Original Article

Pediatric Reference Values for Serum Creatinine and Estimated Glomerular Filtration Rate in Iranians: Tehran Lipid and Glucose Study

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Abstract

Background: Serum creatinine is the most widely used marker for estimating glomerular filtration rate (GFR). The aim of this study was to determine pediatric reference values for serum creatinine levels and eGFR values using data from a population-based study in Iran.

Methods: Serum creatinine of 1594 subjects, aged 3 – 18 years, participating in phase 4 of the Tehran Lipid and Glucose Study (2008 – 2011) was measured using the conventional Jaffe method. The non-parametric method of Schwartz and Counahan-Barratt equations were used to calculate eGFR. CLSI/IFCC guidelines were used to determine reference values.

Results: In both genders, serum creatinine concentration was significantly increased with age and had a positive correlation with age (boys (r = 0.786, n = 778, $P < 0.001$) and girls (r = 0.638, n = 724, $P < 0.001$)). In addition, mean serum creatinine concentration was significantly higher in boys, compared to girls (0.86 ± 0.01 vs. 0.80 ± 0.01 mg/dL, $P < 0.001$). Based on these results, we proposed the following formula: serum creatinine (mg/dL) = k × age (year) + 0.5, where k was 0.03 for boys and 0.02 for girls.

Conclusions: This study presents pediatric reference values in Iranian boys and girls for serum creatinine levels to be 0.6 – 1.20 mg/dL and 0.6 – 1.00 mg/dL and for eGFR values to be 81 – 154 mL/min/1.73 m^2 and 80 – 129 mL/min/1.73 m^2, respectively. These values can be used for diagnostic and therapeutic purposes.

Keywords: Child, Jaffe, reference values, serum creatinine

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Introduction

Chronic kidney disease (CKD) is a growing public health problem throughout the world.[1] An annual incidence of over 2% has been reported for CKD among the Iranian population.[2] In addition, the prevalence of pediatric CKD is higher in Iran, compared to Western countries.[3] Estimated glomerular filtration rate (eGFR) could be used for classification of CKD and adjustment in drug dosage.[4]

Serum creatinine is the most widely used marker for estimating GFR,[5] which is the best index of renal function.[6–8] Estimating GFR from serum creatinine has been emphasized by members of NKF CKD (National Kidney Foundation chronic kidney disease), and their recommended equations are Schwartz and Counahan-Barratt formulas.[9]

Compared to the Jaffe method for serum creatinine measurement, which has a low specificity and overestimates serum creatinine values,[10–13] enzymatic methods for creatinine measurement are more specific[10] and superior, especially in children[14,15] who generally have higher non-creatinine chromogens.[16] However, creatinine measurement with the Jaffe method is still widely used[15,17] mainly for economical reasons.[17]

Renal development could overlay any possible renal damages, which complicates the assessment of renal function in children.[5] Inadequate, pediatric reference values could lead to misdiagnosis and misclassification of disease.[18] Establishing pediatric reference values is a challenging task and very limited data are available.[14,18] Factors such as age, gender, dietary patterns, genetics, and ethnicity affect laboratory indices,[19] possibly due to variations in muscle mass. There is a difference in serum creatinine concentration between Caucasians, Chinese, Indians and Malays.[20] Using normal laboratory values of external populations could result in selection bias; therefore it is essential to develop region-specific reference values for efficient patient management and conducting quality clinical research. To the best of our knowledge, there is no report of pediatric reference values of serum creatinine and eGFR in Iran. Hence, the aim of this study was to determine pediatric age- and sex-specific reference values for serum creatinine levels and eGFR values using data from a population-based study in Iran.

Materials and Methods

Subjects

The Tehran Lipid and Glucose Study (TLGS) initiated in 1999 to determine the prevalence of non-communicable disease risk factors.[21] A multistage stratified cluster random sampling technique was used in this study to select 15,005 persons aged over 3 years, from Tehran.[22] In the current study, 1594 subjects, aged

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3 – 18 years, participants of phase 4 TLGS (June 2008 to September 2011), were included. Excluded were those with cancer and diarrhea or those using cigarette or any medications including steroids, diuretics, betablockers, digitals, calcium channel blockers, angiotensin converting enzyme inhibitors, aspirin and other anticoagulants, lipid lowering drugs, anti-diabetic drugs, male or female hormones, or drugs for thyroid disorders. Moreover, subjects with a history of hospitalization during the past 3 months and those with a history of significant weight loss during past 6 months were also excluded. After application of exclusion criteria, 1502 apparently healthy non-smoker participants (778 boys and 724 girls), aged 3 to 18 years, remained for analysis; none of the female subjects were pregnant or lactating. The ethics committee of the Research Institute for Endocrine Sciences approved the study; and written informed consent was obtained from both parents and adolescents, aged ≥ 15 years; informed assent was obtained from all participants < 15 years.

Anthropometric and clinical assessments

Details of data collection in the TLGS have been previously published;[22] in brief, weight and height were measured according to standard protocols. Body mass index (BMI) was calculated. Blood pressure was measured twice after 15 minutes of rest and the mean of two measurements was reported.

