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Serum Cystatin C Levels in Nigerian Children: Reference Intervals and Relationship to Demographic and Anthropometric Variables

Sérum Cystatine C Chez les Enfants Nigérians: Intervalles de Référence et des Relations Avec les Variables Démographiques et Anthropométriques

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ABSTRACT

BACKGROUND: **Cystatin C has been recognized as a good marker of kidney function but reference ranges have not been determined in Nigerian children.**

OBJECTIVE: **To determine the reference range of serum cystatin C in Nigerian children with no overt signs of kidney disease and to determine and compare the relationship of serum cystatin C and serum creatinine with demographic and anthropometric variables.**

METHODS: **Fifty-nine children aged two years to 16 years with no evidence of overt kidney disease were recruited from the Paediatric Clinics of the Lagos University Teaching Hospital. Serum cystatin C levels were measured using ELISA method while serum creatinine was measured by a rate-blanked and compensated Jaffe method using a Roche/Hitachi 902 auto-analyser. Both were measured using the same serum sample.**

RESULTS: **The mean (±1.96SD) serum cystatin C level was 0.73 (0.41–1.04) mg/L and was similar among male and female children (P=0.640) and between children younger than five years and those five years and older (P=0.596). Unlike cystatin C, serum creatinine was higher among children five years or older. In contrast to serum creatinine, serum cystatin showed no significant correlation with age (r=0.153, P=0.246), weight (r=0.062, P=0.641) and length (r=0.067, P=0.612).**

CONCLUSION: **Serum cystatin C reference range in Nigerian children is similar to that reported for children in other regions of the world and appears to be independent of gender, weight, height, body mass index and age after two years. WAJM 2011; 30(3): 188–192.**

Keywords: **Children, clinic, creatinine, cystatin C, reference interval.**

RÉSUMÉ

CONTEXTE: La cystatine C a été reconnu comme un bon marqueur de la fonction rénale, mais les valeurs de référence n'ont pas été déterminées chez les enfants nigérians.

OBJECTIF: Déterminer la gamme de référence de la cystatine C sérique chez les enfants nigérians sans signes apparents de maladie rénale et de déterminer et de comparer la relation de la cystatine C sérique et de la créatinine sérique avec des variables démographiques et anthropométriques.

MÉTHODES: Cinquante-neuf enfants âgés de deux ans à 16 ans ne présentant aucun signe de maladie rénale manifeste ont été recrutés dans les cliniques pédiatriques de l'hôpital universitaire de Lagos. Sérum taux de cystatine C ont été mesurées en utilisant la méthode ELISA tandis créatinine sérique a été mesuré par un taux-blanchi méthode de Jaffé et compensé en utilisant un analyseur Roche / Hitachi 902 auto-analyseur. Tous deux ont été mesurées en utilisant le même échantillon de sérum.

RÉSULTATS: La moyenne (± 1.96SD) cystatine C sérique était de 0,73 (0.41 à 1.04) mg / L et était similaire chez les enfants mâles et femelles (P = 0,640) et entre les enfants de moins de cinq ans et ceux de cinq ans et plus (P = 0,596). Contrairement à la cystatine C, la créatinine sérique était plus élevé chez les enfants de cinq ans ou plus. Contrairement à créatininémie, la cystatine sérum n'a montré aucune corrélation significative avec l'âge (r = 0,153, P = 0,246), poids (r = 0,062, P = 0,641) et la longueur (r = 0,067, P = 0,612).

CONCLUSION: Sérum plage de référence cystatine C chez les enfants nigérians est similaire à celle rapportée pour les enfants dans d'autres régions du monde et semble être indépendante du sexe, poids, taille, indice de masse corporelle et l'âge après deux ans. **WAJM 2011; 30(3): 188–192.**

Mots-clés: enfants, une clinique, créatininémie, la cystatine C, intervalle de référence.

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Abbreviations: BMI, Body Mass Index; **CV,** Coefficient of variation; **HAZ,** Height for age z score; **IQR,** Interquartile range; **QC,** Quality Control; **WAZ,** Weight for age z score.

