Original Article

Reference Values for Serum Creatinine with Jaffe-compensated Assay in Adult Iranian Subjects: Tehran Lipid and Glucose Study

Asghar Ghasemi PhD•[1,2], Iraj Azimzadeh BSc[3], Saleh Zahediasl PhD[1,2], Fereidoun Azizi MD[2]

Abstract

Background: Chronic kidney disease is a worldwide public health problem and glomerular filtration rate (GFR), the best overall index of renal function, is most commonly estimated from serum creatinine concentrations. The aim of this study was to determine reference values for serum creatinine concentrations using data from a population-based study in Iran.

Methods: Serum creatinine was measured using the Jaffe method in 5247 men and women, aged 20–88 years, participants of the Tehran Lipid and Glucose Study. For calculating Jaffe compensated creatinine values in 382 samples, serum creatinine was measured using both the Jaffe and the enzymatic p-aminophenazone (PAP) methods. Linear regression analysis yielded a regression line equation of Jaffe-creatinine=0.863 × PAP-creatinine + 38.9 μmol/L (r = 0.973, n = 382, $P < 0.001$). CLSI/IFCC guidelines (International Federation of Clinical Chemistry/ Clinical and Laboratory Standards Institute), non-parametric method was used for determining creatinine reference values.

Results: Reference values for serum creatinine ranged between 47–98 μmol/L (0.53–1.11 mg/dL), 37–68 μmol/L (0.42–0.77 mg/dL), and 37–78 μmol/L (0.42–0.88 mg/dL) in men, non-menopausal women, and menopausal women, respectively. Mean serum creatinine concentration was significantly higher in men compared to women for both age ≤ 50 years [70 ± 11 vs. 50 ± 10 μmol/L (0.79 ± 0.12 vs. 0.57 ± 0.11 mg/dL), $P < 0.001$] and age > 50 years [73 ± 12 vs. 55 ± 12 μmol/L (0.83 ± 0.14 vs. 0.62 ± 0.14 mg/dL), $P < 0.001$].

Conclusion: Reference values for serum creatinine using the compensated Jaffe method are presented in Iranian subjects, values that could help assessment of kidney function.

Keywords: Jaffe assay, reference values, serum creatinine

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Introduction

Chronic kidney disease is a worldwide public health problem with increasing prevalence and incidence.[1,2] The incidence of chronic kidney disease has been reported to be above 2% each year in the Iranian population.[3] Glomerular filtration rate (GFR), the best overall index of renal function, is most commonly estimated from serum creatinine concentration,[1,4,5] and any equation using serum creatinine level for estimating GFR is dependent on the serum creatinine assay.[6,7] In addition, serum creatinine values are also used for assessing liver function.[8] Serum creatinine concentration is affected by factors other than creatinine filtration, including sex, age, race, diet, muscle mass, and the analytical method.[4,9,10] The Jaffe method, used in most routine laboratories, has low specificity and overestimates serum creatinine by approximately 20–30% in physiological values, due to non-creatinine chromogens, mainly proteins.[6,9,11,12] Enzymatic creatinine methods are more specific[9] and have been widely adopted for routine clinical laboratory as alternatives to the alkaline picrate methods.[10]

For serum creatinine, like other naturally occurring biochemical compounds, a reference interval needs to be provided.[9,13] Reports of reference intervals for serum creatinine levels in Asian populations, considering IFCC (International Federation of Clinical Chemistry) criteria with accurate description of the measurement method, are scant[9] and to our knowledge, there is no documented report on reference values of serum creatinine in Iran. The aim of this study was therefore to determine age- and sex-specific reference intervals for serum creatinine concentrations using data from a population-based study from Iran.