Creatinine measurement

Blood samples were obtained in a sitting position after 12 – 14 hours overnight fasting and centrifuged within 30 to 45 minutes of collection; all blood analyses were done in the TLGS research laboratory on the day of sample collection. Serum creatinine was measured using the photometric Jaffe method (Pars Azmoon Kit, Tehran, Iran) in which creatinine reacts with picrate in an alkaline medium to yield an orange-red color, read at 505 nm. In 47 samples, creatinine measurement was done with both the Jaffe and enzymatic p-aminophenazone (PAP) methods. Comparison between the Jaffe and PAP methods for measuring serum creatinine concentration showed a good correlation between the two methods, with a regression line of: Cr_{PAP}(mg/dL) = 1.046 × Cr_{Jaffe} (mg/dL) – 0.398 (r = 0.979, n = 47, P < 0.001). This equation was then used for calculating compensated creatinine values for the complete data (Figure 1A). Mean bias between the two methods was 0.35 ± 0.03 mg/dL (95% confidence interval: 0.29 – 0.42) (Figure 1B). Creatinine measurement with the Jaffe method has a low specificity and overestimates serum creatinine by approximately 20% – 30%, due to non-creatinine chromatogens, mainly proteins.[10-13] To overcome the problem, a constant value is subtracted from values obtained by the original Jaffe assay and results are considered compensated Jaffe values.

Intra-assay CVs were 1.7% and 4.4% for the Jaffe and PAP methods respectively (n = 8). Bland-Altman method comparison was used for comparing creatinine measurements by these two methods.

Serum glucose, total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using the enzymatic colorimetric method. For glucose measurement, glucose was oxidized to gluconic acid and H_2O_2 by glucose oxidase. For TC assay, cholesteryl ester was converted to cholesterol by cholesteryl ester hydrolase; and cholesterol was oxidized by cholesterol oxidase to cholesterol-4-en-3-one and H_2O_2. For TG measurement, glycerol was released from TG by lipoprotein lipase, followed by phosphorylation of glycerol to glycerol phosphate by glycerokinase; glycerol phosphate oxidase converted glycerol phosphate to dihydroxyaceton phosphate and H_2O_2. Measurement of HDL-C was done after precipitation of the apolipoprotein B containing lipoproteins with phosphotungstic acid and magnesium ions. In all the above-mentioned assays, the colorimetric indicator is quinoneimine, which is generated from 4-aminoantipyrine and phenol by H_2O_2 and measured at 546 nm. Low-density lipoprotein cholesterol (LDL-C) concentrations in samples with TG < 400 mg/dL (4.52 mmol/L) were calculated with the Friedewald equation:[23] LDL-C = TC – HDL-C – TG/5. The analyses were performed using commercial kits (Pars Azmoon Inc., Tehran, Iran) and a Selectra Pro M auto-analyzer (Vital Scientific, Spankeren, Netherlands). Intra- and inter-assay coefficients of variation (CV) were both < 2.0% for glucose, 1.8% for TC and TG, as well as 2.9% for HDL-C.

Calculating eGFR

Equations of Schwartz

$$[eGFR\ (mL/min/1.73\ m^2) = \frac{K \times Height\ (cm)}{Serum\ creatinine\ (mg/dL)}]$$

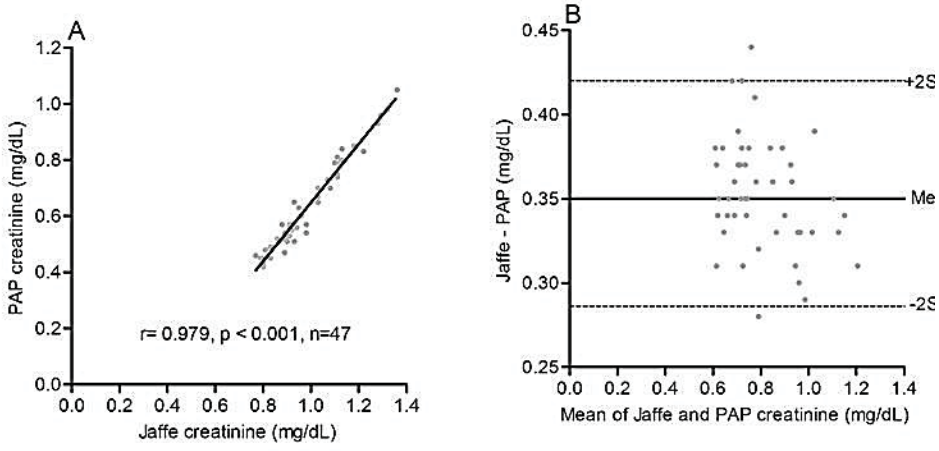

Figure 1. A) Linear regression analysis of serum creatinine measured by the conventional Jaffe method, compared to the PAP enzymatic method. The analysis yielded a regression line equation of: Cr_{PAP} = 1.046 × Cr_{Jaffe}–0.398 mg/dL (r = 0.979, n = 47, P <0.001); **B)** Bland and Altman analysis of serum creatinine comparison data; statistically significant bias was found with a mean difference of 0.35 ± 0.03mg/dL (95% limits of agreement: 0.29–0.42 mg/dL).

and Counahan-Barratt

$$[eGFR\ (mL/min/1.73\ m^2) = \frac{0.43\times Height\ (cm)}{Serum\ creatinine\ (mg/dL)}]$$

have been used for calculating eGFR.[24,25] In the Schwartz formula, the considered *k* values were 0.55 for girls and boys < 12 years and 0.7 for boys ≥ 12 years.[25]

Outliers' determination

The Dixon outlier range statistic was used to determine outliers, as recommended by Clinical and Laboratory Standards Institute (CLSI) for reference values determined by the nonparametric procedure.[26] In the Dixon test, if the ratio D/R exceeds 1/3, the extreme value is considered as an outlier and should be deleted, where D is the absolute difference between the most extreme value and the next most extreme value and R is the range of the values.

