widely considered and reported. Therefore, in this study, we aimed to determine age-specific RIs for some biochemical tests by removing and/or limiting critical gaps including sex, age group, geographical region, measurement device, and type of kits and reagents for routine laboratory biochemical variables in healthy adolescent girls aged 12-19 years.

Materials and Methods: In this cross-sectional study a total of 1026 adolescent girl was recruited. According to the study aim, the levels of serum for some routine biochemical tests were measured on a BT-3000 auto-analyzer. On the basis of the non-parametric rank method and by use of CLSI Ep28-A3 guidelines, we determined age-specific RIs, including confidence interval 90%, under specific exclusion criteria.

Results: Age partitioning was required for none of the upper and lower limits of biochemical markers. All Serum biochemical tests concentration remained relatively constant throughout the age range.

Conclusion: By removing and/or limiting critical gaps involved in reference values of biochemical tests, it has provided an important understanding of metabolic processes and it has facilitated clinical application to track different diseases better.

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Introduction

For better health care delivery and clinical diagnostics, accessing relatively accurate reference intervals adequate, monitoring and selection is the most important factor for wide range of biochemical tests (1, 2). According to the key critical gaps involved in reference intervals (RI), including sex, age, geographical regions, measurement device, kits, and reagents types, accurate determining and classifying RIs from a healthy population are required for ensuring from adequate interpretation of laboratory tests results (3). Additionally, to determine population reference values are related to a selection of large number of healthy subjects. Therefore, RIs can be defined as health-associated benchmarks, used for laboratory test interpretation and clinical decision-making, assisting clinicians in disease diagnosis, management, and treatment (4, 5). RIs are commonly established by determining the central 95% of test result values in a healthy reference population. While there are many direct and indirect statistical methodologies utilized for RI establishment, the Clinical and Laboratory Standard Institute (CLSI) guideline for de novo RIs recommends using the nonparametric method when there is a sufficiently large healthy population (n≥120) (6). Direct RI establishment based on healthy populations can be very challenging, especially in pediatrics, due to the need for stratification by important covariates, including age, sex, ethnicity and instrument method. As a result, critical gaps continue to exist in the establishment of robust RIs for biochemical parameters in health and disease in specific populations. In the past decade, several international initiatives have contributed to closing these critical gaps and improving clinical decision making in the diagnosis of disease in a special age and/or sex group (4, 7). However, further work is required to ensure clinical laboratories have access to accurate RIs for their specific population.

In contrast with the critical gaps-limited RIs values, there are several key factors involved in regulating metabolic processes and physiologic status including hormonal regulators-related sex as well as organ maturity, respectively, which leads to change values of RIs between male, female, and even children (8, 9). Therefore, regarding the affecting agents n values of RIs including sex, age group, geographical region, measurement device, and reagents and consumable kits, determining RIs values for both special gender and age range may be required and it is also inappropriate to use a common reference interval test for children and adults (10). In line with this, findings of the recent studies have shown that specific sex- and age-RIs values of metabolic, renal function, liver function, electrolytic tests were determined in neonates to adults (11, 12). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, γ-glutamyltransferase (GGT), and total bilirubin are known as common liver function tests (LFTs) which are generally used to assess wide spectrum of hepatic disorders and non-liver related diseases (13). Renal function tests (RFTs), including assays for blood urea nitrogen (BUN), creatinine, sodium, and potassium, are generally used to examine both normal kidney function and renal disorders (14).

In the current study, our aim is calculating RIs and serum concentration of 13 biochemical markers including total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride (TG), fasting blood glucose (FBG), hs-CRP, ALT, AST, ALP, creatinine, BUN, calcium, phosphate, and vitamin D in healthy adolescent girls aged 12-19 years, lived in Mashhad and Sabzevar cities, Iran.

Materials and Methods

This study was approved by the ethical committee of Mashhad University of Medical Sciences (MUMS). A total of 1026 healthy adolescent girls aged 12-19 years of Mashhad and Sabzevar cities, Iran, were selected through a cluster random sampling method between January and April 2015. Informed participants and parental written consent were obtained from all study participants.

Participants were excluded if they had a history of chronic illness (i.e., auto-immune disease, cancer, metabolic disorders, cardiovascular disease, malabsorption, hepatic or renal failure, thyroid or

parathyroid or adrenal disease) used prescribe medications including anti-inflammatory, anti-diabetic, anti-depressant, anti-obesity, hormone medications and/or taking vitamin D or calcium supplementation within 6 months pre-collection. After applying the exclusion criteria, Missing data for some participants led to a difference in the sample size for each biomarker

Blood sampling

After 14 hours overnight fast, fasted blood samples were taken via an antecubital vein venipuncture early in the morning between 8:00-10:00 a.m. According to a standard protocol, blood samples were obtained from participants in a sitting position and then were collected into vacuum clot tubes. Samples were instantly centrifuged (Hettich model D-78532) at 14563 g for 10 min at room temperature (25° C). Serum samples were then stored at -80 C until analyses.

Analyses of biochemical markers

Biochemical markers including FBG, TG, TC, HDL, LDL, creatinine, BUN, calcium, vitamin D, phosphate, AST, ALT, ALP, and hs-CRP were tested on serum samples using commercial kits (Pars Azmoun, Karaj, Iran) and were measured by BT-3000 auto-analyzer (Biotechnica, Rome, Italy) (15, 16). The intra- and inter-assay coefficients of variation (CV) were 4% for TG, 5% for LDL and HDL, and 2% for TC, uric acid, and FBG, 4% for AST, 5% for ALT, 5% for creatinine, and 6% for BUN. We applied an immunoturbidity method to estimate serum levels of hs-CRP using Pars Azmoun kits on an Alcyon auto analyzer (Abbott, Chicago, IL, USA), with a detection limit of 0.06 mg/L. Intra- and inter-assay CV were also 17.0% for hs-CRP. The amount of calcium was measured based on the intensity of the purple color for calcium generated by the interaction between Calcium and Cresolphthalein Complex one and phosphate was measured based on the intensity of the color induced by the interaction between phosphate and ammonium molybdate and sulfuric acid (17).

