Establishment of serum Cystatin C reference range for healthy Sri Lankan adults – A preliminary study

Final Report on the NSF grant No: RG/2011/HS/21

Dr. P. P. R. Perera

Dept. of Biochemistry,

Faculty of Medical Sciences,

University of Sri Jayewardenepura.

Section 1

Information regarding Project / Project personnel

1. **Grant number** : RG/2011/HS/21
2. **Title of the project** : Establishment of serum cystatin C reference range for healthy Sri Lankan adults – A preliminary study
3. **Principle investigator** : Dr. P.P.R. Perera
4. **Co-investigator** : Nil
5. **Institutes where research was being carried out :**
   - Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura
   - Colombo South Teaching Hospital
   - Family Practice Center, Faculty of Medical Sciences, University of Sri Jayewardenepura.
6. **Date of the award** : 30/12/2011
7. **Date of completion of Project** : 01. 06 .2013
8. **Total allocation of funds (Rs)** : Rs. 589,250.00
9. **Total spent (Rs)** : Rs. 499,987.12
10. **Number of research students employed** : Nil
11. **Post graduate degree completed within dates : Not applicable**
12. **Number of Technical Assistants and / or labours employed and periods of service** : Nil
13. **Publications / Communications arising from the project during the reporting period :**

Wijayatunga N, Perera R, Peiris H , Wanigasuriya K. Reference intervals for serum cystatin c in healthy sri lankan adults- a preliminary study. Submitted to **4th PGIM Academic Sessions** (Accepted for a poster) – Refer annexure 1

Section 2

Executive summary of the project (200-250 words)

Serum cystatin C (SCys) is a new marker of glomerular filtration rate and it also predicts morbidity and mortality in cardio vascular disease. Our aim was to study to identify associations of gender and age with SCys and to identify reference range for Scys in a selected apparently healthy Sri Lankans adults .

Hundred and eighteen apparently healthy adults which included 44.9 % males and 55.1% females between 30- 60 years were recruited. Those with diseases and on drugs causing an increase in SCys, blood pressure more than 140/90, random blood sugar more than 200mg/dl , and with estimated GFR < 60 ml/min/1.73m ^{2} and with albumin to creatinine ratio>30mg/g were excluded. SCys was measured using particle enhanced immunoturbidimetry using Kone 20XT auto analyzer.

Mean serum cystatin C in the 118 healthy subjects was 0.81 mg/l (± 0.12) and levels ranged from 0.5 to 1.14 mg/L . There was no significant ($p>0.05$) difference in mean age between males and females. A significantly($p<0.05$) higher mean SCys was observed in 51-60 years group and males had a significantly higher mean SCys than females in both 30-50 and 51-60 years groups. Reference ranges for males and females in 30 -50 years were 0.62-1.02 mg/l and 0.55-0.91 mg/l respectively. In the 51-60 years age group the male and female reference ranges were 0.65 – 1.13mg/l and 0.62 – 1.01mg/l respectively.

In selected healthy Sri Lankan adults, age and gender is associated with SCys levels and those need to be considered when interpreting Scys values.

Section 3: Report in detail:

1. Introduction /Back ground

The prevalence of chronic kidney disease (CKD) is rising in Sri Lanka and in the world. Early detection of renal impairment will enable the clinicians to treat the patients effectively and to minimize the complication arising from CKD. (1-2).

CKD is defined as abnormalities of kidney structure or function, present for >3 months. Either one of the following should be present for more than 3 months.

1. Markers of kidney damage (one or more) such as albuminuria (Albumin excretion rate ≥30 mg/24 hours; Albumin to creatinine ratio ≥30 mg/g [≥3 mg/mmol]), urine sediment abnormalities, electrolyte and other abnormalities due to tubular disorders, abnormalities detected by histology, structural abnormalities detected by imaging, history of kidney transplantation
2. Decreased glomerular filtration rate (GFR) less than 60 ml/min/1.73 m2 (3).

GFR is the sum of filtration rate of all functioning nephrons of the kidney and it is accepted as the best overall index of kidney function (1). The reference range of GFR in young individuals varies from 80-130 ml/min/ 1.73m^2(4).

GFR measured by exogenous filtration markers (mGFR) is considered to be the gold standard. But it is costly, time consuming, labour intensive, not entirely free of risk to patients and can be performed only in specialized laboratories. Serum creatinine (SCr) and serum cystatin C(SCys) are endogenous markers of GFR , which are more convenient to measure and are cheaper (5).