Determination of serum creatinine and eGFR reference values

For determining reference values, the CLSI/IFCC guidelines, and non-parametric method were used.[27,28] The retrospective (posteriori) selection of individuals from a population-based study was used as it is considered ideal for the study of exclusion and partitioning criteria according to IFCC.[29] For the IFCC non-parametric method, which is recommended for determining reference values,[30] values were sorted in ascending order and rank numbers were assigned to values. Rank numbers of the 0.025 and 0.975 fractiles were computed as 0.025 × (N + 1) and 0.975 × (N + 1) respectively and considered as reference values.

Statistical analysis

For comparing baseline variables between boys and girls, the independent sample *t*-test was used. The pearson correlation coefficient was used for calculating the correlation between age and serum creatinine. Differences between serum creatinine concentrations in different age groups were compared by one-way analysis of variance. The Tukey post-hoc was used for multiple comparisons. Two-sided *P*-values < 0.05 were considered statistically significant. To obtain a simple equation for estimating serum creatinine values, linear regression analysis was used with age and BMI as independent and serum creatinine as dependent variables. Due to previous reports on the significant associations between these variables and serum creatinine, age and BMI were included.[5,31,32] Pearson correlation coefficient was used to calculate the obtained equation in data of children with normal creatinine values in phase 5 TLGS. The software SPSS (SPSS Inc., Chicago, IL, USA; Version 15) was used for all statistical analyses except for the Bland-Altman method comparison, for which GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA) was used.

Results

This study was conducted on 1502 healthy children and adolescents (778 boys and 724 girls), age range 3 to 18 years. As shown in Table 1, boys had higher values of systolic blood pressure, diastolic blood pressure, and fasting serum glucose, whereas other parameters were comparable between genders.

In both genders, serum creatinine concentrations were significantly increased with age and there were positive correlation between age and serum creatinine in both boys (r = 0.786, n = 778, *P* < 0.001) and girls (r = 0.638, n = 724, *P* < 0.001). In addition, the mean serum creatinine concentration was significantly higher in boys, compared to girls (0.86 ± 0.01 vs. 0.80 ± 0.01 mg/dL, *P* < 0.001). Based on our results, we propose the following formula: serum creatinine (mg/dL) = k × age (year) + 0.5, where k was 0.03 for boys and 0.02 for girls. Including BMI in the equation, following formula has been provided: serum creatinine (mg/dL) = [k × age (year)] + [0.001 × BMI] + 0.5, where k was 0.03 for boys and 0.02 for girls. However, due to the low coefficient corresponding to BMI, it was removed from the final proposed equation. Pearson correlation coefficient between measured and estimated creatinine in children with normal creatinin levels of phase 5 TLGS were 0.76 and 0.64 in boys and girls, respectively. The difference between measured and estimated values was 4.7% (1.1% in boys and 8.5% in girls). Regarding age groups, serum creatinine concentrations in boys were significantly higher in the 9 – 12, 12 – 15, and 15 – 18 year age groups than corresponding age groups in girls (Figure 2).

Reference values for serum creatinine according to age and sex, are presented in Tables 2 and 3 for the conventional and compensated Jaffe methods respectively. In both genders, upper limits of reference values were increased with age. Overall, 95% reference values for serum creatinine concentrations, using the conventional Jaffe method, ranged between 0.60 – 1.20 mg/dL and 0.60 – 1.00 mg/dL in boys and girls respectively. Because reference values of serum creatinine in the 3 – 6 and 6 – 9 year age groups were similar in boys and girls, we determined reference values for both boys and girls < 10 years, which ranged between 0.50 to 0.90 mg/dL, according to the conventional Jaffe method. Reference values for boys and girls ≥ 10 years, according to conventional Jaffe

Table 1. Characteristics of study subjects[a]

	Boys, (n = 778)	Girls, (n = 724)	*P*-value[b]
Age (years)	12.1 ± 3.7	12.3 ± 3.7	0.386
Body mass index, kg/m^2	20.2 ± 4.9	20.6 ± 6.7	0.177
Systolic blood pressure, mm Hg	102 ± 13	98 ± 12	< 0.001
Diastolic blood pressure, mm Hg	66.0 ± 11.3	64.6 ± 11.1	0.027
Total cholesterol, mg/dL	155.9 ± 29.3	157.2 ± 27.6	0.345
Triglycerides, mg/dL	80.2 (77.8–82.8)	82.5 (90.0-85.1)	0.204
HDL-C, mg/dL	51.3 ±11.4	51.2 ± 11.1	0.778
LDL-C, mg/dL	86.7 ± 25.5	87.9 ± 24.2	0.365
Fasting serum glucose, mg/dL	93.2 ± 7.1	90.8 ± 7.2	< 0.001

a: Values are mean (SD), except for triglycerides, for which because of skewed distribution, the geometric mean (95% confidence interval) is presented; b: By independent t-test; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

