ORIGINAL ARTICLE

Reference intervals for serum cystatin C and creatinine of an indigenous adult Nigerian population

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Abstract

Background and Aim: Estimation of the glomerular filtration rate (GFR) is important for the evaluation of patients with kidney disease. Some studies suggest that GFR estimated from serum cystatin C (Cys C) is more accurate than that from serum creatinine (SCr). For Cys C to be used for this purpose, normal values need to be determined for various populations. This study determined the serum Cys C levels and reference intervals (RIs) of a Nigerian population.

Materials and Methods: Three hundred and four healthy adult subjects were analysed. Serum Cys C and SCr were determined by particle enhanced turbidimetric immunoassay and modified Jaffe kinetic method respectively. Data were analysed using the Statistical Package for Social Sciences version 17.0 (SPSS for Windows Inc., Chicago, IL, USA). Estimation of RIs was done as per the International Federation of Clinical Chemistry guidelines.

Results: The RIs for Cys C were 0.65-1.12 mg/L (median 0.86) for males, 0.62-1.12 mg/L (median 0.85) for females and 0.64-1.12 mg/L (median 0.86) for all the subjects. The RIs for SCr were 73-110 μmol/L (median 89) for males, 65-102 μmol/L (median 82) for females and 66-106 μmol/L (median 86) for all the subjects. There was no significant gender difference in the RIs for serum Cys C, ($P > 0.05$). The SCr levels and RI were significantly lower in females than in males ($P < 0.001$).

Conclusion: This study has determined the serum Cys C levels and RI of an indigenous healthy adult black population in Nigeria.

Key words: Cystatin C, reference value, Nigerian population

Date of Acceptance: 25-Sep-2014

Introduction

Current clinical practice guidelines[1,2] recommend the use of estimated glomerular filtration rate (GFR) for the evaluation and classification of renal diseases. The commonly used formulae including Cockcroft and Gault, the Modification of Diet in Renal Disease, and the Chronic Kidney Disease Epidemiology Collaboration formulae[3-5] all use serum creatinine (SCr) for estimating GFR. Creatinine-based measurements of GFR have several limitations,[6,7] including the inability to detect early renal dysfunction due to low sensitivity. As a result of these limitations, plasma cystatin C (Cys C) has been proposed as an alternative endogenous marker for GFR.[8,9] Cys C is freely filtered without tubular secretion and is completely catabolized at the proximal tubule.[10] A number of studies[11,12] have suggested that estimating GFR from serum Cys C values may be more accurate and superior to SCr based estimation. To effectively utilise Cys C for this purpose, the reference value for the population needs to be determined. Reference values differ in many populations and with gender and age.[13,14] Some studies have documented different reference values for Caucasians and African Americans.[15] It is not known whether this difference applies to Nigerian population. To our knowledge, the reference values for Cys C have not been determined for the Nigerian population. This study was designed to determine the reference values for serum Cys C and SCr in an indigenous Nigerian population and to correlate the values with gender.

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Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/1119-3077.151036

Materials and Methods

This was a population based cross-sectional study of a representative sample of the adult population of Enugu metropolis, conducted between April 2010 and August 2010. Enugu is the capital city of Enugu state, South-East Nigeria with a population of 871,438.[16]

Ethical Clearance for the study Ref: ESUTH P/C.MAC/RA/033/124 of 09 June 2010, was obtained from the Health Research Ethics Committee of the Ministry of Health, Enugu state. Adult participants were recruited from 15 out of 20 enumeration areas (EA) chosen to represent the various sectors of the metropolis. From each of the EA, participants were selected using age and gender stratified random sampling to ensure that participants from each Local Government Council matched the overall population structure. This was done using computer-generated list of random numbers (the utility Epi Info: Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia (USA)) as follows: 6 out of 8, 5 out of 7, and 4 out of 5 EAs were chosen from Enugu East, Enugu north and Enugu South local government councils respectively.

Signed written consent was obtained from each study participant after explanation of the study by the investigators.

The inclusion criteria for this study were: Adult age (20 years and above), being black and resident in Enugu metropolis, and being generally of good health with no evidence of a chronic or acute illness on clinical examination. The exclusion criteria were: Hypertension, diabetes mellitus, stroke, kidney disease, family history of kidney disease, significant proteinuria (urine dipstick protein ≥1+ equivalent to approx. 30 mg/dL), liver disease, and pregnancy.