In Sri Lanka, SCr is the most widely used index for non invasive assessment of GFR as it is cheap. But it is not an ideal marker to measure glomerular filtration rate due to several reasons. The amount of creatinine produced by a person per day is relatively constant but it is proportional to the muscle mass (6). Thus SCr values are affected by sex, age , ethnicity, muscle mass, chronic illness and meat consumption(5, 7). Creatinine is freely filtered by the glomerulus and not reabsorbed by the renal tubules but about 15% is secreted by the proximal tubules (5). Thus a decrease in GFR to around 40 ml/min/1.73m^2 does not cause an increase in SCr above the normal upper limit. Therefore the relationship between GFR and SCr is a reciprocal non-linear one. (8).This may cause SCr levels to be within the normal range even with a GFR close to 60ml/min/1.73m^2 and this is called as the "creatinine blind range" (9). Jaffé method is commonly used to measure serum creatinine despite the lack of specificity. Non creatinine chromogens such as bilirubin, ascorbic acid, acetic acid, pyruvate, glucose, proteins can cause color reactions with this method leading to 15-25% overestimation of serum creatinine levels. (7, 9-10).

Thus on the quest for a better marker of GFR, serum cystatin C was discovered. It was discovered in 1961 and since 1985 SCys has been described as a promising endogenous marker of GFR (9). Cystatin C, is a 122-amino acid ,basic, low molecular weight protein

with a molecular mass of 13 kD (7, 11). It is found in all major human biological fluids such as urine, human plasma, CSF, ascitic and pleural fluid(12-13). Cystatin C is the most potent inhibitor of cysteine proteases(11). Cystatin C gene found in chromosome 20, has some features of housekeeping genes and the production of cystatin C occur at a constant rate by all nucleated cells of the body ,(7, 12-13). Cystatin C is positively charged molecule which is freely filtered by the glomerulus, then fully reabsorbed and catabolized in the proximal tubules. Thus the it's concentration in urine is very small (0.03-0.3 mg/l) . Therefore even though urine clearance of cystatin C cannot be used to measure GFR, plasma or SCys concentration is a good measure of GFR(7, 13). SCys , the new marker for GFR testing is currently available in private sector but is not routinely used in clinical practice in Sri Lanka.

SCys also shows an inverse, non linear relationship with GFR but in contrast to SCr , and when GFR falls to 40- 70 ml/min/1.73m^2 the proportional increase of SCys is higher (5) . Thus when there is only a mild reduction in GFR when changes in SCr does not occur, changes in serum cystatin C levels may be observed (8). Thus SCys may be able to identify the gradient of kidney function among patients who are not diagnosed as clinical kidney disease by conventional definition. Those with abnormal Scys levels ($\geq$1mg/l) , but estimated GFR more than 60ml/min/1.73m ^{2} based on SCr, were proposed to be called as patients with "preclinical kidney disease" (9).

By comparison of SCys levels and also cystatin C derived equations with gold standard methods of GFR measurements, it was found that SCys or the reciprocal of SCys is superior or at least equivalent to SCr to detect reduced GFR(7). SCys was found to correlate better with inulin clearance than SCr and to be more accurate in children, patients with malnutrition or chronic diseases who have low levels of creatinine due less muscle mass and also in the elderly who develop physiological reduction in renal function due to age and also have decrease in muscle mass. Many studies found in the diabetic population SCys to be a better indicator of GFR than SCr and showed better best correlation with changes in GFR than SCr and also was identified that SCys is useful for follow up of patients with diabetes(7). In early diabetic nephropathy , SCys more closely followed the measured GFR than SCr (14). In patients with abnormal muscle mass due to myasthenia, leg amputation, paraplegia and in those with liver cirrhosis where there is reduced metabolism of creatinine, detection of reduced renal function may be inaccurate with SCr and creatinine based eGFR measurement of SCys will be useful. Early detection of nephro toxicity of cisplatin is possible with SCys than by creatinine based eGFR(5, 15).

Higher levels of SCys are shown to be associated with increased mortality and cardiovascular outcomes like myocardial infarction, heart failure. Subjects without CKD but with SCys more than 1mg/L had a 4 times higher risk of developing CKD after 4 years(7). In patients with cardio vascular disease, elevated SCys levels has been identified as a marker of poor prognosis and significantly correlated with the severity heart failure according to the New York Heart Association heart failure functional classes (15).

