COMPARING AGE-WISE REFERENCE INTERVALS FOR SERUM CREATININE CONCENTRATION IN A “REALITY CHECK” OF THE RECOMMENDED CUT-OFF

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ABSTRACT

An increase in the communication within the healthcare services, both nationally and internationally, has strengthened the need for harmonization of measurements and reference intervals in laboratory medicine. In the present report, the calculated reference interval for serum creatinine (sCr) levels of healthy normal individuals (n=1121) in different sex and age groups are compared with the established interval. The calculated reference interval for sCr level was 0.4-1.3 mg/dL and 0.6 to 1.3 mg/dL in the age groups of 21-40 and 41-60 years respectively. The difference between the mean sCr values in total males and total females (age range 21-60 years) was statistically significant ($p<0.0001$); When male and female subjects were analyzed age-group wise, the data showed a significant difference in mean sCr values ($p<0.0001$) in three age groups (21-30, 31-40 and 41-50 years) however, in older age group (51-60 years), the difference was non-significant ($p=0.07$). The reference ranges were 0.7-1.3 and 0.4-1.0 mg/dL for males and females respectively where the lower limit was 0.1-0.2 units less than that of standard limits. An increase in the mean value of sCr was observed particularly in females with an increase in age. Hence it is of interest to validate an age specific reference ranges for sCr in our population.

KEY WORDS

sCr, Reference Range, Validation, Glomerular Filtration Rate, Clinical Reference Laboratory.

INTRODUCTION

A low glomerular filtration rate (GFR) leading to a condition of reduced urinary excretion, results in retention and accumulation of various nitrogenous waste products in the plasma and other body fluids. Creatinine is one of those substances detected in serum or plasma; the concentration of which is a primary determinant for estimating the GFR. Physicians usually estimate the level of GFR from prediction equations that take into account the serum creatinine (sCr) concentration and the variables viz., age, sex, race, and body size. Other nitrogenous substance of diagnostic relevance is the blood urea nitrogen

(BUN). However sCr estimate has been used as an important marker for the kidney function test (1).

Reference intervals in biochemical parameters are commonly based on the measurements in a reference population, which is representative of a defined group of individuals (2). The reference limits in use are usually referred from text books or article or values provided by the kit manufacturers. To our knowledge, there is no population-based study so far on reference limits of sCr in India. Hence the standard reference interval, particularly of international origin, needs to be validated in our population. In the present study, in order to improve the diagnostic accuracy, we have estimated the reference interval for sCr level in different sex and age group of normal healthy population tested in a Clinical Reference Laboratory.

MATERIALS AND METHODS

Study population

We have reviewed retrospectively records of healthy individuals from Western part of the country who had

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undergone health check at SRL Ranbaxy Clinical Reference Laboratory. Record of 1327 individuals was analyzed of which 206 individuals were excluded based on the following criteria (Table 1). The reference range was calculated on rest 1121 healthy subjects (age range 20 to 60 years). There was a male predominance (n=848) over females (n=273). And these subjects were considered healthy on the basis of physical and biochemical examinations along with questionnaire details as evaluated by a physician.

Table 1
Exclusion criteria for defining reference individuals

History of	Risk factors
Diabetes mellitus,	Pregnancies,
Liver dysfunction,	Strenuous exercise,
Hypertension or other cardiovascular abnormalities, Tuberculosis,	Alcohol consumption,
Acute inflammatory conditions	and Smoking

From National Committee for Clinical Laboratory Standards: How to define, determine and utilize reference intervals in the clinical laboratory: Proposed guidelines (2000) 2nd ed., NCCLS document, Villanova PA, NCCLS [Above details were obtained from the health check reports and from questionnaire filled by the individuals.]

Estimation of sCr concentration

Creatinine level in serum samples of all the 1121 subjects were detected by using same enzymatic creatinine method on auto analyzer (DADE Behring Limited, USA) in order to maintain the uniformity in the test results.

Data analysis and calculation of reference range

The reference interval for sCr level was calculated from the 95% confidence limits computed as recommended in NCCLS guidelines (3). The 97.5th percentile and 2.5th percentile formed the upper and lower reference limits of the population respectively. Reference interval was calculated for males and females, and for two age groups i.e., 21-40 and 41-60 years separately. The differences between the values in different age groups and sexes were calculated statistically in order to know the significance of the differences. The P values were derived from the z-statistic values calculated by MS-Excel.