Statistics

Harris and Boyd method was done for determining age partition of each factor (18). Outliers were excluded by Tukey's method if normally distributed (19) and the adjusted Tukey method if skewed (20). RIs were calculated using the nonparametric rank method, as recommended by CLSI Ep28-A3 guidelines (6). Confidence interval (CI) 90% were also determined around the lower and upper limits of RIs.

Results

Distribution of key biochemical markers across the adolescent girls ages has been shown in Figure 1. All 14 biochemical markers and lower and upper limits of age-specific reference intervals were calculated and are also provided in Table 1.

The scatter-plot results did not show age partitioning for age-specific lower and upper limits of all biochemical markers reference intervals. Concentrations of the macro-minerals (calcium and phosphate), vitamin D, RFTs, LFTs, and metabolic markers (including lipid profile and FBG) and hs-CRP remained relatively fixed across the adolescent girls age range.

Discussion

The study is aimed to evaluate age-specific RIs for several routine biochemical markers including a lipid profile, FBG, hs-CRP, liver and renal function tests. This study has enabled generation of an age specific reference interval database, indicative of the Iranian population. The reference intervals were established from a representative of the Iranian adolescent girls with common lifestyle, environmental factors, and the current health status of them. Additionally, appropriate disease diagnosis and monitoring affected by the accuracy of laboratory test results, as well as the age-sex and method specific RIs were reported for test interpretation. According to the International Federation of Clinical Chemistry (IFCC), it is necessary to determine specific RI for clinical biomarkers in a specific population with different age, sex and ethnicities or to validate a preexisting RI (21). The current study aimed to determine RIs for 13 biochemical markers including lipid profile, FBG and hs-CRP, AST, ALT, ALP, creatinine, calcium, phosphate, vitamin D and BUN in the healthy Iranian adolescent girls.