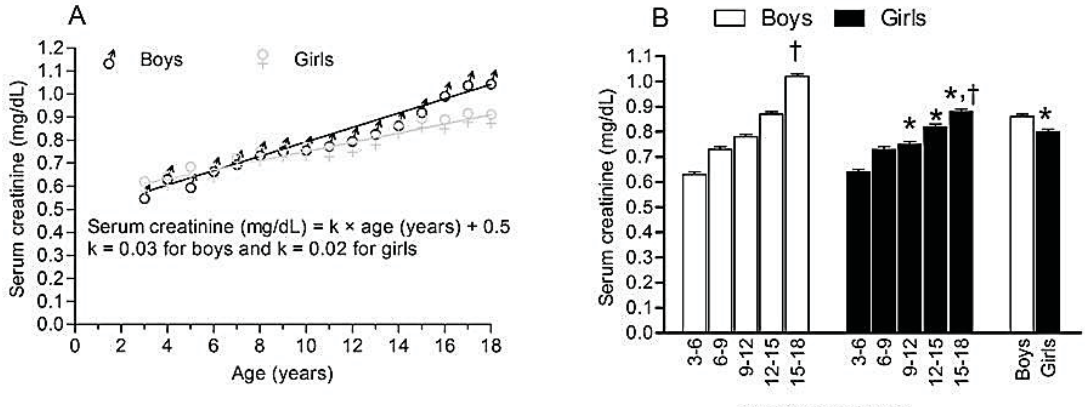

Figure 2. A) Correlation between serum creatinine values and age; **B)** Comparison of serum creatinine concentration according to sex and age. In both genders, serum creatinine concentrations increased significantly with age (†, *P* for trend < 0.001) and in both genders, there was significant (*P* < 0.001) difference between all age groups except for the two, the 6–9 and the 9–12 year age groups in girls. *: Significant difference compared to boys.

Table 2. Reference values for serum creatinine concentration (mg/dL) in healthy children, according to age and sex using the conventional Jaffe method[a]

	Age (years)	n	95% Reference intervals	Mean ± SD	Median	IQR	Min	Max
Boys	3–6	47	0.50–0.80	0.63 ± 0.08	0.60	0.60–0.70	0.50	0.80
	6–9	125	0.60–0.90	0.73 ± 0.08	0.70	0.70–0.80	0.60	1.00
	9–12	185	0.60–1.00	0.78 ± 0.09	0.80	0.70–0.80	0.50	1.20
	12–15	195	0.70–1.10	0.87 ± 0.10	0.90	0.80–0.90	0.60	1.20
	15–18	226	0.80–1.20	1.00 ± 0.11	1.00	1.00–1.10	0.70	1.30
	All	778	0.60–1.20	0.86 ± 0.16	0.80	0.70–1.00	0.50	1.30
Girls	3–6	46	0.50–0.80	0.65 ± 0.08	0.60	0.60–0.70	0.50	0.80
	6–9	108	0.60–0.90	0.73 ± 0.08	0.70	0.70–0.80	0.50	0.90
	9–12	166	0.60–0.90	0.75 ± 0.08	0.75	0.70–0.80	0.50	0.90
	12–15	183	0.60–1.00	0.82 ± 0.10	0.80	0.80–0.90	0.60	1.20
	15–18	219	0.70–1.10	0.88 ± 0.09	0.90	0.80–0.90	0.70	1.10
	All	724[b]	0.60–1.00	0.80 ± 0.12	0.80	0.70–0.90	0.40	1.20

a: According to Clinical and Laboratory Standards Institute (CLSI)/ International Federation of Clinical Chemistry (IFCC) criteria, non–parametric method; b: Outliers were excluded in each age group separately; therefore the total number is not equal to sum of number of subjects in each group; IQR: interquartile range; To convert creatinine values from milligram per deciliter to micromole per liter, multiply by 88.4.

method were 0.70 – 1.20 and 0.60 – 1.00 mg/dL, respectively.

Reference values for eGFR are presented in Table 4; the highest values were documented among age groups of 12 – 15 and 9 – 12 years for boys and girls respectively. Overall, their 95% reference values for eGFR ranged between 81 – 154 mL/min/1.73 m^2 and 80 – 129 mL/min/1.73 m^2.

Discussion

This study presents reference values for serum creatinine concentrations and eGFR in apparently healthy Iranian children and adolescents from a population-based study; values which could be used for accurate interpretation of laboratory results in disease diagnosis and treatment.