RESULTS

The mean sCr values and SD in males and females were compared in four age groups (21-30, 31-40, 41-50, and 51-60 years) as summarized in Table 2. In females, the mean values of sCr level were observed to be less than that in males of all the age groups. The results showed a statistically significant difference in mean values ($p < 0.0001$) in three age groups except in the older age group (51-60 years), where the difference was non-significant ($p = 0.07$).

For calculation of age-group specific reference intervals in our population the study subjects were divided into two age groups (i.e., 21-40 years and 41-60 years). Age group specific reference ranges were calculated combining male and female subjects together. The results are summarized in Table 3. The reference ranges in these two age groups were also within

Table 2
Mean values and ranges of sCr concentration in males and females of different age groups

sCr concentration (mg/dL) showing the mean value (range) and SD in different age groups of healthy males and female subjects					
Sex	21-30 years	31-40 years	41-50 years	51-60 years	Total
Male	0.979 (0.2-1.2) ± 0.161 n=574	1.022 (0.6-1.6) ± 0.162 n=146	1.021 (0.7-1.4) ± 0.152 n=90	0.994 (0.7-1.3) ± 0.176 n=37	0.998 (0.2-1.6) ± 0.1622 n=848
Female	0.721 (0.3-1.2) ± 0.161 n=203	0.673 (0.3-1.0) ± 0.162 n=34	0.7 (0.4-0.9) ± 0.152 n=21	0.9 (0.6-1.3) ± 0.17 n=16	0.725 (0.3-1.3) ± 0.1615 n=273
Z value	19.625	11.293	8.717	1.839	23.686
P value	<0.0001	<0.0001	<0.0001	0.071614	<0.0001

[SD- Standard deviation; n- Number of subjects; range- Actual range observed.]

Table 3
Age wise distribution of serum creatinine levels observed in the healthy normal subjects

Age groups in years (n)	Total range (mg/dL)	Mean (mg/dL)	SD	Calculated R range (mg/dL)	* Established R range (mg/dL)
21-40 (n=957)	0.2-1.6	0.921	± 0.2	0.4-1.3	0.6 to 1.3
41-60 (n=164)	0.4-1.4	0.964	± 0.182	0.6-1.3	
Total (n=1121)	0.2-1.6	0.927	± 0.19	0.5-1.3	

[* As per manufacturer's (DADE Behring, USA) recommendation; SD- Standard deviation; n=Number of subjects; Statistically significant ($p=0.005$) difference between the values in two age groups]

the standard range (*i.e.*, 0.6-1.3 mg/dL). The reference interval of the total study population ($n=1121$) was estimated to be 0.5-1.3 mg/dL. The calculated reference range for 21-40 years age group ($n=957$) was 0.4-1.3 mg/dL and that in 41-60 years age group ($n=164$) was 0.6-1.3 mg/dL. In the age group 21-40 years, the lower limit was observed to be less than the lower limit of the standard range with a difference of 0.2 units. The mean values were found statistically significant ($p=0.005$) when calculated for these two age groups (21-40 and 41-60 years) separately.

Reference ranges were calculated separately for males and females and the values are summarized in Table 4. The established reference interval, which has been utilized by the laboratory, is also tabulated along with the results so as to enable us to compare the results obtained from the study. The mean values of sCr level in males of 21-40 and 41-60 years age groups were 0.987 mg/dL and 1.013 mg/dL respectively.

In females, the mean values of sCr level were calculated to be 0.714 mg/dL and 0.8 mg/dL in 21-30 and 31-40 years age groups respectively (Table 4). The differences were statistically significant in both the age groups ($p<0.0001$). The difference between the mean sCr values in total males and total females (age range 21-60 years) was also significant ($p<0.0001$). Though the reference ranges were falling within the limits of the standard ranges for both males and females, the lower limit was 0.1-0.2 units less than the standard limits in either case.

DISCUSSION

Creatinine is a small, freely filtered solute whose production varies little from day to day since it is derived from the metabolism of muscle creatine. Creatinine can be secreted by the proximal tubule through an organic cation pathway. The secreted creatinine component confounds GFR

measurements because it can vary within individuals over time; moreover the proportion of secreted creatinine increases as GFR decreases (1). In the present study we have taken the data of sCr level for validating the reference range applicable to our own population.