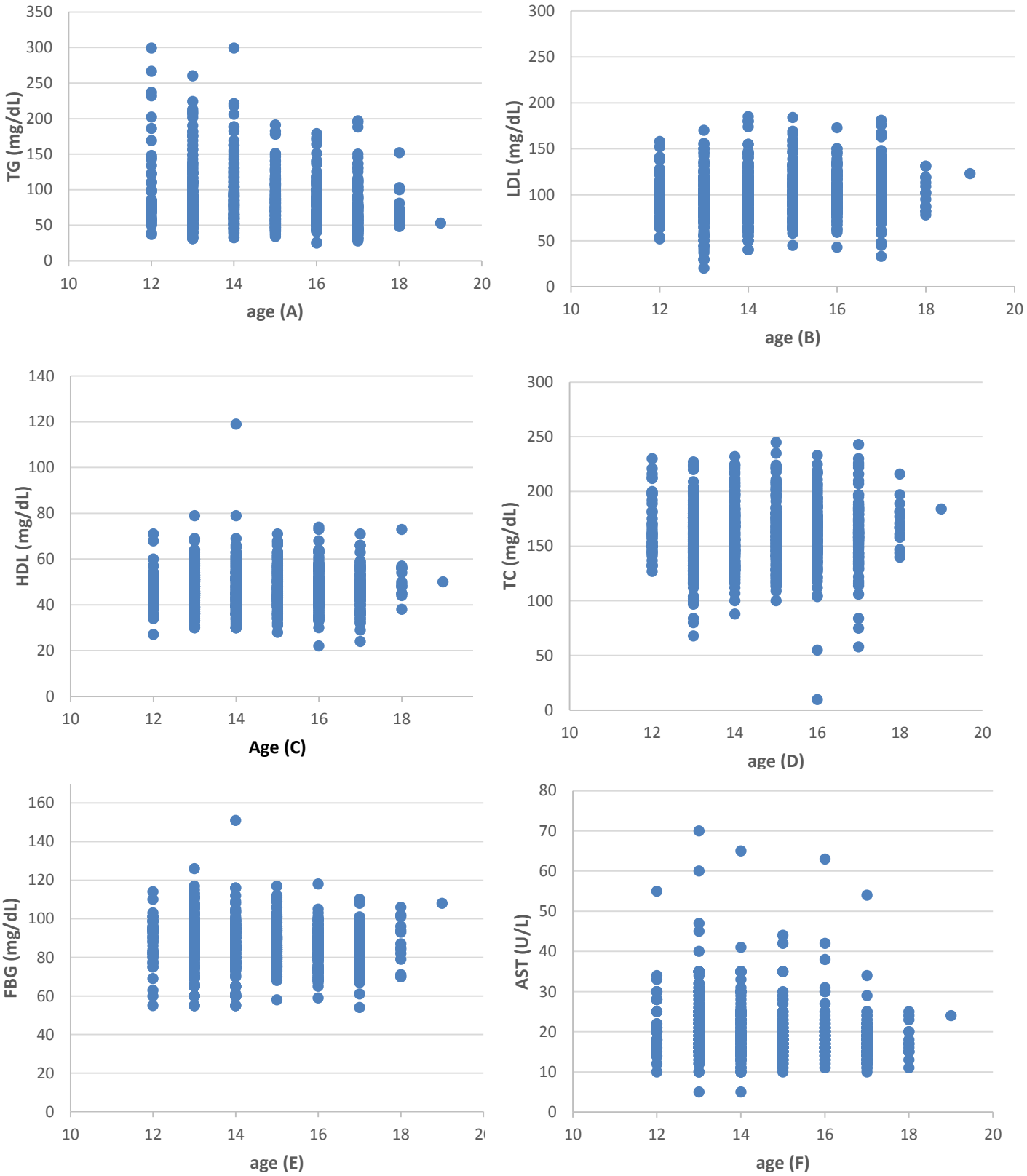
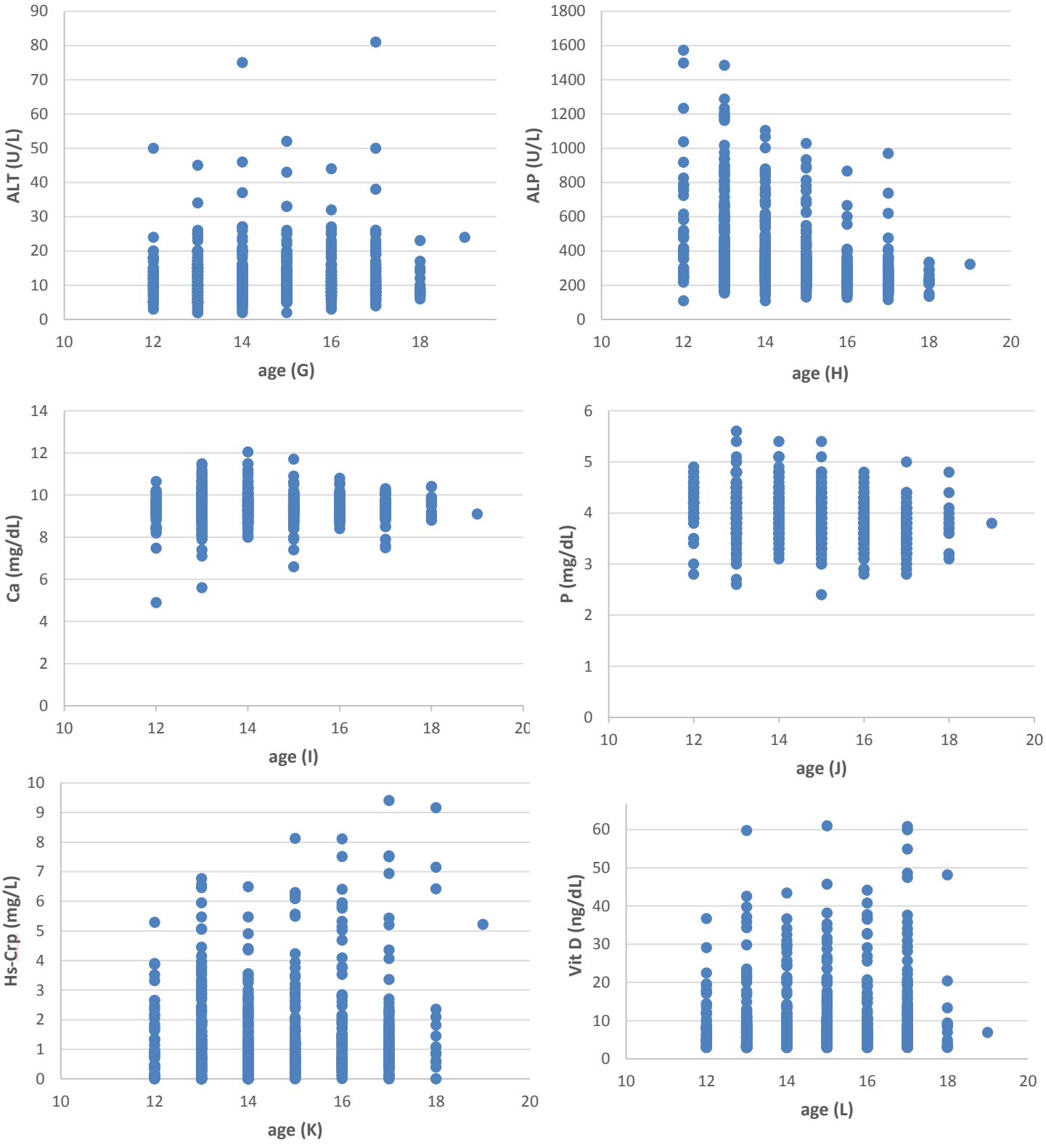
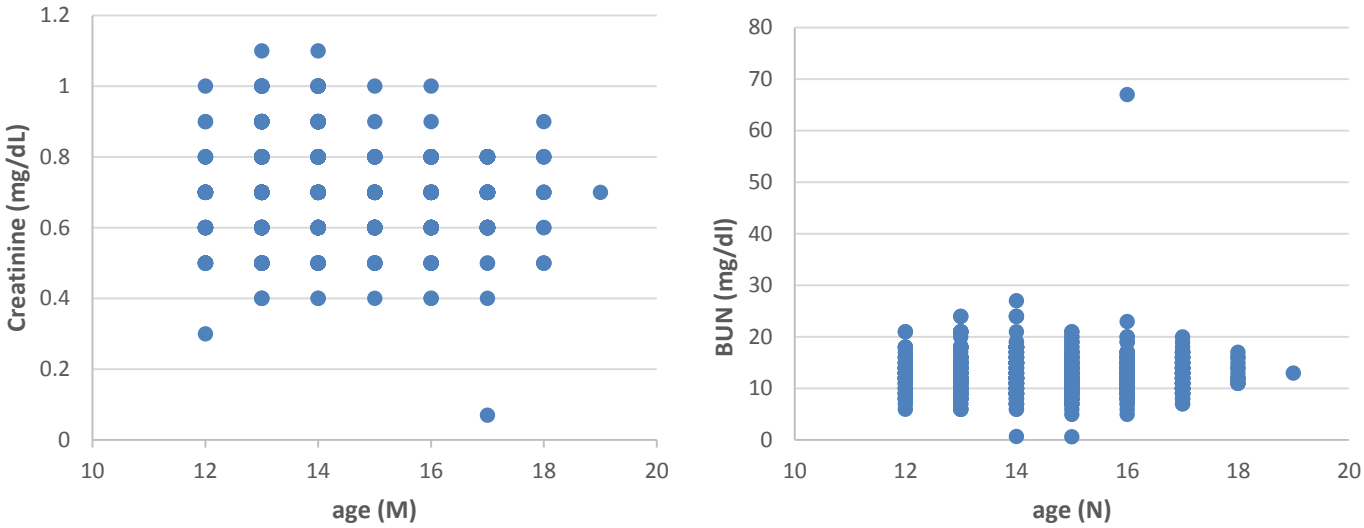