The participants were screened by history and physical examination and those who satisfied the study criteria were recruited. An investigator-administered, structured questionnaire was completed on behalf of each recruited participant. Anthropometric measurements were taken: Standing height was measured without footwear to the nearest centimetre (cm) using a stadiometer. Weight was measured to the nearest kilogram (kg) with a manual Seca 761 scale (Vogel and Halke, Hamburg, Germany) in light indoor clothing. Blood pressure was measured in the nondominant arm with appropriate cuff size, in the sitting position after 5 min of rest with an automated sphygmomanometer (OMRON HEM705CP, Omron Matsusaka Co., Matsusaka city, Mie-Ken, Japan). Three consecutive readings were taken 1 min apart, and the mean of the three readings was determined. Body mass index (BMI) was calculated using weight in kilogram divided by the square of the height in metre, while body surface area (BSA) was calculated using duBois and duBois formula.[17]

Three millilitres of fasting venous blood was drawn and preserved in appropriate bottles for serum Cys C, SCr, and blood glucose. All laboratory analyses were conducted at the Medical Research Laboratory, Department of Medicine, College of Medicine, University of Nigeria Enugu Campus, using Humalyzer 2000 Chemistry analyzer (Human GmBH Diagnostica Worldwide, Wiesbaden, Germany). SCr concentration was measured by the modified Jaffe kinetic method,[18] using reagent kit by Linear Chemicals, Montgat, Spain. Serum Cys C concentration was determined by particle (latex) enhanced turbidimetric immunoassay (PETIA) method,[19] (Randox Laboratories Ltd., Antrim, United Kingdom).

The assays were done in duplicates and the mean of two results determined.

Urine sample was collected for dipstick urine analysis for protein, glucose, and bilirubin (Combur 10, Roche Diagnostics GmBH, Mannheim, Germany). Female participants <55 years had a urine dipstick (ACON Laboratories Inc., San Diego, U.S.A.) examination for β-human chorionic gonadotrophin hormone to exclude pregnancy.

Statistical analysis

Data entry and analyses were done using the Statistical Package for Social Sciences (SPSS for Windows Inc., Chicago, IL, USA) version 17.0. Results were expressed where appropriate as mean ± standard deviation and median (inter quartile range). Since all the data were not evenly distributed, the Mann–Whitney U-test was used to compare the variables. Age, weight, height, BMI and BSA were correlated with Spearman Rho for Cys C and SCr. Reference intervals (RIs) were determined by the nonparametric method as described in the International Federation of Clinical Chemistry (IFCC) guidelines.[20] This method was used to determine the 2.5 and 97.5 percentiles and the respective 90% confidence limits around these estimates. This consists of cutting off values from each tail of the distribution and essentially eliminating the outliers. Briefly, reference values were sorted in ascending order of magnitude, after which consecutive inter rank numbers are given to each value. The limits of the conventional 95% RI have rank numbers equal to $0.025 \times (n + 1)$ and $0.975 \times (n + 1)$. The values corresponding to these rank numbers are the RI.

All tests were two-tailed and $P < 0.05$ considered statistically significant.

Results

A total of 181,490 adults were randomly selected from the sampling frame and 935 (0.52%) gave consent to participate in the study. Of this number, 631 were disqualified for various reasons; 149 hypertensive, 98 diabetic, 124 proteinuria,

Table 1: Characteristics of the study population

Parameter	Mean±SD			*P*
	All (*n*=304)	Males (*n*=151)	Females (*n*=153)	
Age (years)	49.5±17.0	49.4±16.8	49.5±17.3	0.958
Weight (kg)	79.5±13.5	79.7±12.2	79.3±14.7	0.738
Height (m)	1.68±0.09	1.72±0.08	1.63±0.06	<0.001
BMI (kg/m^2)	28.3±4.9	26.8±3.6	29.8±5.5	<0.001
BSA (m^2)	1.94±0.19	1.96±0.18	1.91±0.19	0.016
SCr (μmol/L)	86.0±10.1	91.0±9.0	82.0±9.1	<0.001
Cys C (mg/L)	0.88±0.13	0.88±0.13	0.87±0.13	0.830

BMI=Body mass index; BSA=Body surface area; SCr=Serum creatinine; Cys C=Cystatin C; SD=Standard deviation

Table 2: Reference intervals for serum Cys C (mg/L)