A triple marker approach using SCr , SCys and albuminuria may improve diagnosis , help to determine prognosis and identify risk and of death and end stage renal failure than using SCr or albuminuria alone. This may minimize unnecessary referrals but at the same time help to take necessary actions for individuals at a higher risk (16).

Reviews have described Scys as not having circadian rhythm and that SCys not being affected by age, sex, muscle mass and ethnicity (9, 15, 17). Several earlier studies have shown that SCys range in the normal population is constant after the age of 1year(11). Some describe that SCys rises in an age dependent manner and also correlates with decrease in GFR after 50years of age (5). An increase in prevalence of abnormal SCys with age occurs and Köttegen et al. showed that only 1% in 20-40 yrs and over 50 % in the >80yrs group had abnormal SCys levels(14). In the literature, some studies report differences between genders(18-19) while others do not report such a difference and they had reported reference ranges only considering the age(20). Knight et al found that older age, male gender, greater weight, greater height, current smoking, and high CRP levels were independently positively associated with SCys levels(21). There is wide variation in reference ranges studied in different countries. The 2 major methods that are used to measure SCys are particle enhanced immunoturbidimetry (PETIA) and particle enhanced immunonephelometry (PENIA) methods. ELISA kit which is used has a poor performance. A wide variation in SCys reference ranges is seen when different assays are used (9).

So far data on factors affecting SCys and its reference range in adult Sri Lankans has not been elucidated. In order to use SCys in our clinical setup more accurately, it is essential for the clinicians to have an insight regarding the association of Scys with gender and age and to have a reference range for SCys in healthy Sri Lankans. Clinicians can use the knowledge of non GFR determinants of SCys to assist interpretation of serum levels of cys C and eGFR based on it. Thus, we conducted a preliminary cross sectional study to investigate reference ranges for SCys in a group of healthy volunteers in Sri Lanka to establish reference ranges and to identify association of age and gender with SCys levels.

2. Scientific scope of the project

General Objective

- To identify adult reference intervals for serum Cystatin C in Sri Lankans.

Specific Objectives

1. To identify if gender is affecting the serum cystatin C levels.
2. To identify if age is affecting serum cystatin C values.
3. To identify adult reference range for serum cystatin C

3. Materials and methods

Apparently healthy persons were recruited for the study from Colombo South Teaching hospital and Family Practice Center, Faculty of Medical Sciences, University of Sri Jayewardenepura. Exclusion criteria for the controls were, previously diagnosed diabetes, renal, hypertension, chronic lung disease, liver disease, cardio vascular disease , rheumatoid arthritis, hyperthyroidism, hypothyroidism , pregnancy, menstruating females at the time of urine collection, history of fever, infection within one week , received vaccination or immunization in previous 3 weeks and on drugs that may influence renal function or cystatin C concentrations (i.e. antihypertensive, diuretics, anti-inflammatory agents, hypoglycemic agents, anti-convulsants, anti-cancer or anti-viral drugs and antibiotics). Subjects with features of hypo or hyperthyroidism, peri-orbital oedema, pitting ankle edema, jaundice, signs of arthritis, pyrexia and infected wounds were also excluded after a general examination as well as those with body mass index (BMI) more than 30. Random blood glucose levels were checked using One Touch Ultra glucometer and those with values more than 200mg/ dl were excluded (22).

Using an interviewer administered questionnaire information on age, sex, ethnicity, level of education, past medical history and drug history were recorded from the subjects. General physical examination was carried out. Height was measured using a stadiometer and recorded to the nearest 0.1 cm. Weight was measured with a digital scale and recorded to the nearest 0.1kg. Waist was measured by positioning the measuring tape midway between the lower rib margin and the iliac crest at the end of normal expiration. Hip circumference was measured at the maximum circumference over the buttocks. Blood pressure was measured in the seated position resting for at least 5 minutes using mercury column sphygmomanometer. Two readings were taken and the mean was used for analysis.

Random spot urine samples were collected into sterilized plastic 30ml urine collector for determination of albumin and creatinine. Three ml blood was drawn for analysis of serum creatinine and serum cystatin C. All specimen of blood were transported to the lab within

4hours of collection and blood was centrifuged at 3500 rpm for 10 minutes and serum was separated and 2 aliquots were stored at -20^{0}C for analysis . Biochemical analysis was carried out on Kone 20XT auto analyzer at the Clinical Laboratory at the Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura.