Figure 1. Scatterplot distribution for serum total cholesterol (mg/dL), triglyceride (mg/dL), low-density lipoprotein (mg/dL), high-density lipoprotein (mg/dL), fasting blood sugar (mg/dL), AST (U/L), ALT (U/L), ALP (U/L), Hs-CRP (mg/L), calcium (mg/dL), phosphate (mg/dL), vitamin D (ng/dL), creatinine (mg/dL), blood urine nitrogen (mg/dL) (A-N). Blue horizontal lines depict adolescent girls-specific lower and upper reference limits.



Continue Figure 1. Scatterplot distribution for serum total cholesterol (mg/dL), triglyceride (mg/dL), low-density lipoprotein (mg/dL), high-density lipoprotein (mg/dL), fasting blood sugar (mg/dL), AST (U/L), ALT (U/L), ALP (U/L), Hs-CRP (mg/L), calcium (mg/dL), phosphate (mg/dL), vitamin D (ng/dL), creatinine (mg/dL), blood urine nitrogen (mg/dL) (A-N). Blue horizontal lines depict adolescent girls-specific lower and upper reference limits.



Continue Figure 1. Scatterplot distribution for serum total cholesterol (mg/dL), triglyceride (mg/dL), low-density lipoprotein (mg/dL), high-density lipoprotein (mg/dL), fasting blood sugar (mg/dL), AST (U/L), ALT (U/L), ALP (U/L), Hs-CRP (mg/L), calcium (mg/dL), phosphate (mg/dL), vitamin D (ng/dL), creatinine (mg/dL), blood urine nitrogen (mg/dL) (A-N). Blue horizontal lines depict adolescent girls-specific lower and upper reference limits.

Table1. Lower and upper limits of RI of some routine biochemical tests with 90% confidence interval

Analyte	Units	Sample size	Reference intervals			
			Lower limit	Upper limit	Lower 90% CI	Higher 90% CI
TC	mg/dL	492	118.3	219.6	114-120.6	216.3-223.3
TG	mg/dL	550	37	182	34.2-41	74.9-198.8
LDL	mg/dL	486	55	146	49.4-59.6	142-151
HDL	mg/dL	511	32	66	30-33	64-69
FBG	mg/dL	524	70	109.8	69-71	108.7-111.8
hs-CRP	mg/L	445	0.1	4.4	0-0.1	3.5-4.9
AST	U/L	551	12	35	11.2-12	35-36.8
ALT	U/L	486	6	25	6-6	24-26
ALP	U/L	519	180	918	174-188	770-973
Creatinine	mg/dL	582	0.5	1	0.5-0.5	1-1.1
BUN	mg/dL	431	11	21	11-11	21-22
Ca	mg/dL	515	8.4	10.5	8.2-8.4	10.3-10.6
Phosphate	mg/dL	471	3.5	5	3.5-3.5	4.9-5.2
Vitamin D	mg/dL	718	3	14.2	3-3	13.8-15

Abbreviation: TC: total cholesterol, **TG:** triglyceride, **FBG:** fasting blood sugar, **HDL-C:** high density lipoprotein cholesterol, **LDL-C:** low density lipoprotein cholesterol, **Cr:** creatinine, **BUN:** blood urine nitrogen, **Ca:** calcium, **P:** phosphorous, **AST:** aspartate transaminase, **ALT:** alanine transaminase, **ALP:** alkaline phosphatase, **hs-CRP:** high sensitive C-reactive protein and **CI:** confidence interval.

Based on the statistical methodology of the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) (22) and CLSI Ep28-A3 guidelines (6), we calculated RIs of all biochemical markers

The large sample size was collected for calculation of limited confidence intervals and detection of accurate biochemical changes ages in these subjects. Under examination of scatterplots for the electrolytes such as, calcium and potassium, limited intervals with minimal changes were observed. This concept supports that the feedback mechanisms of electrolytes, hepatic markers, and metabolic elements possibly remain stable throughout life. In line with this, calculation of reference intervals shows no age partitioning for all biochemical markers. In contrast with the current findings, in the most recent studies have proved that several age and/or sex partitions reference intervals for biochemical analytes are closely correlated with high volume of sample size and sporadic age range. As shown in Table 2, the affecting factors on RIs including volume of sample size, wide age gap, type of reagent and measurement device led to recording different results between our study and other studies.

RIs for macro-minerals and vitamin D:

In the current study, RIs for macro-minerals including calcium and phosphate which both had no age partition and relatively stable serum levels in the age gap were calculated. In contrast with our results, some of recent studies have showed dissimilar results of age- and sex-specific RIs for these markers in children aged 0-20 years.

RIs have been previously determined for calcium in Canada (12) which are presented in Table 2. Partitioning based on age was required for calcium in Canadian children and adolescent girl participants while it was not required for age range of our study participants. Regarding the determining age partitioning for calcium in Canadian population, an increased lower limit RI was reported between age partitions aged 0 to <1 and 1 to 19 years whereas serum calcium concentration remained relatively constant throughout the age range.

RIs were also determined for phosphate in children and adolescent from India (23) and Croatia (24) which are reported in Table 2. Age partition was required for phosphate in Croatian and Indian participants whereas it was not required for this element in our adolescent girl population. The upper and lower limits of phosphate decreased and increased during increasing age, respectively, while serum phosphate concentration increased with increasing percentiles of phosphate from P3th to P97.5th throughout the age range.