INTRODUCTION

Cystatin C, a 30 kilodalton 120-amino acid peptide, has recently been proposed and increasingly been recognised as a better endogenous marker of glomerular filtration compared to the commonly used creatinine.[1,2] Unlike creatinine, it is produced at a constant rate by the house-keeping genes of all nucleated cells of the body and cleared from the plasma almost entirely by glomerular filtration.[3] Hence in conditions of reduced glomerular filtration the plasma level of cystatin C rises. Adult levels of cystatin C are reported to be reached at one year of life and similar levels are maintained across childhood and between male and female gender, allowing for a single reference range across a wide age range.[1,4] Being constitutively produced it is unaffected by muscle mass and diet, unlike creatinine.[3]

Reference range for serum cystatin C has been determined for adults and children but not in African children and there are reports that race might affect serum cystatin C level.[4–7] Because of these reasons and the growing recognition of the utility of cystatin C over creatinine in the evaluation of children for renal disease, there is a need to determine the reference range of serum cystatin C in Nigerian children with no signs of overt kidney disease. Secondly, there is a need to compare the correlation of cystatin C and creatinine with age, height, weight and body mass index.

SUBJECTS, MATERIALS, AND METHODS

The subjects were apparently healthy children aged two years to 16 years with no overt evidence of kidney disease attending the various paediatric clinics of the Lagos University Teaching Hospital, Lagos, Nigeria between May 2008 and June 2008. The children were recruited from the Paediatric Respiratory Clinic (those with bronchial asthma but not taking systemic steroid), Paediatric Neurology Clinic (children with seizure disorders attending the clinic for the first time and not yet on anticonvulsants), Paediatric Surgical Clinic (children prior to or after herniotomy or post appendicectomy) and Ear, Nose and Throat Clinic (those with speech or hearing disorder). Consecutive children who met the study criteria during the study time span were enrolled.

Excluded were children with cardiac disease, sickle cell disease, HIV infection, proteinuria ≥ 1+ or suspected of having kidney disease. Also excluded were those children with febrile illness within the preceding 48 hours, diarrhoea within the past week, hospitalisation within the past two weeks or needing hospitalisation and those on systemic steroids or non steroidal anti-inflammatory drugs. Haemolysed samples were excluded.

Informed consent from the caregiver (with assent from the children aged seven years and older) was obtained. The study was approved by the Hospital's Research and Ethics Committee.

The weight and height of each child were measured with the children wearing light clothing and bare footed. Urinalysis was done using dipstick to exclude those with proteinuria. From each participating child about five millilitre of blood was obtained. Out of this, three millilitre were allowed to clot and the resulting serum after centrifugation was frozen at -80^0C until analysis. The remaining blood was tested for HIV antibodies (using Determine rapid kit™) and haemoglobin genotype using cellulose acetate electrophoresis.

Cystatin C and Creatinine Assays

The same serum sample from each child was analyzed for cystatin C and creatinine. The serum cystatin C was measured by a sandwich enzyme immunoassay method using kits manufactured by BioVendor - Laboratorni medicina a.s, Czech Republic. According to the manufacturer, the analytical limit of detection for cystatin C was 0.2 ng/ml.

Serum creatinine was determined by a rate-blanked and compensated Jaffe method using a Roche/Hitachi 902 auto-analyser. To enable us calculate the coefficient of variations 20 control sera were analyzed.

Fifty-nine children were studied, namely: children with isolated hearing and speech disorder, 5 (8.5%); post herniotomy, 10 (16.9%); bronchial asthma not on steroids, 17 (28.8%) and seizure disorder not yet on anticonvulsants, 27 (45.8%).

Statistical Analysis

Statistical analysis was performed using SPSS version 14.0 (for Windows version) statistical package. Continuous data are summarised as means (SD) or median (interquartile range) while categorical data were presented as proportions. Reference range for parametric variable is reported as mean (±1.96SD) and for non-parametric variable as median (with 2.5^{th} and 97.5^{th} percentiles) according to the guidelines of the International Federation of Clinical Chemistry.[8] Tests of difference were determined using Student's t-test, Mann Whitney U test, ANOVA or Kruskal-Wallis H test as appropriate. Pearson and Spearman correlation coefficients were calculated for parametric and non-parametric data respectively. Statistical significance was set at $p<0.05$. In this study cystatin C and height were normally distributed while creatinine, age, weight and BMI were not normally distributed.

RESULTS

The children were aged 2–14.5 years with a median (IQR) age of 5.59 (4.58) years. The male-female ratio was 1.57:1. The weight for age, height for age and BMI for age z scores were –0.44, –0.04 and –0.66 respectively (Table 1).