Serum creatinine and urine creatinine levels were measured using Biolabo Creatinine Kinetic method kit (Jaffe reaction). Urine albumin was measured using Konelab Microalbuminuria MST kit (Immunoturbidimetric method). Albumin to creatinine ratio was calculated as Albumin (mg)/ Creatinine (g). Estimated glomerular filtration rate ($eGFR_{MDRD}$) was calculated using Modification of Diet in Renal Disease (MDRD) equation for standardized creatinine . BMI was calculated as weight / height^2 as kg/cm^2. After exclusion of participants with $eGFR_{MDRD}$ as less than 60 ml/min/1.73m^2 and ACR more than 30mg/g, 118 were enrolled for the study since eGFR < 60/ml/min/1.73m^2 is the threshold defining Chronic Kidney Disease (CKD) by the international Kidney Disease Improving Global Outcomes Organization (KDIGO) using the simplified Modification of Diet in Renal Disease Study (MDRD) equation and ACR>30mg /g indicates albuminuria(3).

Serum cystatin C was measured using Konelab cystatin C kit (Particle enhanced Immunoturbidimetry). Detection limit was 0.18 mg/l whilst measuring range for serum cystatin c was from 0.44- 7 mg/l. Two levels of cystatin C quality controls , cystatin C control (code 981913) and cystatin C control high (code 981914) were used before assay of each batch of samples to ensure that assay values were within ± 2SD of the specified concentration. This assay shows a within run percent coefficient of variation of (CV%s) of 1.4% when mean is 0.7mg/l, 2.6% when mean is 1.49mg/l and 1.2% when 0.5% when mean is 71mg/l. The %CV of between runs was 1.1% for low and 0.7% for high cystatin C. The study was approved by the Ethical Review Committee of Faculty of Medical Sciences, University of Sri Jayewardenepura and also by the Ethical Review Committee of Colombo South Teaching Hospital. Written informed consent was taken from each and every participant before the interview, examination, measurement of anthropometry and collection of blood and urine samples.

Statistical analysis.

Data were entered to Microsoft Excel (Richmond, Washington) and analyzed using SPSS version 17 (Chicago, Illinois). Continuous variables are presented as mean ± standard deviation(SD). Normal distribution of data was confirmed using the Kolmogorov-Smirnov (K-S) test and $p < 0.05$ was considered statistically significant. Pearson correlation analysis was used to determine correlation between continuous variables. Comparisons of continuous variables between groups of study were performed using the independent samples *t*-test and the Analysis of Variance (ANOVA). Values of $p<0.05$ were considered significant. Subgroups analyses were performed by gender, age category (i.e., 30–40, 41–50, and 51-60years old). Reference intervals were derived using the mean± 2 SD as estimates of the 2.5th and 97.5th percentiles and it covers 95% of the central observations and is considered as the normal interval for SCys.

4. Results / outputs

The study group comprised of 118 participants with 53 (44.9%) males and 65 (55.1%) females and there was no significant difference in mean age between the two groups. (48.4± 8.60 years for males and 49.9 ± 7.97 years for females).

CysC values showed a normal distribution when considering all healthy participants (K-S p = 0.2) (Figure 1A).When considering all the 118 healthy participants , the mean SCys level was 0.81 mg/L with a SD of 0.12 and levels ranged from 0.5 to 1.14 mg/L .