In the present study, reference values for serum creatinine concentration according to the conventional Jaffe method were found to be 0.60 – 1.20 mg/dL and 0.60 – 1.00 mg/dL in boys and girls, respectively. According to the compensated Jaffe method, these values were 0.23 – 0.86 mg/dL and 0.23 – 0.65 mg/dL, respectively. The creatinine assay according to the conventional Jaffe method yields higher values due to interference with this assay.[18] To compensate for the nonspecific reaction of non-creatinine chromogens, a constant value is subtracted and the results are considered as compensated Jaffe values. The constant value which ranges between 0.17 and 0.32 mg/dL in adults,[33] may be higher in children due to higher non-creatinine chromogens;[16] this value in our study was 0.35 mg/dL. Finney, et al. studying182 children and adolescents, aged 1 – 17 years, in the UK have reported serum creatinine reference values of 0.4 – 1.03 mg/dL and 0.37 – 0.97 mg/dL in boys and girls, respectively.[5] Schlebusch, et al. studying 257 German children, aged 3 – 15 years, have reported reference values for serum creatinine to be 0.31 – 0.87. According to the compensated Jaffe method;[17] the corresponding range was 0.23 – 0.65 mg/dL in our study. Upper limits of our reference

Table 3. Reference values for serum creatinine concentration (mg/dL) in healthy children according to age and sex using the compensated Jaffe method[a]

	Age (years)	n	95% Reference intervals	Mean ± SD	Median	IQR	Min	Max
Boys	3–6	47	0.13–0.44	0.26 ± 0.08	0.23	0.23–0.33	0.13	0.44
	6–9	125	0.23–0.54	0.37 ± 0.08	0.33	0.33–0.44	0.23	0.65
	9–12	185	0.23–0.65	0.42 ± 0.10	0.44	0.33–0.44	0.13	0.86
	12–15	195	0.33–0.75	0.51 ± 0.11	0.54	0.44–0.54	0.23	0.86
	15–18	226	0.44–0.86	0.67 ± 0.12	0.65	0.65–0.75	0.33	0.96
	All	778	0.23–0.86	0.50 ± 0.16	0.44	0.33–0.65	0.13	0.96
Girls	3–6	46	0.13–0.44	0.28 ± 0.08	0.23	0.23–0.33	0.13	0.44
	6–9	108	0.23–0.54	0.36 ± 0.08	0.33	0.33–0.44	0.13	0.54
	9–12	166	0.23–0.54	0.38 ± 0.09	0.39	0.33–0.44	0.13	0.54
	12–15	183	0.23–0.65	0.46 ± 0.11	0.44	0.44–0.54	0.23	0.86
	15–18	219	0.33–0.75	0.52 ± 0.09	0.54	0.44–0.54	0.33	0.75
	All	724[b]	0.23–0.65	0.44 ± 0.12	0.44	0.33–0.54	0.02	0.86

a: According to Clinical and Laboratory Standards Institute (CLSI)/ International Federation of Clinical Chemistry (IFCC) criteria, non–parametric method; b: Outliers were excluded in each age group separately; therefore the total number is not equal to sum of number of subjects in each group; To convert creatinine values from milligram per deciliter to micromole per liter, multiply by 88.4; IQR: interquartile range.

Table 4. Reference values for estimated glomerular filtration rate (mL/min/1.73 m^2) in healthy children according to age and sex[a]

			Shwartz equation						Counahan–Barrattequation					
	Age (years)	n	95% Reference intervals	Mean ± SD	Median	IQR	Min	Max	95% Reference intervals	Mean ± SD	Median	IQR	Min	Max
Boys	3–6	44	73–121	94 ± 12	94	86–103	72	122	57–95	73 ± 9	73	68–81	56	96
	6–9	124	76–122	95 ± 10	95	88–100	73	129	59–95	74 ± 8	74	69–79	57	101
	9–12	181	81–137	100 ± 13	99	91–108	59	150	63–107	78 ± 10	77	71–85	46	117
	12–15	192	108–164	131 ± 15	129	120–140	97	171	66–101	81 ± 9	79	74–86	60	105
	15–18	221	98–142	121 ± 13	119	112–130	92	179	60–92	74 ± 8	73	69–80	57	110
	All	762	81–154	113 ± 19	112	97–126	59	179	61–99	77 ± 9	76	70–82	46	117
Girls	3–6	45	67–124	93 ± 13	92	81–103	66	125	53–97	73 ± 10	72	64–81	52	98
	6–9	107	79–117	96 ± 11	95	87–103	75	128	62–91	75 ± 8	74	68–80	59	100
	9–12	164	86–134	106 ± 13	105	96–114	79	158	67–105	83 ± 10	82	75–89	62	124
	12–15	182	85–130	106 ± 12	106	97–114	73	147	66–102	83 ± 10	83	76–89	57	115
	15–18	217	81–125	100 ± 10	99	93–108	77	131	63–97	79 ± 8	77	73–84	60	103
	All	717b	80–129	102 ± 13	101	93–110	66	158	62–101	80 ± 10	79	73–86	52	124

a: According to Clinical and Laboratory Standards Institute (CLSI)/ International Federation of Clinical Chemistry (IFCC) criteria, non–parametric method; b: Outliers were excluded in each age group separately; therefore the total number is not equal to sum of number of subjects in each group; IQR: interquartile range; To convert creatinine values from milligram per deciliter to micromole per liter, multiply by 88.4.