Subjects and Methods

Subjects

The Tehran Lipid and Glucose Study (TLGS) was initiated in 1999, aiming to determine the prevalence of non-communicable disease risk factors.[14] A multistage stratified cluster random sampling technique was used to select 15,005 persons, aged over 3 years, from District 13 of Tehran, which is representative of Tehran's population.[15] In the current study, subjects (n = 10,795) were participants, aged ≥ 20 years, of phase 4 TLGS (June 2008 to September 2011). Excluded were pregnant women, hypertensive subjects, those with diabetes, history of cardiovascular disease, cancer, and diarrhea or those using any medications including steroids, diuretics, beta-blockers, digitals, calcium channel blockers, angiotensin converting enzyme inhibitors, aspirin and other anticoagulants, lipid lowering drugs, male or female hormones,

Authors' affiliation: [1]Endocrine Physiology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [2]Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. [3]Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

•Corresponding author and reprints: Asghar Ghasemi PhD, Endocrine Physiology Research Center, Research Institute for Endocrine Sciences
Shahid Beheshti University of Medical Sciences, No. 24, Parvaneh St., Velenjak, Tehran, Iran. P.O. Box: 19395-4763, Tehran, Iran.
Tel: +98 21 22409309; Fax: +98 21 22416264; E-mail: Ghasemi@endocrine.ac.ir

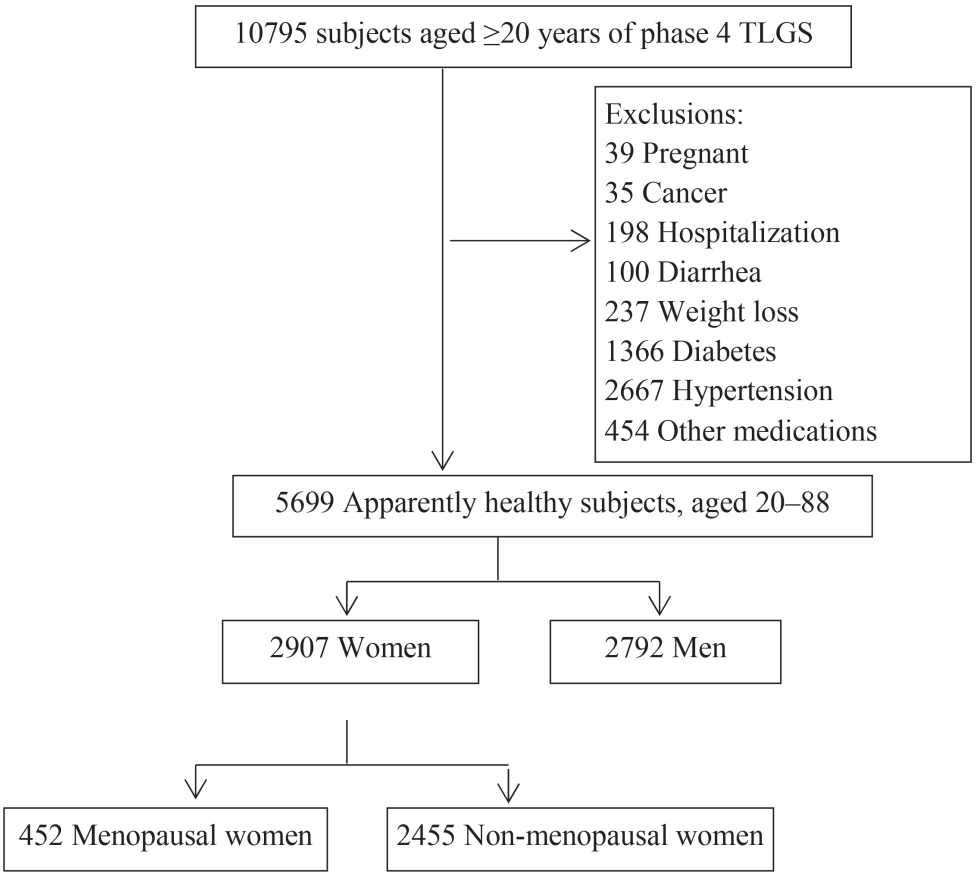

Figure 1. Study population and exclusions

contraceptives (oral or injection), or drugs for thyroid disorders. Subjects with a history of hospitalization during the past 3 months and those with a history of significant weight loss during the past 6 months were also excluded. After application of the exclusion criteria, 5247 apparently healthy participants (2792 men and 2455 women), aged 20 to 88 years, remained for analysis. A separate analysis was performed for healthy menopausal women (n = 452; age range 51–84 years) (Figure 1). The study was approved by the ethics committee of the Research Institute for Endocrine Sciences and written informed consent was obtained from each participant.