RIs of vitamin D with 6 age partition was determined in Canadian children population. Results of this study demonstrated that 2 age partition with different concentrations was required for this marker between subjects aged 9- <14 and 14- <19 years which is presented in Table 2 (25). In contrast with findings, our results showed that no age partition with relatively constant for its concentration was determined for this element.

RIs for RFTs:

In this study, RIs for RFTs (i.e., creatinine and BUN) had no age partition and their concentrations remained relatively fixed with increasing age in participants of 12 to 19 years.

RIs were previously calculated for creatinine in adolescent participants from Denmark (26) and Canada (12) which are presented in Table 2. Partitioning based on age was required for creatinine in both Denmark and Canadian female participants while it was not necessary for this marker in our study. The upper and lower limits of creatinine increased continually from 15 days to < 19 years of age in Canadian children and adolescent population while value percentiles of creatinine with 90% confidence intervals (CI) increased with increasing P97.5th percentile in Denmark adolescent girl subjects at the age of 13 to 17 years. Linear range for serum creatinine concentration, showing that upper limit increased from 0.40 to 0.95 and decreased from 0.95 to 0.88 in Denmark adolescent girls aged 13- 15 and 15- 17 years old, respectively, while its concentration remained relatively stable in our study.

RIs were also examined for BUN in healthy adolescent and adult participants from Kenya (27) which are reported in Table 2.

Table 2. Comparison of reference intervals of the current study population with reference intervals of other countries.

Analyte	Reference intervals – CLSI (P2.5th -P97.5th percentile)– CI 90% (lower and upper limit)								
TC (mg/dL)	**Canada (29)**			**Brazil (38)**			**Current study**		
	Subject (G)	AR	RR	Subject (G)	AR	RR	Subject (G)	AR	RR
	Children and adolescent	3 – 5	120 – 216	Children and adolescent	9 – 12	103– 212	Adolescent	12 – 19	118.3 – 219.6
		6 – 15	116 – 205						
		16– 19	100 – 182						
TG (mg/dL)	Children and adult	6 – 29	35 – 186		6 – 12	17 – 134		12 – 19	37– 182
LDL (mg/dL)	Children and adult	6 – 24	46 – 143		9 – 12	45–140		12 – 19	55– 146
HDL (mg/dL)	Children and adolescent	6 – 14	35 – 81		4 – 12	27 – 69		12 – 19	32– 66
SS	>500			1,866			1,026		
RT	N.M			N.M			Pars Azmoun		
MD	Ortho VITROS 5600 Fs analyzer			Cobas 6000 analyzer			BT-3000		
FBG (mg/dL)	**Canada (29)**			**Ethiopia (28)**			**Current study**		
	Subject (G)	AR	RR	Subject (G)	AR	RR	Subject (G)	AR	RR
	Children and adolescent	12 –19	75 – 93	Adolescent	12 – 17	65.33 – 111.35	Adolescent	12 – 19	70.00– 109.50
ALT (U/L)	Children and adolescent	AR	RR	Adolescent	AR	RR	Adolescent	AR	RR
		9–11	18 – 39		12 – 17	5.10 – 20.00		12 – 19	6 – 25
ALP (U/L)	Subject (G)	AR	RR		AR	RR		AR	RR
	Adolescent and adult	11 – 15	64 – 359		12 – 17	63.56 – 253.34		12 – 19	180 – 918
		16 – 29	44 – 107						
SS	>500			172			1,026		
RT	N.M			N.M			Pars Azmoun		
MD	Ortho VITROS 5600 Fs analyzer			Biosystem 25 A			BT-3000		
AST (U/L)	**China (27)**			**Ethiopia (28)**			**Current study**		
	Subject (G)	AR	RR	Subject (G)	AR	RR	Subject (G)	AR	RR
	Adolescent	10~<18	13.50– 28.8	Adolescent	12 – 17	13.30– 28.50	Adolescent	12 – 19	12 – 35
SS	1,613			172			1,026		
RT	N.M			N.M			Pars Azmoun		
MD	Ortho VITROS 5600			Biosystem 25 A			BT-3000		

Abbreviation: RR: reference range, **G:** girl, **B:** boy, **AR:** age range, **SS:** sample size, **RT:** reagent type, **MD:** measurement device, **N.M:** not mentioned.

Continue Table 2. Comparison of reference intervals of the current study population with reference intervals of other countries.

Analyte	Reference intervals – CLSI (P2.5th -P97.5th percentile)– CI 90% (lower and upper limit)								
hs-CRP (mg/L)	**Austria (33)**			**Canada (12)**			**Current study**		
	Subject (G)	AR	RR	Subject (G)	AR	RR	Subject (G)	AR	RR
	Adolescent	14 – 17	0.06 – 6.79	Children and adolescent	15 days - <15	0.1 – 1.0	Adolescent AR: 12 – 19	12- 19	0.10 – 4.4
					15 - <19	0.1 – 1.7			
SS	39			849			1,026		
RT	Roche			N.M			Pars Azmoun		
MD	Cobas c311			Abbott Architect c8000 analyzer			BT-3000		
Creatinine (mg/dL)	**Denmark (25)**			**Canada (12)**			**Current study**		
	Subject (G)	AR	RR	Subject (G)	AR	RR	Subject (G)	AR	RR
	15	0.0 – 0.95	0.0 – 0.40	Children and adolescent	5 - 12	0.31 – 0.61	Adolescent	12 – 19	0.5 – 1.0
					12 – 15	0.45 – 0.81			
		17	0.0 – 0.88		15 - <19	0.49 – 0.84			
SS	959			665			1,026		
RT	N.M			N.M			Pars Azmoun		
MD	Cobas c501			Abbott Architect c8000 analyzer			BT-3000		
BUN (mg/dL)	**Kenya (26)**			**Current study**					
	Subject (G)	AR	RR	Subject (G)	AR		RR		
	Adolescent and adult	13 – 17	1.2 – 4.8	Adolescent	12 – 19		11 – 21		
		18 – 34	1.8 – 5.3						
SS	140			1,026					
RT	N.M			Pars Azmoun					
MD	Cobas Integra 400 plus biochemistry analyzer			BT-3000					
Calcium (mg/dL)	**Canada (12)**			**Current study**					
	Subject (G)	AR	RR	Subject (G)	AR		RR		
	Children and adolescent	0 - <1	8.5 – 11.0	Adolescent	12 – 19		8.4 – 10.5		
		1 – 19	9.2 – 10.5						
SS	1,156			1,026					
RT	N.M			Pars Azmoun					
MD	Abbott Architect c8000 analyzer			BT-3000					