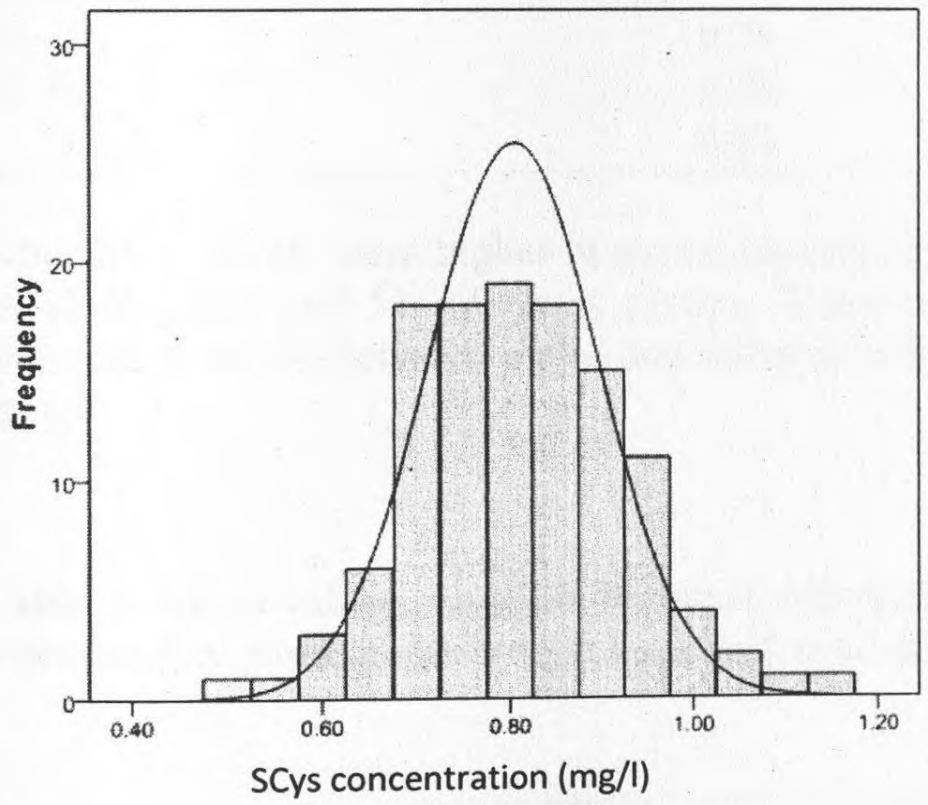

Figure 1: Frequency distribution of values of serum cystatin C in 118 study subjects.

A significant difference in mean values of SCys levels was observed between age groups of 30-40 and 41-50 years and 51- 60 years when considering all the participants. Also the males were having a higher mean SCys value than females.

Table 1: Mean values and standard deviation of serum cystatin C levels among subgroups of apparently healthy participants in the study.

	Mean Cystatin C (mg/L)	SD	*P*
All participants	0.81	0.12	
Gender			
Male	0.85	0.11	<0.001
Female	0.77	0.10	
Age group according to decades (years)			
30-40	0.75	0.11	<0.05
41-50	0.78	0.02	
51-60	0.84	0.01	

Since SCys levels were higher in above 50 years old participants, they were dichotomized as 30-50 years and 51- 60 years groups. There was a significant ($p<0.05$) difference in mean SCys levels between males and females in both 30-50 years group and 51-60 years group.

Table 2: Mean values, standard deviation and reference ranges of serum cystatin C levels when healthy participants were categorized according to age and gender.

	Mean Cystatin C (mg/L)	SD	*P*	Reference range
In 30- 50 years age group				
Males	0.82	0.10	<0.05	0.62 – 1.02
Females	0.73	0.09		0.55 – 0.91
In 51-60 years age group				
Males	0.89	0.12	<0.05	0.65 – 1.13
Females	0.81	0.10		0.62 – 1.01

A significant ($p<0.05$) but weak correlation was observed between age and SCys in the healthy subjects ($r= 0.34$).