The calculated intra-assay co-efficient of variation (CV %) of cystatin C were as follows: low quality control (QC), 3.9%; high QC, 3.1%. For the inter-assay, CV was as follows: low QC, 6.8% and high QC, 11.8%. For serum creatinine the intra-assay and inter-assay CV were 0.7% and 2.3% respectively.

The mean (±1.96SD) serum cystatin C level was 0.73 (0.41-1.04) mg/L. It was similar between male and female children (p=0.640) and between children younger than 5 years and those 5 years or older (p=0.596). For creatinine, its median value (2.5^{th} and 97.5^{th} percentile) was 0.51 (0.28–1.21). Unlike serum cystatin C, serum creatinine was higher in children 5 years and older (0.58 mg/dl versus 0.41 mg/dl, p=0.001) with a higher but not statistically significant level in female children compared to male children (Table 2 and 3).

Table 1: Age and Anthropometric Characteristics of the Children Studied by Sex

Variable	All	Male	Female
N	**59**	**36**	**23**
Median age (IQR), years	5.59 (4.58)	5.09 (5.0)	5.6 (4.9)
Weight, kg			
Median (IQR)	19.50 (11.50)	20 (10.9)	18.5 (10.3)
WAZ score	–0.44 (1.17)	–0.22(1.58)	–0.67 (1.19)
Height, cm			
Mean (SD)	116.0 (19.1)	116.8 (20.3)	111.9 (16.9)
HAZ score	–0.04 (1.30)	0.19 (1.31)	–0.40 (0.98)
BMI, kg/m^2			
Median (IQR)	14.6 (1.47)	14.6 (1.34)	14.4 (1.48)
BMI z score	–0.66 (1.20)	–0.70 (1.55)	–0.85 (1.18)

IQR, Interquartile range; WAZ, Weight for age z score; HAZ, Height for age z score; BMI, Body mass index

Table 2: Reference Ranges for Serum Cystatin C and Creatinine by Sex and Age

Characteristic (n)	Serum Cystatin C*	Serum Creatinine†
Sex		
All (59)	0.73 (0.41–1.04)	0.51 (0.28–1.21)
Male (36)	0.72 (0.39–1.05)	0.45 (0.30–1.00)†
Female (23)	0.74 (0.44–1.04)	0.55 (0.28–1.26)
Age in Years		
All Ages (59)	0.73 (0.41–1.04)	0.51 (0.28–1.21)
< 5 years (25)	0.71 (0.44–0.99)	0.41 (0.28–1.15)‡
≥ 5 years (34)	0.74 (0.40–1.08)	0.58 (0.32–1.26)†

**Values are mean (±1.96SD); † values are median (2.5th & 97.5th); ‡ Significant difference between Under-fives and older, other differences not significant*

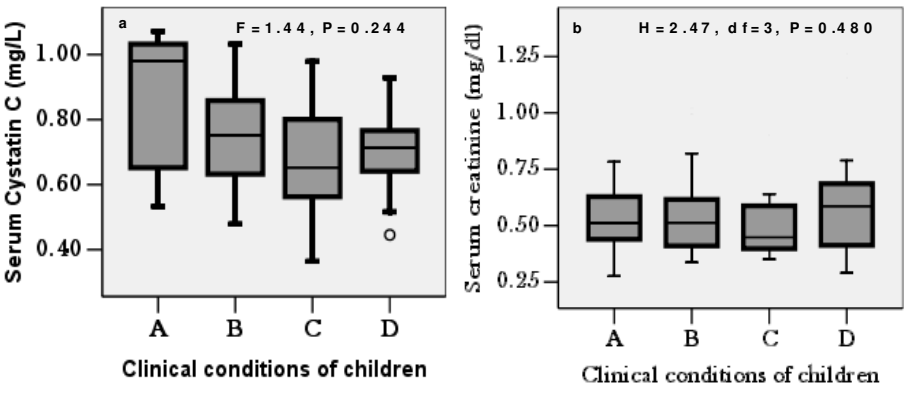

Figure 1: Box plot of Serum cystatin C (a) and serum creatinine (b) ranges by cohort of children studied. A, Children with isolated hearing & speech disorder; B, Children with seizure disorders; C, Children with post herniotomy; D, Children with bronchial asthma. *Note:* Depicts the similar serum cystatin C and creatinine levels between groups of children with different clinical conditions.