Parameter	All (*n*=304)	Males (*n*=151)	Females (*n*=153)	*P*
Median serum Cys C (mg/L)	0.86	0.86	0.85	>0.05
2.5th percentile (90% CI)	0.64 (0.61-0.68)	0.65 (0.61-0.68)	0.62 (0.58-0.69)	>0.05
97.5th percentile (90% CI)	1.12 (1.10-1.13)	1.12 (1.09-1.14)	1.12 (1.10-1.12)	>0.05

Cys C=Cystatin C; CI=Confidence interval

Table 3: Reference intervals for SCr (μmol/l)

Parameter	All (*n*=304)	Males (*n*=151)	Females (*n*=153)	*P*
Median SCr (μmol/)	86.0	89.0	82.0	<0.001
2.5th percentile (90% CI)	66 (64-67)	73 (63-76)	65 (62-66)	<0.001
97.5th percentile (90% CI)	106 (104-113)	110 (106-114)	102 (98-103)	<0.001

CI=Confidence interval; SCr=Serum creatinine

22 stroke, 133 other diseases including kidney disease, liver disease, and mental illness and 105 withdrew consent. The remaining 304 (151 male, 153 female) participants were analysed.

There were no differences in the mean age and weight between the males and female participants [Table 1].

There was no difference in the mean serum Cys C levels between the males (0.88 ± 0.13 mg/L) and the females (0.87 ± 0.13 mg/L), $P = 0.830$. However the males had higher mean SCr levels than females (91.0 ± 9.1 μmol/L vs. 82.0 ± 9.1 μmol/L; $P < 0.001$).

The nonparametric reference intervals (NPRI) for Cys C were 0.65-1.12 mg/L (median 0.86 mg/L) in males, 0.62-1.12 mg/L in females (median 0.85 mg/L) and 0.64-1.12 mg/L (median 0.86 mg/L) in all the subjects [Table 2]. The NPRI for SCr were 73-110 μmol/L (median 89 μmol/L) in males, 65-102 μmol/L (median 82 μmol/L) in females and 66-106 μmol/L (median 86 μmol/L) in all the subjects [Table 3]. There was no significant difference between males and females in the

Table 4a: Correlation of serum Cys C with age, weight, height, BMI, and BSA

Parameter	All subjects (*n*=300)		Male subjects (*n*=150)		Female subjects (*n*=150)	
	r	*P*	*r*	*P*	*r*	*P*
Age (years)	0.89	<0.01	0.91	<0.01	0.89	<0.01
Weight (kg)	0.09	>0.05	−0.08	>0.05	0.24	<0.01
Height (m)	−0.04	>0.05	−0.11	>0.05	−0.04	>0.05
BMI (kg/m^2)	0.15	<0.05	0.01	>0.05	0.24	<0.01
BSA (m^2)	0.06	>0.05	−0.12	>0.05	0.23	<0.01

r is Spearman's coefficient of variation. BMI=Body mass index; BSA=Body surface area; Cys C=Cystatin C

Table 4b: Correlation of SCr with age, weight, height, BMI and BSA

Parameter	All subjects (*n*=300)		Male subjects (*n*=150)		Female subjects (*n*=150)	
	r	*P*	*r*	*P*	*r*	*P*
Age (years)	−0.01	>0.05	−0.18	<0.05	0.11	>0.05
Weight (kg)	0.50	<0.01	0.56	<0.01	0.54	<0.01
Height (m)	−0.40	<0.01	0.36	<0.01	0.04	>0.05
BMI (kg/m^2)	0.28	<0.01	0.42	<0.01	0.54	<0.01
BSA (m^2)	0.56	<0.01	0.56	<0.01	0.54	<0.01

r is Spearman's coefficient of variation. BMI=Body mass index; BSA=Body surface area; SCr=Serum creatinine

Cys C levels and RIs ($P > 0.05$), whereas SCr levels and RI were strongly associated with gender, with males having about 7.5% higher values than the females.

Correlation analysis [Tables 4a and 4b] of age, gender, weight, height, BMI and BSA and serum Cys C showed positive correlation with age and minor positive correlation with BMI in all subjects. However in females age, weight, BMI and BSA correlated with Cys C.