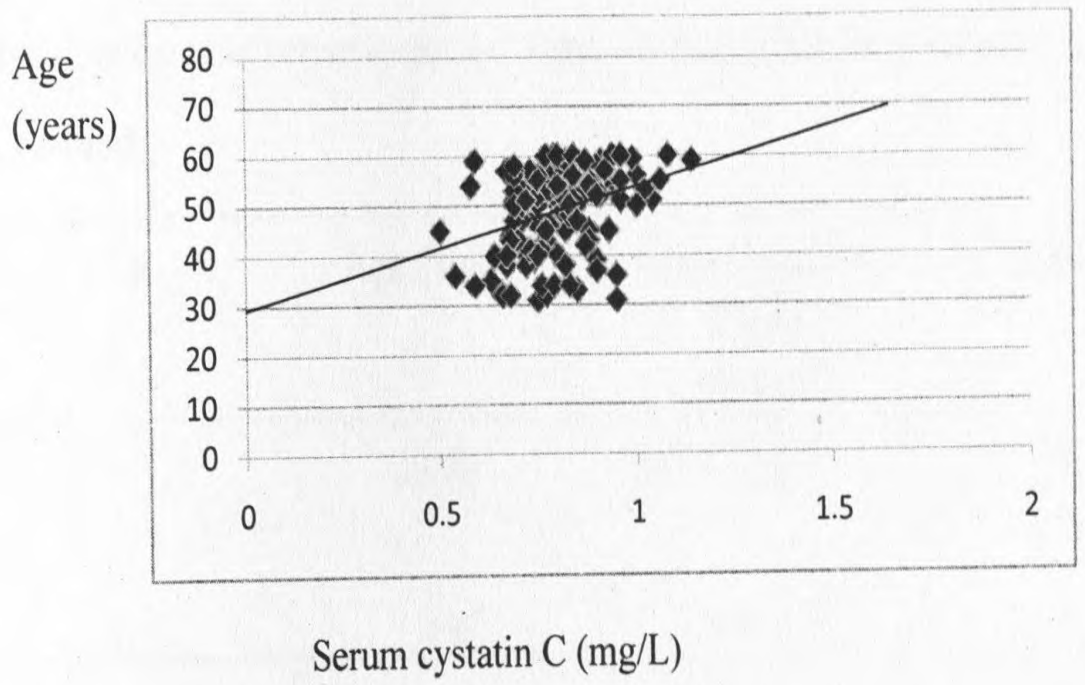

Figure 2 : Serum cystatin C levels (both sexes) in relation to age.

We studied regarding the effects of age and sex on SCys levels and to report on reference intervals for Sri Lankan healthy adults. These are the first preliminary reference intervals for SCys in healthy adults reported in Sri Lanka. In our study we have carefully recruited apparently healthy individuals who are not with any renal impairment or other medical conditions that would affect SCys levels.

Some studies have found at age, gender, weight , height, smoking and high CRP levels were independently positively associated with SCys levels(21). In our study we found that SCys C levels were affected by age. SCys was higher in those above the age of 50 years than the participants below the age of 50 years. Also a significant but weak correlation was found between age and SCys levels.

There is wide variation in reference ranges studied in different countries. A wide variation in SCys reference ranges is seen when different assays are used. (Table 1) This could be due to ethnic differences and also due to the methods used to measure SCys.

Table 3: Comparison of serum cystatin C values in healthy adults as reported in literature

PETIA method

Gender	n	Age (Years)	Serum Cystatin C (mg/L) 95% CI	Reference
Both		20-50	0.70-1.21	(19)
Both		>50	0.84-1.55	
Both	162		0.41–0.92	(23)

PENIA method

Gender	n	Age (Years)	Serum Cystatin C (mg/L) 95% CI	Reference
Males	216	20-59	0.74±0.100	(24)
Females	216	20-59	0.65 ± 0.085	
Both	92	≥60	0.83±0.103	
Both		60-79	0.93 - 2.38	(25)
Both		>80	1.07 - 3.35	
Both	139	18-78	0.51 - 0.92	(26)
Males		31.2±9.2	0,71-1.02	(27)
Females		31.2±9.2	0.58 – 0.98	
Males		30-50	0.60 – 0.95	(18)
Females		30-50	0.55- 0.84	
Both		51-75	0.64 – 1.05	
Both		<50	0.53 - 0.92	(20)
Both		>50	0.58 - 1.02	
Both		<45	<0.95 mg/L	(28)
Both		>45	<1.20 mg/L	

Table 4 : Summary of Scys reference ranges in our study .

Gender	Age (Years)	Serum Cyststain C (mg/L) 95% confidence interval
Males	30-50	0.62 – 1.02
Females	30-50	0.55 – 0.91
Males	30-50	0.65 – 1.13
Females	30-50	0.62 – 1.01

Gender difference has not been consistently observed in the past studies. In some studies higher SCys was found in males similar to our study (24) (27) (18). In our study, there was a significant difference between males and female in both 30-50 years and 51-60 years age groups. In contrast the mean cys levels were higher in healthy Saudi females than males (23).

The manufacturer of the cystatin C immunotrubidimetric kit had suggested the reference ranges according to age as 0.70-1.21mg/L for between 20-50 years and 0.84-1.55mg/L for >50 years of age. But our reference ranges were lower than those.

6. Conclusions

This is the first study for determination of cystatin C reference values in a Sri Lankan healthy adult population. Age and gender needs to be considered when interpreting serum cystatin C.

Our findings demonstrate that serum cystatin C levels are higher in males and in healthy adults above the age of 50 years. We obtained reference values lower than previously published reference ranged using PEITA.