Abbreviation: RR: reference range, **G:** girl, **B:** boy, **AR:** age range, **SS:** sample size, **RT:** reagent type, **MD:** measurement device, **N.M:** not mentioned.

Continue Table 2. Comparison of reference intervals of the current study population with reference intervals of other countries.

Analyte	Reference intervals – CLSI (P2.5th -P97.5th percentile)– CI 90% (lower and upper limit)								
Phosphate (mg/dL)	India (22)			Croatia (23)			Current study		
	Subject (G)	AR	RR	Subject (G and B)	AR	RR	Subject (G)	AR	RR
	Children and adolescent	6 – 17	1.24 – 1.55	Children and adolescent	8 -13	1.11 – 1.73	Adolescent	12 – 19	3.5 – 5
					14 - 15	1.07 – 1.64			
					16 – 18	0.83 – 1.42			
SS	1,620			998			1,026		
RT	N.M			Boehringer Mannheim GmbH			Pars Azmoun		
MD	Hitachi 902			Molybdenum blue			BT-3000		
Vitamin D (ng/dL)	Canada (24)			Current study					
	Subject (G)	AR	RR	Subject (G)	AR		RR		
	Children and adolescent	9 - <14	12.68 – 46.52	Adolescent	12 – 19		3 – 14.		
		14 - <19	4.8 – 42.32						
SS	764			1,026					
RT	N.M			Pars Azmoun					
MD	Abbott Architect i2000 system			BT-3000					

Abbreviation: RR: reference range, **G:** girl, **B:** boy, **AR:** age range, **SS:** sample size, **RT:** reagent type, **MD:** measurement device, **N.M:** not mentioned.

Partitioning based on age required for ALT with 5 age partitions in Canadian childhood and adulthood participants aged 3-79 years old while age partition was not necessary both for adolescent female in Ethiopia and our study. Adeli et al. also reported that female upper limits remain relatively stable throughout the age range. Value female percentiles of ALT increased with increasing percentile from 25th - 75th to 2.5th – 97.5th percentiles in Ethiopian adolescent girl participants. Serum levels of enzymatic activity of ALT remained comparatively fixed for the adolescent girl population constant in Ethiopia, Canada, and our study.

RIs were determined for ALP in adolescent female population from Canada (30) and Ethiopia (29) which are reported in Table 2. 5 Age partition required for ALP in wide age range of 3-79 years of age years old while it was not required for this marker in Ethiopian and our adolescent girls. The lower and upper limits of ALP decreased continually in the Canadian female participants above the age of 11 years while its concentration remained relatively unchanged in adolescent female individuals from Ethiopia and in our study.

Hs-CRP is an acute phase protein of pentraxin family which is produced by liver in response to both releasing interleukine-1β (IL-1β) and IL-6 induced by inflammatory signals, as well as overweight and obesity, respectively (31, 32). Since the value of serum CRP concentration averagely is < 1 mg/L in healthy subjects, it is necessary to determine specific reference range for any sex and age group to elevate management and control of inflammatory disease in these subjects (33). RIs of hs-CRP were determined both in adolescent girl population aged 14-17 years in Austria (34) as well as Canadian children and adolescent girl individuals 0- < 19 years of age (12). 2 age partitions required for RIs of adolescent subjects from Canada while no age partition required both for Austrian and our adolescent girl population. The upper limit of hs-CRP increased in children and adolescent girl participant from Canada while value CRP percentiles with 90% CI enhanced during increasing percentile from 2.5th to

97.5th in Austrian adolescent girl subjects. Furthermore, Serum hs-CRP concentration ranged from 0.06 to 6.79 in Austrian adolescent girls of 14-17 years of age.

RIs for metabolic markers:

In the current study, concentrations of metabolic markers including TC, TG, LDL, HDL, and FBG remained relatively constant during advancing age. The different results of several studies concerning RI values of metabolic tests have showed that there are influenced various elements on these RIs markers including board age range (35), sex (36), consumable kit type (36, 37), analyzer machine type (38). In accordance with these findings, it has been demonstrated that concentrations of metabolic markers significantly increase in adulthood versus childhood (30). Findings of a study about sex as another main factor influencing on RI values of metabolic factors showed that serum levels of TG increase in male compared with females between 30 to 79 years of age, while concentration of HDL increase during increasing age in women in comparison with men (30).

RIs for FBG were calculated both in Canadian childhood and adulthood of 3-79 years of age (30) and healthy female adolescent aged 12-17 years in Ethiopia (29). 5 age partition are required for FBG in subjects of 3-79 years of age while no age partition was applied both in Ethiopian adolescent female and our study. In Adeli and colleague's study has been shown that lower limit of FBG remained comparatively constant during the age gap but its upper limit increased at the age of 3-19 years in Canadian female subjects. In Ethiopian subject and our study, serum FBG concentration remained relatively unchanged.