Figure 1 shows that the serum cystatin C level was similar in children with different underlying pathologies; the same pattern was observed for creatinine. There was no significant linear relationship between serum cystatin C and creatinine (r=0.039, p=0.769). Serum cystatin C showed no significant correlation with age, weight, length and body mass index while serum creatinine was statistically higher with older age, higher weight and higher length (Figure 2).

DISCUSSION

The study documented a mean (95% confidence interval) serum cystatin C level of 0.73 (0.41–1.04) mg/L among a clinic cohort of Nigerian children with no overt sign of kidney disease. Serum cystatin C level was similar between male and female children and between children less than 5 years and those older than 5 years. This was in contrast to creatinine which was significantly higher among older children and showed a higher trend among females. Similarly, unlike creatinine, there was no significant correlation between serum cystatin C with age, weight, length and body mass index.

The documented reference range for cystatin C in this study is similar to the ranges reported by most studies[4,9–12] in children. Although, some studies[13,14] have reported ranges significantly different from our results, difference in methods of analysis of cystatin C, subjects' characteristics, such as proportion of children younger than one year and level of kidney function could be responsible. The similarity of our serum cystatin C reference range with those reported in a large number of studies[4,9–12] involving children from different races implies no association between cystatin C and race; unlike creatinine which in adults have been shown to be higher in blacks.[15]

Consistent with other works[1,4,9,13] serum cystatin C level in this study, contrary to serum creatinine, was similar among male and female children and there was no significant linear relationship with age, height and weight. Explicably, this is due to the constitutive production of cystatin C by the house-keeping genes of all nucleated cells of the body and not