Anthropometric and clinical assessments

Details of data collection in the TLGS have been published previously[15]; in brief, weight and height were measured according to standard protocols. Body mass index (BMI) was calculated as weight (Kg) divided by square of height (m^2). 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 at 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 382 samples, creatinine measurement was done with both Jaffe and enzymatic p-aminophenazone (PAP) methods. Intra-assay CVs were 2.2% and 3.1% for the Jaffe and PAP method, respectively (n = 72). Inter-assay CVs for normal creatinine concentration were 4.1% and 6.1% for Jaffe and PAP method, respectively and for high creatinine concentration were 1.3% and 1.7% for the Jaffe and PAP method, respectively (n = 17). Bland-Altman method comparison was used for comparing creatinine measurements by the Jaffe and PAP methods.

Determining outliers

The Dixon outlier range statistic was used for determining outliers as it has been recommended by Clinical and Laboratory Standards Institute (CLSI) for reference intervals determined by the nonparametric procedure.[16] In the Dixon test, if the D/R ratio exceeds 1/3, the extreme value is considered as 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.

Determining serum creatinine reference values

We used the CLSI/IFCC guidelines, non-parametric method, to determine reference values.[17,18] The retrospective (*a 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.[19] For the IFCC non-parametric method, which is recommended for determining reference values,[20] 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 intervals.