7. References

1. Stevens LA, Coresh J, Greene T, Levey AS. Assessing Kidney Function — Measured and Estimated Glomerular Filtration Rate. New England Journal of Medicine. 2006;354(23):2473-83.
2. James MT, Hemmelgarn BR, Tonelli M. Early recognition and prevention of chronic kidney disease. The Lancet. 2010;375(9722):1296-309.
3. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney International. 2013;Suppl(3):1-150.
4. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic Nephropathy: Diagnosis, Prevention, and Treatment. Diabetes care. 2005;28(1):164-76.
5. Thomas C, Thomas L. Renal Failure—Measuring the Glomerular Filtration Rate. Deutsches Ärzteblatt International . 2009;106(51-52):849-54.
6. Burtis C.A. AER, Bruns D.E. Tietz fundamentals of clinical chemistry.363-36.
7. Chew JS, Saleem M, Florkowski CM, George PM. Cystatin C-A Paradigm of Evidence Based Laboratory Medicine Clincal Biochemistry Reviews. 2008;29(2):47-62.
8. Ferguson MA, Waikar SS. Established and Emerging Markers of Kidney Function. Clinical Chemistry. 2012;58(4):680-9.
9. Salgado JV, Neves FA, Bastos MG, França AK, Brito DJ, Santos EM, et al. Monitoring renal function: measured and estimated glomerular filtration rates - a review. Brazilian Journal of Medical and Biological Research. 2010;43:528-36.
10. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for Improving Serum Creatinine Measurement: A Report from the Laboratory Working Group of the National Kidney Disease Education Program. Clinical Chemistry. 2006;52(1):5-18.
11. Brguljan PM, Cimerman N. Human Cystatin C. Türk Biyokimya Dergisi [Turkish Journal of Biochemistry-Turk J Biochem] . 2007; 32(3):95-103.
12. Abrahamson M, Olafsson I, Palsdottir A, Ulvsbäck M, Lundwall A, Jensson O, et al. Structure and expression of the human cystatin C gene. Biochemistry Journal. 1990;268(2):287-94.
13. Randers E, Erlandsen EJ. Serum Cystatin C as an Endogenous Marker of the Renal Function â€“ a Review. Clinical Chemistry and Laboratory Medicine. 1999;37(4):389-95.
14. Shlipak MG. Cystatin C: Research Priorities Targeted to Clinical Decision Making. American Journal of Kidney Disease. 2008;51(3):358-61.
15. Abdelmalek JA, Rifkin DE. Cystatin C, Creatinine, and Albuminuria: Bringing Risk Into 3 Dimensions. American journal of kidney diseases. 2012;60(2):176-8.
16. Peralta CA, Katz R, DeBoer I, Ix J, Sarnak M, Kramer H, et al. Racial and Ethnic Differences in Kidney Function Decline among Persons without Chronic Kidney Disease Journal of the American Society of Nephrology. 2011 22 (7):1327-34
17. Dabla PK. Renal function in diabetic nephropathy. World Journal of Diabetes. 2010;1(2):48-56.
18. Ichihara K, Saito K, Itoh Y. Sources of variation and reference intervals for serum cystatin C in a healthy Japanese adult population. Clinical Chemistry Laboratory Medicine.2007;45(9):1232-6.

19. Norlund L, Fex G, Lanke J, von Schenck H, Nilsson JE, Leksell H, et al. Reference intervals for the glomerular filtration rate and cell-proliferation markers: serum cystatin C and serum Î²2-microglobulin/cystatin C-ratio. Scandinavian Journal of Clinical & Laboratory Investigation. 1997;57(6):463-70.
20. Finney H, Newman DJ, Price CP. Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. Annals of Clinical Biochemistry. 2000; 37 (1):49-59
21. Knight EL, Verhave JC, Spiegelman D, Hillege HL, De Zeeuw D, Curhan GC, et al. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kideny International. 2004;65(4):1416-21.
22. Executive Summary: Standards of Medical Care in Diabetes—2012. Diabetes care. 2012;35(Supplement 1):S4-S10.
23. Al Wakeel JS MN, Chaudhary AR, Mitwalli AH, Tarif N, Isnani A, Hammad D. Normal Reference Levels of Serum Cystatin C in Saudi Adults. Saudi Journal Kidney Disease and Transplant. 2008(19):361-70.
24. Galteau M-M, Guyon M, Gueguen R, Siest Gr. Determination of Serum Cystatin C: Biological Variation and Reference Values. Clinical Chemistry and Laboratory Medicine. 2001;39(9):850-7.
25. Finney H, Bates CJ, Price CP. Plasma cystatin C determinations in a healthy elderly population. Archives of Gerontology and Geriatrics. 1999;29(1):75-94.
26. Uhlmann EJ, Hock KG, Issitt C, Sneeringer MR, Cervelli DR, Gorman RT, et al. Reference Intervals for Plasma Cystatin C in Healthy Volunteers and Renal Patients, as Measured by the Dade Behring BN II System, and Correlation with Creatinine. Clinical Chemistry. 2001;47(11):2031-3.
27. Croda-Todd M, Juarez E, HernÃ¡ndez P, Flores G, Rivera G, Bocanegra-Garcia V. Reference intervals for serum cystatin C in healthy Mexican adults. Clinical Chemistry and Laboratory Medicine. 2007;45(7):925-7.
28. Ognibene A, Mannucci E, Caldini A, Terreni A, Brogi M, Bardini G, et al. Cystatin C reference values and aging. Clinical Biochemistry. 2006;39(6):658-61.