In the present study the observed reference range for sCr is 0.4-1.3 mg/dL for age group 21-40 years and 0.6-1.3 in age group 41-60 years. Where as the standard range as per the recommended guidelines is 0.6-1.3 mg/dL. Though the estimated range was within the standard range, the lower limit of the observed range was found 0.2 units less than that of the standard range. This indicates sCr level may vary in the healthy population to different extent. In other words, sCr concentration is affected by factors other than GFR, such as creatinine secretion and generation and extra renal excretion as observed in earlier studies (4, 5).

The standard ranges for male and female is 0.8-1.3 and 0.6-1.3 mg/dL respectively. The lower limit of the range was 0.1 and 0.2 units less in males and females respectively when compared to that of the standard range. In the higher age group (*i.e.*, 41-60 years), the reference interval was calculated only for males since there was no adequate number of female subjects in this age group. The lower limits of the reference intervals in both males and females were less than that of the corresponding standard values with a difference of 0.2 units as observed in the present data. Males of age range 41-60 years showed the range falling within the standard. The reference intervals for males and females of different age groups was found deviating from the reference intervals in total males and total females also (Table 4). Ranges for males and females are need to be verified further, particularly in elder age group, as the present series does not have adequate number of female samples particularly in the 41-60 years age group.

The differences between lower limits of the observed ranges

Table 4
Calculation of reference ranges for serum creatinine (sCr) levels separately for male and female healthy subjects in 21-40 and 41-60 years age groups

Age groups	Sex	Total range (mg/dL)	Mean (mg/dL) $\pm$ SD	P value	Calculated R range (mg/dL)	* Standard R range (mg/dL)
21-60 years	Male n=848	0.2-1.6	0.991 $\pm$ 0.1622	<0.0001	0.8-1.3	0.7-1.3
	Female n=273	0.3-1.3	0.725 $\pm$ 0.1615		0.6-1.0	0.4-1.0
21-40 years	Male n=721	0.2-1.6	0.987 $\pm$ 0.1625	<0.0001	0.8-1.3	0.6-1.3
	Female n=236	0.3-1.2	0.714 $\pm$ 0.1602		0.6-1.0	0.4-1.0
41-60 years	Male n=127	0.7-1.4	1.013 $\pm$ 0.1601	<0.0001	0.8-1.3	0.7-1.3
	Female n=37	0.4-1.3	0.8 $\pm$ 0.1544		0.6-1.0	**

[* As per manufacturer's (DADE Behring, USA) recommendation; SD- Standard deviation; n=Number of subjects; P value <0.05 is statistically significant; ** Number insufficient for calculation of reference range as per NCCLS guidelines]

compared to that of the recommended established range (DADE Behring Limited, USA) could not be evaluated statistically since no reference data (viz., mean and SD) was available for an appropriate statistical test. However, same method, as recommended by NCCLS, was followed for estimating the reference range as it was done for the standard range.

According to international standards, reference values for biochemical tests should be obtained in disease-free subjects and for therapies that may influence measurement values. An impaired renal function may be expected in older age and thus the normal creatinine level may be expected to vary as the age increases (6). In the present series, the median value of the sCr level was found more in age group 41-60 than 21-40 years. But the variation was not prominent. An alteration of sCr level in serum in normal individuals may be affected by diet. Low creatinine may result from inadequate dietary protein as well as reduced muscle mass (7). In other hand, an increased sCr level may occur from noncreatinine substances, including meat ingestion, glucose, pyruvate, uric acid, fructose, guanidine, acetoacetate, and ascorbic acid (8). Falsely high sCr concentration is also associated with severe methanol intoxication (9).

CONCLUSION

The present study has shown a variation in mean values of normal sCr level when estimated in different age groups separately and also between males and females of different age groups. The differences were found statistically significant. In this preliminary study we have observed deviation of the lower limit of the calculated reference ranges compared to that of the standard ranges even though they fall within the ranges. The clinical conditions with low sCr level are not known. However, an age group specific reference ranges taking a population with different social status may need to be validated. Every laboratory may establish reference ranges on their own population for a "reality check" of the internationally referred values.

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