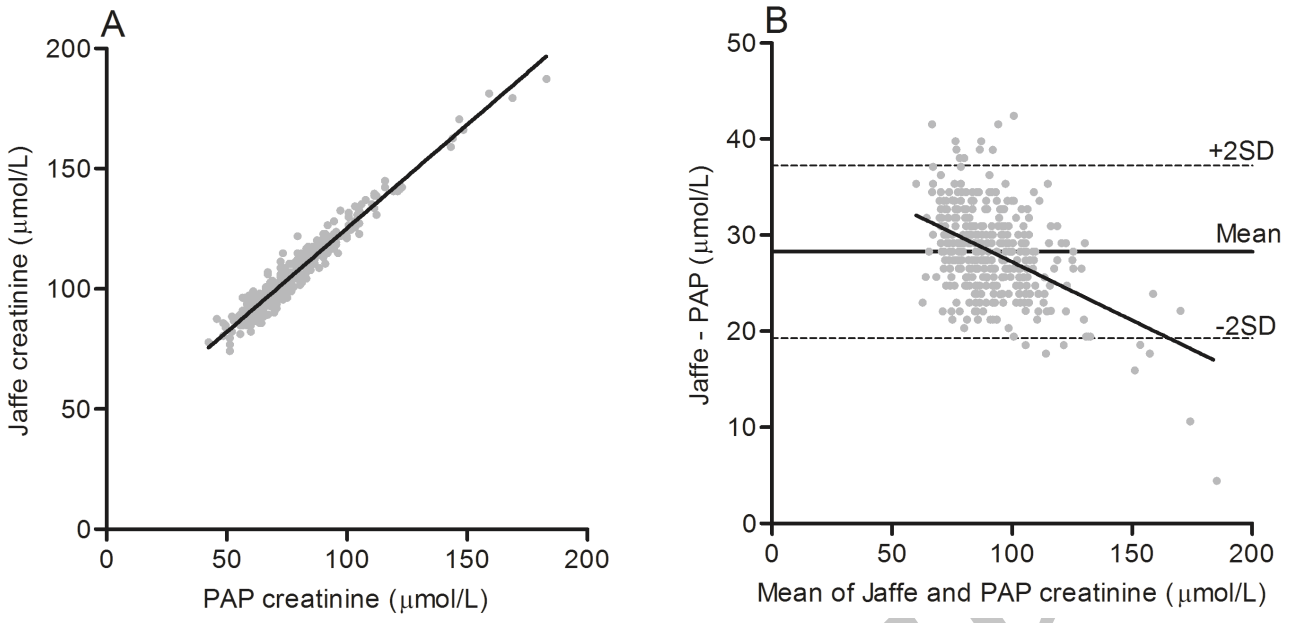

Figure 2. (A) Linear regression analysis of serum creatinine measured by conventional Jaffe method, compared to the PAP enzymatic method. The analysis yielded a regression line equation of: y= 0.863x + 38.9 μmol/L (r= 0.973, n = 382, p<0.001). **(B)** Bland and Altman analysis of serum creatinine comparison data: correlation r = - 0.460 (P<0.001), slope = - 0.121 (P<0.001), intercept = 39.4. Statistically significant bias was found with a mean difference of 28.3 ± 4.6 μmol/L. To convert creatinine values from micromole per liter to milligram per deciliter divide by 88.4.

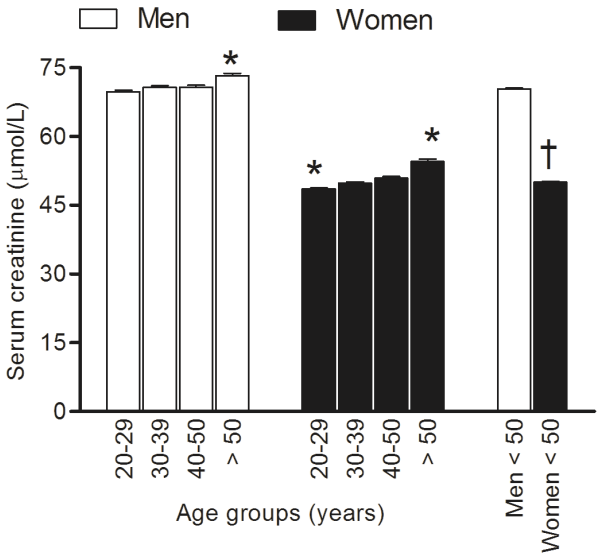

Figure 3. Comparison of serum creatinine concentration by sex and age. Serum Creatinine concentrations were significantly lower in women compared with men in all age groups ($P < 0.001$). *: significant difference with other groups. †: significant difference compared to men. To convert creatinine values from micromole per liter to milligram per deciliter divide by 88.4.

Definitions of variables

Diabetes was defined according to the American Diabetes Association as fasting serum glucose ≥7.0 mmol/L or 2-hour serum glucose ≥11.1 mmol/L and/or medical treatment.[21] Hypertension was defined as blood pressure ≥140/90 or using antihypertensive medication. Smoking was defined as using ≥1 cigarettes per day or using the water pipe. Women were considered menopausal if they had had no menstrual bleeding in the previous 12 months. History of cardiovascular disease included coronary heart disease (myocardial infraction, history of heart surgery, angioplasty, and hospitalization in the coronary care unit) and cerebrovascular attack.

Statistical analysis

For comparing baseline variables between men and women, the independent sample *t*-test was used. Pearson correlation coefficient was used for calculating correlation between age and serum creatinine concentrations. Differences between serum creatinine concentrations in different age groups were compared by one-way analysis of variance and Tukey post hoc test was used for multiple comparisons. Two-sided p values less than 0.05 were considered statistically significant. SPSS (SPSS Inc., Chicago, IL, USA; Version 15) software 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 5247 healthy subjects (2792 men and 2455 women), aged 20 to 88 years. Men were older than women (39.9 ± 14.3 vs. 33.3 ± 8.5 years). Comparison between Jaffe and PAP method for measuring serum creatinine concentration showed a good correlation between the two methods with a regression line of: y (Jaffe-creatinine) = 0.863 × (PAP-creatinine) + 38.9 μmol/L (r = 0.973, n = 382, $P < 0.001$); this equation was then used for calculating compensated creatinine values for the entire data (Figure 2A); although the mean difference was 28.3 ± 4.6 μmol/L (0.32 ± 0.05 mg/dL), a statistically significant bias was found and the magnitude of difference was lower with higher concentrations of serum creatinine (Figure 2B).