RIs for lipid profiles including TG, TC, LDL, HDL were also determined both in Canadian childhood and adulthood of 3-79 years of age (30) and Brazilian children and adolescent participants aged 1-12 years (39). 2 and 3 age partition were required for TG in Canadian and Brazilian participants while it was not essential in our study. The upper limit of TG peaked for Canadian female participants after 30 years of age while it's both lower limit and upper decreased after 1 years of age in children and adolescent subjects in Brazil. For RIs of TC required 6 and 3 age partition in Canadian and Brazilian subjects, respectively. The upper limit and lower limit of TC decreased and increased in younger participants aged 3-20 years and Brazilian subjects aged 1-12 years old. Partitioning based on age was also required for LDL both in Brazilian children and adolescent aged 1-12 years and Canadian subjects while it was not required in our study. Serum LDL concentration remained relatively unchanged in Canadian younger participants aged 6-24 years, Brazilian subjects, and our study. 3 and 4 age partition were required for HDL in Canadian and Brazilian participants, respectively. The lower and upper of TC increased both in Canadian female subjects and Brazilian participants while serum HDL concentration remained relatively constant in our study.

Strengths and limitations

This paper is the first study that assessed the age-specific RIs for biochemical tests specifically BUN concentration with a large sample size. Although, we only considered girls aged 12 – 19 years old; therefore, lack of information of other ages and also boys in children and adolescents in this study makes the comparison limited. However, we gathered the population sample only from north-eastern of Iran, so generalizing the results to all Iranians is needed to more studies. Established RI by using the non-parametric rank method is another advantage of study that it is robust against outliers in calculating RIs.

Conclusion

We determined the age-specific reference intervals for some biochemical tests by using the direct method. In this study, our results accompanied with 1 board age partition for all biochemical markers. Concentration of all biochemical tests were relatively stable by increasing age across the adolescent girl age range.

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Conflict of interest

The authors have no conflict of interest to disclose .

Ethical approval

This study was approved by the Ethics Committee of Mashhad University of Medical Sciences (Mums), Mashhad, Iran.

References

1. Bohn MK, Lippi G, Horvath A, Sethi S, Koch D, Ferrari M, et al. Molecular, serological, and biochemical diagnosis and monitoring of COVID-19: IFCC taskforce evaluation of the latest evidence. Clinical Chemistry and Laboratory Medicine (CCLM). 2020;58(7):1037-52.
2. Kornacki J, Wender-Ożegowska E. Utility of biochemical tests in prediction, diagnostics and clinical management of preeclampsia: a review. Archives of Medical Science. 2020 Aug 3;16(1).
3. Jung B, Adeli K. Clinical laboratory reference intervals in pediatrics: the CALIPER initiative. Clinical biochemistry. 2009 Nov 1;42(16-17):1589-95.
4. Karbasy K, Ariadne P, Gaglione S, Nieuwesteeg M, Adeli K. Advances in pediatric reference intervals for biochemical markers: Establishment of the CALIPER database in healthy children and adolescents. Journal of Medical Biochemistry. 2015 Jan;34(1):23.
5. Friedberg RC, Souers R, Wagar EA, Stankovic AK, Valenstein PN. College of American Pathologists The origin of reference intervals. Arch Pathol Lab Med. 2007;131:348-57.
6. Horowitz GL, Altaie S, Boyd JC. Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline. CLSI; 2010.
7. Tahmasebi H, Higgins V, Fung AW, Truong D, White-Al Habeeb NM, Adeli K. Pediatric reference intervals for biochemical markers: gaps and challenges, recent national initiatives and future perspectives. Ejifcc. 2017 Feb;28(1):43.
8. Liu J, Dai Y, Lee Y, Yuan E, Wang Q, Wang L, et al. Pediatric reference intervals of liver and renal function tests from birth to adolescence in Chinese children as performed on the Olympus AU5400. Clinica Chimica Acta. 2019 Mar 1;490:142-6.
9. Lai DS, Chen SC, Chang YH, Chen CY, Lin JB, Lin YJ, et al. Pediatric reference intervals for several biochemical analytes in school children in Central Taiwan. Journal of the Formosan Medical Association. 2009 Dec 1;108(12):957-63.
10. Li X, Wang D, Yang C, Zhou Q, Zhuoga SL, Wang LQ, et al. Establishment of age-and gender-specific pediatric reference intervals for liver function tests in healthy Han children. World Journal of Pediatrics. 2018 Apr;14(2):151-9..
11. Gomez P, Coca C, Vargas C, Acebillo J, Martinez A. Normal reference-intervals for 20 biochemical variables in healthy infants, children, and adolescents. Clinical chemistry. 1984 Mar 1;30(3):407-12.
12. Colantonio DA, Kyriakopoulou L, Chan MK, Daly CH, Brinc D, Venner AA, et al . Closing the gaps in pediatric laboratory reference intervals: a CALIPER database of 40 biochemical markers in a healthy and multiethnic population of children. Clinical chemistry. 2012 May 1;58(5):854-68.
13. Mu R, Chen W, Pan B, Wang L, Hao X, Huang X, et al. First definition of reference intervals of liver function tests in China: a large-population-based multi-center study about healthy adults. PloS one. 2013 Sep 13;8(9):e72916.
14. Muller F, Dommergues M, Bussieres L, Lortat-Jacob S, Loirat C, Oury JF, et al. Development of human renal function: reference intervals for 10 biochemical markers in fetal urine. Clinical chemistry. 1996 Nov 1;42(11):1855-60.
15. Rabiee B, Roozafzai F, Hemasi GR, Poustchi H, Keyvani H, Khonsari MR, et al. The prevalence of non-alcoholic fatty liver disease and diabetes mellitus in an Iranian population. Middle East journal of digestive diseases. 2017 Apr;9(2):86.
16. Shabani N, Khodabandeh K, Bohn K, Ghazizadeh H, Saberi-Karimian M, Emamian M, et al. Normative serum lipid profiles in the Iranian adult population. International Journal of Clinical Practice. 2020 Nov 11:e13829-.
17. Jafari-Giv Z, Avan A, Hamidi F, Tayefi M, Ghazizadeh H, Ghasemi F, et al. Association of body mass index with serum calcium and phosphate levels. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2019 Mar 1;13(2):975-80.
18. Tukey JW. Exploratory Data Analysis. Mass: Addison-Wesley Publishing Company. Reading. 1977;6:131-60.
19. Tukey JW. Exploratory data analysis addision-wesley. Reading, Ma. 1977;688:581-2.
20. Hubert M, Van der Veeken S. Outlier detection for skewed data. Journal of Chemometrics: A Journal of the Chemometrics Society. 2008 Mar;22(3-4):235-46.
21. Ozarda Y, Sikaris K, Streichert T, Macri J, IFCC Committee on Reference intervals and Decision Limits (C-RIDL). Distinguishing reference intervals and clinical decision limits–A review by the IFCC Committee on Reference Intervals and Decision Limits. Critical reviews in clinical laboratory sciences. 2018 Aug 18;55(6):420-31.