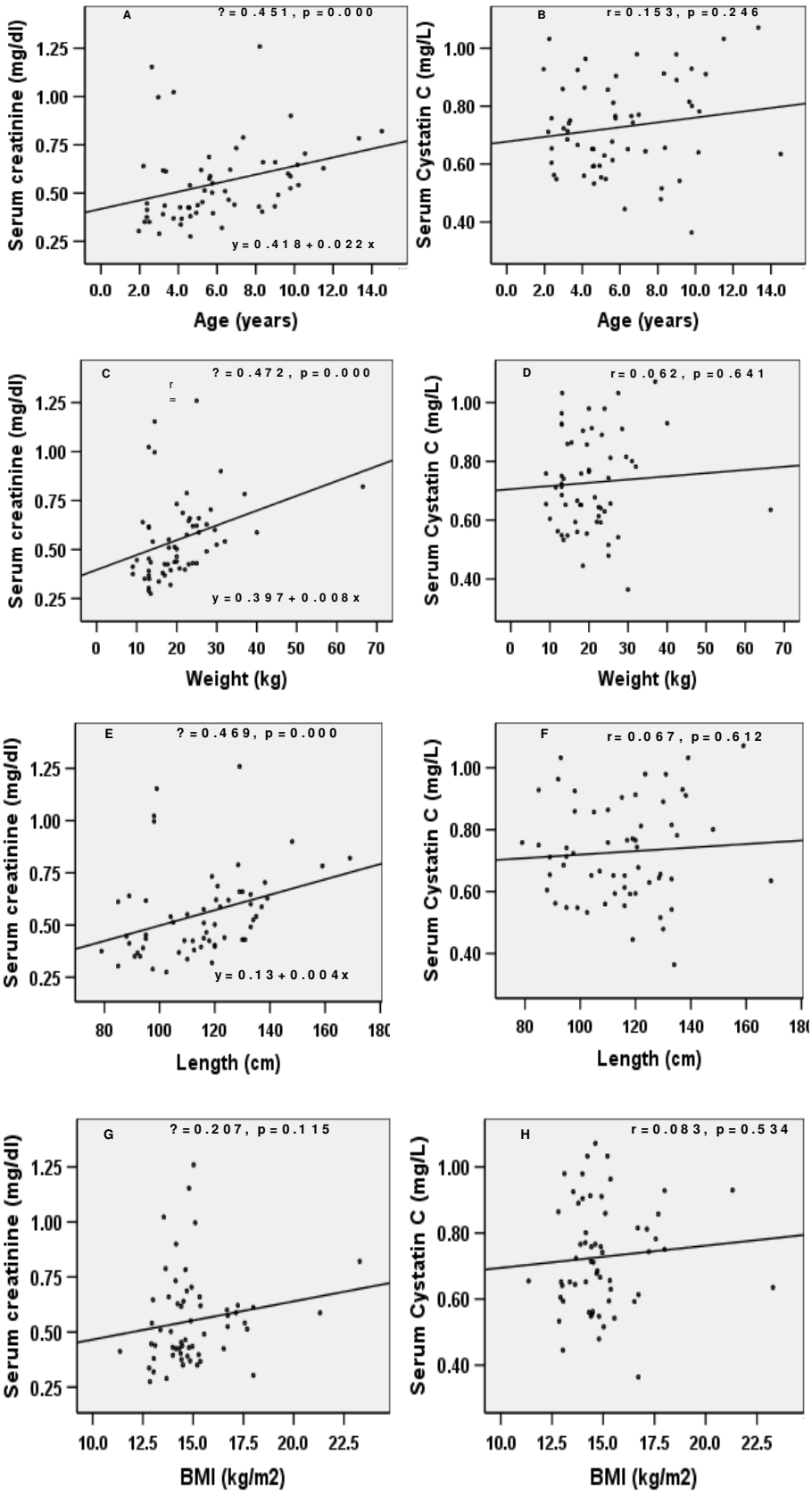

Fig. 2: Correlation of serum creatinine (A, C, G) and serum cystatin C (B, D, H) with age and anthropometric variables. There is shows a strong and significant correlation between serum creatinine with age, weight and length whereas the correlations of cystatin C with age and anthropometric variables are not significant.

by muscle cells alone as in creatinine.[3] These attributes, together with non-tubular secretion, recommend cystatin C over creatinine in the estimation of glomerular filtration rate and allows for the use of the same reference range for children aged two year to 14.5 years and children of both genders. The single reference range for cystatin C in our study and those published by other workers makes it easier for the clinician to recognize deranged kidney function unlike the experience with creatinine which requires knowledge of more than one reference range for different ages and gender. Furthermore, the weak correlation between cystatin C and anthropometric characteristics favours its use over creatinine in the assessment of kidney function in Nigerian children in whom malnutrition is common. The strong positive linear relationship between creatinine and common anthropometric variables in this study and the high prevalence of malnutrition among children in Nigeria implies that detection of kidney disease using serum creatinine has the potential of underestimating the true burden of kidney diseases in our children with significant public health implications.

The lack of a linear association between serum cystatin C and serum creatinine in this study is not unexpected and has been previously reported.[13] This observation is due to the contrasting correlations of the cystatin C and creatinine with age, gender and anthropometric variables as demonstrated in our study. This point was made by Bokenkamp and colleagues,[13] who showed that when the influence of height on creatinine was minimised by dividing creatinine level by height there was a strong correlation between cystatin C and creatinine and not before.

Limitations of our study included the use of clinic-based children rather than the general population of children and the measurement of cystatin C using ELISA technique. This was primarily informed by the perceived reluctance of parents to agree to their children participating in studies that involve bloodletting, moreso when the child is not sick. Not surprisingly, most of the published works on reference range of

serum cystatin C in children used those attending the hospital and some were acutely ill.[4,11] However, the similarity of serum cystatin C level in children with varying clinical conditions but no overt kidney disease further validates our results. ELISA technique was used because particle enhanced nephelometric immunoassay and particle enhanced turbidometric assay are not available in this region of the world and if cystatin C would be measured in the evaluation of children suspected of having kidney disease in this part of the world, the ELISA technique would be used.

We acknowledge that our small sample size might have influenced some of our results especially the confidence interval. We also were not able to ascertain that the children all had normal glomerular filtration rate as this would have entailed the use of inulin clearance, the gold standard technique for GFR, which is cost and labour intensive and not available in our region. However, the criteria for eligibility were robust enough to limit this shortcoming. Alternatively, the use of creatinine clearance would not have been ideal as this is fraught with several shortcomings.[15,16]

In conclusion, we have determined the reference range of serum cystatin C in Nigerian children with no overt kidney disease and have shown that it is similar across gender and across a wide age range after two years of age.

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DUALITY OF INTEREST

None declared.

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