values for serum creatinine are almost similar to those recently reported by the CALIPER (Canadian Laboratory Initiative in Pediatric Reference Intervals) study for 839 boys (0.39 – 1.10 mg/dL) and 850 girls (0.39 – 0.88 mg/dL), aged 1 – 18 years.[18]

In line with previous reports,[5,17,18,31,34,35] in our study, reference values for serum creatinine, in particular the upper limits, which are medically more important than the lower ones,[36] increased with age. It has been reported that the largest creatinine increase occurs between 9 – 17 years of age with the onset of the puberty.[5] Our results indicated that, compared to girls, boys had higher values of serum creatinine concentrations; a finding in agreement with results of the CALIPER study[18] and other reports,[31] but contradictory to others that have reported no sex differences for serum creatinine values in pediatrics.[5,17,35] Endogenous creatinine production and serum creatinine values are proportional to muscle mass,[5,31,32] which could explain the increase in serum creatinine with age and higher values in males.

In our study, according to the Schwartz equation, refer-

ence values for eGFR were 81 – 154 mL/min/1.73 m^2 and 80 – 129 mL/min/1.73 m^2 in boys and girls, respectively. Normal GFR in children and adolescents has been reported to be 79 – 187 mL/min/1.73 m^2 in 2 – 12 year old males and females, and 80 – 200 mL/min/1.73 m^2 and 82 – 170 mL/min/1.73 m^2 in 13 – 21 year old males and females, respectively.[37] In addition, it has been reported that the correct interpretation of GFR values in children and adolescents require awareness that normal GFR values vary according to age, gender, and body size.[37]

As a rule of thumb, we proposed a regression equation to estimate serum creatinine in Iranian children. Initially, BMI was also included in the regression analysis due to the reported relationship between body mass and serum creatinine levels,[5,31,32] but since the corresponding coefficient was low (0.001) it was removed from the final equation in order to propose a simple and easy to remember equation. When we applied this equation on creatinine data of children of the same age in phase 5 TLGS, only 4.7% difference was observed between estimated and measured values. Hence, this equation is applicable for simply screening renal function in Iranian children.

One final point that needs to be clear is the difference between reference values and cut-off points. Reference values are defined as the intervals between and including two numbers, the lower and upper limits, which enclose a specified percentage, usually 95%, of a healthy reference population;[38] they differ from cut-offs, which may be lower than the upper limit of reference values[39,40] and should not be confused for the upper reference limits.[41] Cut-off points or clinical decision limits define thresholds as values above or below those are considered diagnostic for a specific disease and associated with higher risk of adverse clinical outcomes.[42] Reference values have high specificity for health, while cut-off values consider as both sensitivity and specificity of a disease.[42]

Strengths of this study include the large sample size that permits a more accurate estimate of reference values. In addition, our samples were obtained from a population-based study which could provide the best reference values for use in preventive medicine.[43] Finally, this study reports reference values for eGFR alongside creatinine which has been reported to provide a useful tool for clinicians in the detection and management of CKD.[44]

This study has some limitations; first, the fixed compensation factor used in this study for the compensated Jaffe method may produce overcorrection and underestimation of creatinine values[45] and does not take into account the normal variation in serum matrix components.[15] In addition, there is a high variation of proteins as the major part of pseudocreatinines in children.[46] Second, we used the Schwartz[25] and Counahan-Barratt[24] formulas for estimating GFR as recommended by NKF CKD.[9] The gold standard of measuring GFR is however insulin clearance, using an inulin-type marker such as iohexol. Since measuring GFR is a very difficult procedure in children; some equations have been suggested for estimating GFR in clinical medicine.[47–49] The Schwartz and Counahan-Barratt formulas have been shown to have the best performance for GFR ≥ 60 mL/minute/1.73 m^2.[50] In addition, at higher GFR values, the error of eGFR calculations increases and underestimates the results.[48] It has been reported that for GFR < 60 mL/minute/1.73 m^2, Counahan-Barratt equation outperforms the Schwartz one.[50]

In conclusion, this study demonstrates pediatric reference values for serum creatinine levels to be 0.6 – 1.20 mg/dL and 0.6 – 1.00 mg/dL and for eGFR values to be 81 – 154 mL/min/1.73 m^2 and 80 – 129 mL/min/1.73 m^2 in Iranian boys and girls respectively. It can be said that for Iranian children aged 3 – 18 years, serum creatinine (mg/dL) = k × age (year) + 0.5, where k = 0.03 for boys and k = 0.02 for girls. These findings can be used for diagnostic and therapeutic purposes.