22. Jia K, Zhang C, Huang X, Wang L, Hao X, Mu R, et al. Reference intervals of serum sodium, potassium, and chlorine in Chinese Han population and comparison of two ISE methods. Journal of Clinical Laboratory Analysis. 2015 May;29(3):226-34.
23. Marwaha RK, Khadgawat R, Tandon N, Kanwar R, Narang A, Sastry A, et al. Reference intervals of serum calcium, ionized calcium, phosphate and alkaline phosphatase in healthy Indian school children and adolescents. Clinical biochemistry. 2010 Oct 1;43(15):1216-9.
24. Jagarinec N, Flegar-Meštrić Z, Šurina B, Vrhovski-Hebrang D, Preden-Kereković V. Pediatric reference intervals for 34 biochemical analytes in urban school children and adolescents. 1998.
25. Bailey D, Colantonio D, Kyriakopoulou L, Cohen AH, Chan MK, Armbruster D, et al. Marked biological variance in endocrine and biochemical markers in childhood: establishment of pediatric reference intervals using healthy community children from the CALIPER cohort. Clinical chemistry. 2013 Sep 1;59(9):1393-405.
26. Søeby K, Jensen PB, Werge T, Sørensen S. Mining of hospital laboratory information systems: a model study defining age-and gender-specific reference intervals and trajectories for plasma creatinine in a pediatric population. Clinical Chemistry and Laboratory Medicine (CCLM). 2015;53(10):1621-30.
27. Zeh C, Amornkul PN, Inzaule S, Ondoa P, Oyaro B, Mwaengo DM, Vandenhoudt H, Gichangi A, Williamson J, Thomas T, DeCock KM. Population-based biochemistry, immunologic and hematological reference values for adolescents and young adults in a rural population in Western Kenya. PLOS one. 2011 Jun 21;6(6):e21040.
28. Guo W, Zhou Q, Jia Y, Xu J. Division of Myocardial Enzyme Reference Intervals in Population Aged 1 to. Computational and Mathematical Methods in Medicine.;2020.
29. Belay G, Teklehaymanot G, Gebremariam G, Kaleaye K, Haileslasie H, Gebremichail G, et al. Community based reference interval of selected clinical chemistry parameters among apparently healthy Adolescents in Mekelle City, Tigrai, Northern Ethiopia. PloS one. 2020 Apr 7;15(4):e0231017.
30. Adeli K, Higgins V, Nieuwesteeg M, Raizman JE, Chen Y, Wong SL, et al. Biochemical marker reference values across pediatric, adult, and geriatric ages: establishment of robust pediatric and adult reference intervals on the basis of the Canadian Health Measures Survey. Clinical chemistry. 2015 Aug 1;61(8):1049-62.
31. Marnell L, Mold C, Du Clos TW. C-reactive protein: ligands, receptors and role in inflammation. Clinical immunology. 2005 Nov 1;117(2):104-11.
32. Ebrahimi M, Heidari-Bakavoli AR, Shoeibi S, Mirhafez SR, Moohebati M, Esmaily H, et al. Association of serum hs-CRP levels with the presence of obesity, diabetes mellitus, and other cardiovascular risk factors. Journal of clinical laboratory analysis. 2016 Sep;30(5):672-6.
33. Shine B, De Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. Clinica chimica acta. 1981 Nov 25;117(1):13-23.
34. Bogner B, Schwenoha K, Vogl M, Weghuber D, Roth C, Kipman U,et al. Evaluation of reference intervals of haematological and biochemical markers in an Austrian adolescent study cohort. Clinical Chemistry and Laboratory Medicine (CCLM). 2019 Jun 1;57(6):891-900.
35. Alves CX, Brito NJ, Vermeulen KM, Lopes MM, França MC, Bruno SS, et al. Serum zinc reference intervals and its relationship with dietary, functional, and biochemical indicators in 6-to 9-year-old healthy children. Food & nutrition research. 2016 Jan 1;60(1):30157.
36. Lockitch G, Halstead AC, Albersheim S, MacCallum C, Quigley G. Age-and sex-specific pediatric reference intervals for biochemistry analytes as measured with the Ektachem-700 analyzer. Clinical chemistry. 1988 Aug 1;34(8):1622-5.
37. Christopher MM, Berry KH, Wallis IR, Nagy KA, Henen BT, Peterson CC. Reference intervals and physiologic alterations in hematologic and biochemical values of free-ranging desert tortoises in the Mojave Desert. Journal of wildlife diseases. 1999 Apr;35(2):212-38.
38. Šimpraga M, Šmuc T, Matanović K, Radin L, Shek-Vugrovečki A, Ljubičić I, et al. Reference intervals for organically raised sheep: Effects of breed, location and season on hematological and biochemical parameters. Small ruminant research. 2013 May 1;112(1-3):1-6.
39. Slhessarenko N, Jacob C, Azevedo RS, Fontes CJ, Novak GV, Andriolo A. Serum lipids in Brazilian children and adolescents: determining their reference intervals. BMC Public Health. 2015 Dec;15(1):1-8.