Mean serum creatinine concentration was significantly higher in men compared to women for both age ≤ 50 years [70 ± 11 vs. 50 ± 10 μmol/L (0.79 ± 0.12 vs. 0.57 ± 0.11 mg/dL), $P < 0.001$] and age > 50 years [73 ± 12 vs. 55 ± 12 μmol/L (0.83 ± 0.14 vs.

Table 1. Reference intervals for serum creatinine concentration (μmol/L) in healthy adult subjects by age and sex using the compensated Jaffe method[a].

	Age (years)	*n*	95% Reference intervals	Mean ± SD	Median	IQR	Min	Max
Men								
	29–20	856	88–47	70 ± 10	68	79–68	37	98
	39–30	633	88–47	71 ± 11	68	79–68	37	98
	50–40	701	98–47	71 ± 11	68	78–68	27	98
	>50	602	98–47	73 ± 12	78	78–69	27	98
	All	2792	98–47	72 ± 11	68	79–68	27	98
Women								
	29–20	972	68–27	49 ± 9	47	57–47	27	88
	39–30	818	68–37	50 ± 10	47	57–47	27	78
	50–40	665	68–37	51 ± 10	47	57–47	27	88
	>50	452[b]	78–37	55 ± 12	57	57–47	27	98
	All	2455[c]	68–37	50 ± 10	47	57–47	27	88

a = According to Clinical and Laboratory Standards Institute (CLSI)/ International Federation of Clinical Chemistry (IFCC) criteria, non-parametric method; b = Healthy menopausal women; c = Menopausal women are not included; IQR = interquartile range. To convert creatinine values from micromole per liter to milligram per deciliter divide by 88.4.

0.62 ± 0.14 mg/dL), $P < 0.001$]. In addition, in both genders, serum creatinine concentrations were significantly higher in subjects aged above 50 years (Figure 3). A weak but significant correlation was found between serum creatinine levels and age in both men (r = 0.128, n= 2792, $P < 0.001$) and women (r = 0.196, n = 2907, $P < 0.001$).

Reference values for serum creatinine according to age and sex are presented in Table 1. Overall, 95% reference values for serum creatinine concentration ranged between 47–98 μmol/L (0.53–1.11 mg/dL) and 37–68 μmol/L (0.42–0.77 mg/dL) in men and women, respectively. Upper reference limits of serum creatinine levels were higher in men, aged over 40 years and in post-menopausal women. In addition, median serum creatinine level was higher in men older than 50 years and in post-menopausal women.

Discussion

This study presents reference values for serum creatinine concentrations according to the Jaffe compensated method in apparently healthy Iranian subjects from a population-based study. These values could be used for diagnostic and therapeutic purposes.

In our study, the mean difference between creatinine measurement by the conventional Jaffe method and the enzymatic one, as the most specific routine method commercially available,[22] was 28.3 μmol/L (0.32 mg/dL). By subtracting this value, results were considered Jaffe compensated. In line with this result, subtracting a constant of 26 μmol/L (0.29 mg/dL) or 27 μmol/L (0.31 mg/dL) of creatinine as an average value has been done by some kit producers to solve the interference problem in Jaffe method[22,23]. Subtractions of 15 μmol/L (0.17 mg/dL),[24] 18 μmol/L (0.20 mg/dL),[8] or 21 μmol/L (0.24 mg/dL)[12] have also been reported. In addition, creatinine-free serum samples, when measured by the Jaffe kinetic method, have on the average 26.5 μmol/L (0.30 mg/dL) creatinine[12,25] and a positive difference of about 27 μmol/L (0.31 mg/dL) has been reported between the Jaffe and the HPLC method for creatinine measurement.[26,27]