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Declaration of interest

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References

1. Assadi F. The epidemic of pediatric chronic kidney disease: the danger of skepticism. *J Nephropathol*. 2012; 1: 61– 64.
2. Tohidi M, Hasheminia M, Mohebi R, Khalili D, Hosseinpanah F, Yazdani B, et al. Incidence of chronic kidney disease and its risk factors, results of over 10 year follow up in an Iranian cohort. *PLoS One*. 2012; 7: e45304.
3. Gheissari A, Kelishadi R, Roomizadeh P, Abedini A, Haghjooy-Javanmard S, Abtahi SH, et al. Chronic Kidney Disease Stages 3-5 in Iranian Children: Need for a School-based Screening Strategy: The CASPIAN-III Study. *Int J Prev Med*. 2013; 4: 95 – 101.
4. Miller WG. Estimating equations for glomerular filtration rate in children: laboratory considerations. *Clin Chem*. 2009; 55: 402 – 403.
5. Finney H, Newman DJ, Thakkar H, Fell JM, Price CP. Reference ranges for plasma cystatin C and creatinine measurements in premature infants, neonates, and older children. *Arch Dis Child*. 2000; 82: 71 – 75.
6. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Annals of Internal Medicine*. 1999; 130: 461 – 470.
7. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Annals of Internal Medicine*. 2003; 139: 137 – 147.
8. Selvin E, Manzi J, Stevens LA, Van Lente F, Lacher DA, Levey AS, et al. Calibration of serum creatinine in the National Health and Nutrition Examination Surveys (NHANES) 1988-1994, 1999-2004. *Am J Kidney Dis*. 2007; 50: 918 – 926.
9. Mattman A, Eintracht S, Mock T, Schick G, Seccombe DW, Hurley RM, et al. Estimating pediatric glomerular filtration rates in the era of chronic kidney disease staging. *J Am Soc Nephrol*. 2006; 17: 487 – 496.
10. Ceriotti F, Boyd JC, Klein G, Henny J, Queralto J, Kairisto V, et al. Reference intervals for serum creatinine concentrations: assessment of available data for global application. *Clin Chem*. 2008; 54: 559 – 566.
11. Chromy V, Rozkosna K, Sedlak P. Determination of serum creatinine by Jaffe method and how to calibrate to eliminate matrix interference problems. *Clin Chem Lab Med*. 2008; 46: 1127 – 1133.
12. Coresh J, Astor BC, McQuillan G, Kusek J, Greene T, Van Lente F, et al. Calibration and random variation of the serum creatinine assay as