Discussion

This is the first report from Nigeria of normal RIs for serum Cys C in adult Nigerian subjects. Serum Cys C in healthy adults has been studied by other authors and wide variations exist in its reference values. The median serum Cys C concentration (0.86 mg/l) and RIs (0.64-1.12 mg/l) obtained in this study are in agreement with those of some studies.[13,21,22] For example in a similar study of Saudi subjects by Al Wakeel *et al*.,[22] the RI for Cys C was 0.50-1.09 mg/L. Although many studies have reported that Cys C is independent of race/ethnicity,[23,24] some few other studies have indicated that serum Cys C levels are not entirely independent of race/ethnicity.[25-27] Groesbeck *et al*.,[25] and Kottgen *et al*.,[26] have variously reported that non-Hispanic whites have higher serum Cys C levels than non-Hispanic blacks and Mexican Americans individuals, whereas Stevens *et al*.,[27] found out that in patients with impaired kidney function, blacks have higher Cys C levels for the same GFR than whites. Although, to

the best of our knowledge, no study has been conducted in a typical indigenous adult Nigerian population on Cys C levels and RI, a few of the studies that have been undertaken (on other parameters) have indicated differences in the levels and RI values of African populations, compared to those derived from Caucasians.[28,29] However, among the plurality of opinions, there is a consensus that these factors (gender and race) exert a very modest effect at most, which will not significantly influence the use of Cys C as a biomarker of kidney function. There was no statistical difference in the median serum Cys C levels and the RIs between males and females in this study. This has previously been documented.[30] However other studies,[31,32] had documented difference in these values between gender. The reason for this may be the assay method or the cut off points for the RI. Although, the (latex) PETIA and particle-enhanced nephelometric immunoassay (PENIA) are considered the most practical approaches, the PETIA method provided significantly higher results compared with PENIA.[26] Consequently, proposed ranges for serum Cys C concentrations in both paediatric and adult populations in existing literature are inconsistent, with several studies reporting RI that have varied widely between 0.58 and 1.38 mg/L. There is thus need for an international standard for Cys C measurement. Furthermore, the IFCC guideline on the establishment of RI recommends the use of the 2.5th and 97.5th percentiles as cut offs for the lower and upper limits of the RI respectively; the use of different cut offs to demarcate the lower and upper limits of the RI contributes to the discrepancies in the reported RI for Cys C.

There was significant gender difference in the SCr levels, with the median male SCr level being 7.5% higher than those in the females. Gender-specific RIs were also recorded, with males having about 18 μmol/L higher than the females. This is an expected finding and agrees with previous studies.

Result of this study showed a strong positive correlation between Cys C and age. Cys C levels are reported to decrease from birth until the 1st year of life, remaining relatively stable before they rise again, especially beyond age 50 years.[25,31] These findings are similar to ours.

Another notable finding from this study was the association of Cys C with body composition which is also in agreement with the results of other studies[23] Although, weight, BMI and BSA showed slight significant correlations with Cys C in the females, it is unclear why this is so. Males and females recruited for this study were of similar age and weight, and there is no gender difference in the Cys C concentrations between the males and females. It is possible that underlying genetic factors that alter the physiologic generation and/or elimination of Cys C, or different hormonal variations, or factors that are present in the females and interfere with the Cys C assay may account for this. However, it seems likely that these confounding factors, which may cause a variation in these individuals, play only a minor role. This assertion is supported by the views of Galteau *et al*.,[21] who observed a moderate correlation between Cys C and BMI, but concluded that this might not be biologically significant. Conversely, SCr levels showed a consistent and very significant correlation with body composition in this study in consonance with what is obtained in the literature.[23,33] The main advantage of Cys C over creatinine as a GFR marker is that it is less dependent upon the body composition of an individual than creatinine.[23] For instance, while muscle mass strongly influences creatinine, it does not or only marginally affects Cys C. If a person's muscle mass deviates from the mean of that of a person of his/her age, sex, or ethnic origin in a population the use of creatinine as a GFR marker may be spurious.

Conclusion

This study has determined the serum Cys C levels (mean 0.88 ± 0.13 mg/L) and RIs (0.64-1.12 mg/L) of a healthy adult Nigerian population. There was no gender difference in the serum levels and RIs of Cys C.

The study established that while Cys C levels are independent of gender and other body indices, the reverse is the case with SCr, and thus suggests that Cys C may be a better surrogate for determining estimated GFR.

A multicentre study involving a larger population size that will be more representative of the whole country is advocated.

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How to cite this article: Okonkwo IN, Ogbu II, Ijoma UN, Ulasi II, Ijoma CK. Reference intervals for serum cystatin C and creatinine of an indigenous adult Nigerian population. Niger J Clin Pract 2015;18:173-7.
Source of Support: Nil, **Conflict of Interest:** None declared.