In our study, serum creatinine levels were higher in men compared to women in all age groups; a finding similar to those of other reports,[24,28,29] which may be due to muscle mass.[28] In addition, in our study, serum creatinine levels were higher in subjects aged >50 year, revealing positive correlation between age and serum creatinine levels. In line with this result, Wang et al., reported higher serum creatinine values in subjects > 60 years[24] and it has been reported that serum creatinine increases as a function of age with 0.22 μmol/L (0.003 mg/dL) per year in men and 0.14 μmol/L (0.002 mg/dL) per year in women.[22]

We found reference values for serum creatinine concentration to be 47–98 μmol/L (0.53–1.11 mg/dL) and 37–68 μmol/L (0.42–0.77 mg/dL) in men and women, respectively. Reference values for serum creatinine concentrations in some countries are summarized in Table 2.[12,22-25,28-31] Our values, especially the upper reference limit which is medically more important than the lower limit,[23] are very close to those of China[24] and Kenya[28] and lower than those of European countries. In line with these results, serum creatinine concentrations in Asians have been reported to be less than those of the Caucasians, which may be due to less muscle mass in the former.[32] This issue raises the need for inclusion of an Asian ethnic factor for calculation of estimated GFR according to the MDRD formula.[32] Although the Nordic group suggest that common reference intervals for serum creatinine could be used,[33] differences have been reported in Asians between cities.[34] Upper limits of our reference values were higher in men and in menopausal women, aged over 50 years. Partition age for reporting creatinine reference values has been reported to be 50, 60, or 70 years and in line with our results, higher values have been reported with increasing age.[24]

The strengths of this study include a relatively large sample size used for determining reference values. In addition, our samples were obtained from individuals of a population-based study, which could provide the best reference intervals for use in preventive medicine.[35] As a limitation, we used the compensated Jaffe creatinine assay while the reference method for serum creatinine measurement is isotope dilution mass spectrometry[7]; in the compensated Jaffe creatinine assay recalibration consists of subtracting a constant value from the results of conventional Jaffe creatinine assay for non-specific chromogens; however, the levels of chro-

Table 2. Serum creatinine reference values (μmol/L) in selected countries.

Country	Year	*n*	Sex (M/F)	Age (years)	Creatinine reference values (μmol/L)		Creatinine measurement method	Reference number
					Males	Females		
South Australia (Adelaide)	2000	562	293/269	18–70	62–106	44–80	Compensated Jaffe	25
Germany	2004	240	120/120	18–74	63–103	48–85	Compensated Jaffe	12
Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden)	2004	1802	858/944	≥18	62–106	47–88	Compensated Jaffe	30
India	2006	1121	848/273	20–60	71–115	53–88	Enzymatic	31
Spain	2007	468	248/220	19–65	64–106	52–85	Compensated Jaffe	23
Belgium	2008	32658	14312/18346	20–70	56–103	42–82	Enzymatic	29
Italy	2010	16939	ND	18–79	57–103	40–88	Compensated Jaffe	22
China	2011	778	433/345	20–91	58–98	43–70	Enzymatic	24
Kenya	2012	360	206/154	18–50	56–99	48–85	ND	28
Iran	Present study	5247	2792/2455	20–88	47–98	37–68	Compensated Jaffe	Present study

ND = not determined. To convert creatinine values from micromole per liter to milligram per deciliter divide by 88.4.

mogens vary from one subject to another and low protein levels in samples lead to underestimation of creatinine values; these factors impact the measured levels of creatinine.[8]

In conclusion, the results of this study present reference intervals for serum creatinine concentration, according to the compensated Jaffe method, derived from a population-based study in Iran to be 47-98 μmol/L (0.53–1.11 mg/dL) and 37–68 μmol/L (0.42–0.77 mg/dL) in men and women, respectively. These values could be used for diagnostic and therapeutic purposes.

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