critical elements of using equations to estimate glomerular filtration rate. *Am J Kidney Dis*. 2002; 39: 920 – 929.
13. Junge W, Wilke B, Halabi A, Klein G. Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffe method. *Clinica Chimica Acta; International Journal of Clinical Chemistry*. 2004; 344: 137 – 148.
14. Ceriotti F. Establishing pediatric reference intervals: a challenging task. *Clin Chem*. 2012; 58: 808 – 810.
15. Cobbaert CM, Baadenhuijsen H, Weykamp CW. Prime time for enzymatic creatinine methods in pediatrics. *Clin Chem*. 2009; 55: 549 – 558.
16. Panteghini M. Enzymatic assays for creatinine: time for action. *Clin Chem Lab Med*. 2008; 46: 567 – 572.
17. Schlebusch H, Liappis N, Kalina E, Klein C. High Sensitive CRP and Creatinine: Reference Intervals from Infancy to Childhood/Hochsensitives CRP und Kreatinin: Referenzbereich fÃ¼r Neugeborene und Kinder. *LaboratoriumsMedizin*. 2002; 26: 341 – 346.
18. 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. *Clin Chem*. 2012; 58: 854 – 868.
19. Zeh C, Amornkul PN, Inzaule S, Ondoa P, Oyaro B, Mwaengo DM, et al. Population-based biochemistry, immunologic and hematological reference values for adolescents and young adults in a rural population in Western Kenya. *PLoS One*. 2011; 6: e21040.
20. Hawkins RC. Differences in serum creatinine concentration between Caucasians, Chinese, Indians and Malays. *Clinica Chimica Acta; International Journal of Clinical Chemistry*. 2010; 411: 1393.
21. Azizi F, Ghanbarian A, Momenan AA, Hadaegh F, Mirmiran P, Hedayati M, et al. Prevention of non-communicable disease in a population in nutrition transition: Tehran Lipid and Glucose Study phase II. *Trials*. 2009; 10: 5.
22. Azizi F, Rahmani M, Emami H, Mirmiran P, Hajipour R, Madjid M, et al. Cardiovascular risk factors in an Iranian urban population: Tehran lipid and glucose study (phase 1). *Sozial- und Praventivmedizin*. 2002; 47: 408 – 426.
23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972; 18: 499 – 502.
24. Counahan R, Chantler C, Ghazali S, Kirkwood B, Rose F, Barratt TM. Estimation of glomerular filtration rate from plasma creatinine concentration in children. *Arch Dis Child*. 1976; 51: 875 – 878.
25. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. *Pediatric Clinics of North America*. 1987; 34: 571 – 590.
26. Horn PS, Feng L, Li Y, Pesce AJ. Effect of outliers and nonhealthy individuals on reference interval estimation. *Clin Chem*. 2001; 47: 2137 – 2145.
27. Horn PS, Pesce AJ, Copeland BE. A robust approach to reference interval estimation and evaluation. *Clin Chem*. 1998; 44: 622 – 631.
28. Solberg HE. Approved recommendation (1987) on the theory of reference values Part 5. Statistical treatment of collected reference values. Determination of reference limits. *Journal of Clinical Chemistry and Clinical Biochemistry*. 1987; 25: 645 – 656.
29. PetitClerc C, 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 (1987) on the theory of reference values. Part 2. Selection of individuals for the production of reference values. *Journal of Clinical Chemistry and Clinical Biochemistry*. 1987; 25: 639 – 644.
30. Henny J, Petitclerc C, Fuentes-Arderiu X, Petersen PH, Queralto JM, Schiele F, et al. Need for revisiting the concept of reference values. *Clin Chem Lab Med*. 2000; 38: 589 – 595.
31. Schwartz GJ, Haycock GB, Spitzer A. Plasma creatinine and urea concentration in children: normal values for age and sex. *The Journal of Pediatrics*. 1976; 88: 828 – 830.
32. Hetu PO, Gingras ME, Vinet B. Development and validation of a rapid liquid chromatography isotope dilution tandem mass spectrometry (LC-IDMS/MS) method for serum creatinine. *Clinical Biochemistry*. 2010; 43: 1158 – 1162.
33. Ghasemi A, Azimzadeh I, Zahediasl S, Azizi F. Reference Values for Serum Creatinine with Jaffe-compensated Assay in Adult Iranian Subjects: Tehran Lipid and Glucose Study. *Arch Iran Med*. 2014; 17: 394 – 399.
34. Cohen M. What is the normal serum creatinine concentration in children? *Pediatr Radiol*. 2008; 38: 1265; author reply 1266.
35. Donckerwolcke RA, Sander PC, van Stekelenburg GJ, Stoop JW, Tiddens HA. Serum creatinine values in healthy children. *Acta Paediatr Scand*. 1970; 59: 399 – 402.
36. Fuentes-Arderiu X, Alvarez-Funes V, Coca-Fabregas L, Cruz-Placer M, Diaz-Fernandez J, Herrero-Bernal P, et al. Multicentre physiological reference values for the concentration of creatininium in plasma and diagnostic specificity of glomerular filtration rate estimated with the MDRD equation. *Clin Chem Lab Med*. 2007; 45: 531 – 534.
37. Hogg RJ, Furth S, Lemley KV, Portman R, Schwartz GJ, Coresh J, et al. National Kidney Foundation's Kidney Disease Outcomes Quality Initiative clinical practice guidelines for chronic kidney disease in children and adolescents: evaluation, classification, and stratification. *Pediatrics*. 2003; 111: 1416 – 1421.
38. Juma AA, Ngeranwa JJ, Njagi EN. Reference values for some renal function parameters for adult population in north-rift valley, kenya. *Indian J Clin Biochem*. 2012; 27: 40 – 45.
39. Waise A, Price HC. The upper limit of the reference range for thyroid-stimulating hormone should not be confused with a cut-off to define subclinical hypothyroidism. *Ann Clin Biochem*. 2009; 46: 93 – 98.
40. Ghasemi A, Tohidi M, Derakhshan A, Hasheminia M, Azizi F, Hadaegh F. Cut-off points of homeostasis model assessment of insulin resistance, beta-cell function, and fasting serum insulin to identify future type 2 diabetes: Tehran Lipid and Glucose Study. *Acta Diabetologica*. 2015.
41. Schellenberg F, Wielders JP. Evaluation of capillary electrophoresis assay for CDT on SEBIA's Capillarys System: intra and inter laboratory precision, reference interval and cut-off. *Clinica Chimica Acta; International Journal of Clinical Chemistry*. 2010; 411: 1888 – 1893.
42. Sikaris KA. Physiology and its importance for reference intervals. *Clin Biochem Rev*. 2014; 35: 3 – 14.
43. Hansen AM, Garde AH, Eller NH. Estimation of individual reference intervals in small sample sizes. *Int J Hyg Environ Health*. 2007; 210: 471 – 478.
44. Abdi Z, O'Donoghue D. Estimated glomerular filtration rate: why we need it. *Ann Clin Biochem*. 2012; 49: 417 – 418.
45. Kuster N, Bargnoux AS, Pageaux GP, Cristol JP. Limitations of compensated Jaffe creatinine assays in cirrhotic patients. *Clinical Biochemistry*. 2012; 45: 320 – 325.
46. Arzideh F, Wosniok W, Haeckel R. Reference limits of plasma and serum creatinine concentrations from intra-laboratory data bases of several German and Italian medical centres: Comparison between direct and indirect procedures. *Clinica Chimica Acta; International Journal of Clinical Chemistry*. 2010; 411: 215 – 221.
47. Deng F, Finer G, Haymond S, Brooks E, Langman CB. Applicability of estimating glomerular filtration rate equations in pediatric patients: comparison with a measured glomerular filtration rate by iohexol clearance. *Transl Res*. 2015; 165: 437 – 445.
48. Zachwieja K, Korohoda P, Kwinta-Rybicka J, Miklaszewska M, Moczulska A, Bugajska J. Which equations should and which should not be employed in calculating eGFR in children? *Advances in Medical Sciences*. 2015; 60: 31 – 40.
49. Schwartz GJ, Munoz A, Schneider MF, Mak RH, Kaskel F, Warady BA, et al. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol*. 2009; 20: 629 – 637.
50. Lee CK SR, Cerda RD, Portman RJ, Hwang W, Furth SL. Evaluation of Serum Creatinine Concentration–Based Glomerular Filtration Rate Equations in Pediatric Patients with Chronic Kidney Disease. *Pharmacotherapy*. 2012; 32: 642 – 648.