8. Problems if any encountered during implementation of the project

- None (except for delay in receiving funds from the NSF initially)

9. Major findings and follow up activities

Age and gender needs to be considered when interpreting serum cystatin C.
Our findings demonstrate that serum cystatin C levels are higher in males and in healthy adults above the age of 50 years.

Section 4

Impact of Research results:

1. **Relevance of results achieved to scientific advancement**

Identification of impact of age and sex on serum cystatin C and identification of normal reference ranges accordingly will enable to distinguish abnormal values of cystatin C I future research.

Also this is the starting point for the development of more extensive research on non glomerular factors affecting serum cystatin C levels in Sri Lankans.

2. **Relevance of results achieved to national/socio-economic development**

These findings would help the clinicians in better diagnosis and risk prediction of renal impairment and cardio vascular diseases using serum cystatin C normal reference ranges. Early diagnosis would prevent deterioration of renal function and may on long term help to reduce cost of dialysis in Sri Lanka.

3. **Dissemination / application of research output**

Wijayatunga N, Perera R, Peiris H , Wanigasuriya K. Reference intervals for serum cystatin c in healthy Sri Lankan adults- a preliminary study. Accepted for a poster presentation at 4th **PGIM Academic Sessions** – July 2013

Section 5

Miscellaneous

1. **List of major equipments acquired during the project period and their functionality**

- Nil

2. **List of publications / communications arising from the project and / or presentation made at seminars , workshops , etc (Please attach copies)**

Abstract
Wijayatunga N, Perera R, Peiris H , Wanigasuriya K. Reference intervals for serum cystatin c in healthy Sri Lankan adults- a preliminary study. Submitted to 4th **PGIM Academic Sessions- July 2013**
(Accepted for poster presentation)

Section 6

Summary statement of expenditure (indicate Personnel, equipment, consumables, travel, subsistence and miscellaneous)

Attached (Annexure 2)

Section 7

1. Grantee's signature

.....

Dr. P. Pradeep Rasika Perera
Senior Lecturer
Department of Biochemistry
Faculty of Medical Sciences
University of Sri Jayewardenepura

2. Comments of head of the Department

.....Recommended.....

.....

Signature: .....

HEAD, DEPARTMENT OF BIOCHEMISTRY
FACULTY OF MEDICAL SCIENCES
UNIVERSITY OF SRI JAYEWARDENEPURA
GANGODAWILA
NUGEGODA
SRI LANKA.

3. Head of the institution's signature :

.....

Dr. N. L. A. Karunaratne
Vice-Chancellor
University of Sri Jayewardenepura
Nugegoda, Sri Lanka.

Annexure 1

REFERENCE INTERVALS FOR SERUM CYSTATIN C IN HEALTHY SRI LANKAN ADULTS- A PRELIMINARY STUDY

Wijayatunga N[1]; Perera R[1]; Peiris H[1] ; Wanigasuriya K[2].

[1] *Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka*

[2] *Department of Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka*

Introduction and objectives: Serum cystatin C(ScysC) is a better marker of Glomerular Filtration Rate(GFR) than serum creatinine. ScysC reference values for Sri Lankans are not available. Our objective was to determine ScysC reference values for healthy Sri Lankan adult population.

Methods: Healthy volunteers between 30-60years were selected from Colombo South Teaching Hospital and Family Practice Center, University of Sri Jayewardenepura. SCysC and urine albumin were measured by Immunoturbidimetry and Creatinine Kinetic method used for serum and urine creatinine . GFR was estimated ($eGFR_{MDRD}$) using Modification of Diet in Renal Disease Study equation and Albumin to Creatinine Ratio (ACR) (mg/g) was calculated. Subjects with eGFR less than 60ml/min/1.73m^2 and ACR more than 30mg/g were excluded. Subjects were categorized into age groups of 30-39, 40-49 and 50-60 years.

Results: Of the selected 116 volunteers (55.2% females and 44.8% male), ScysC was significantly($p<0.05$) higher in 51-60years group than 31-40 and 41-50years groups. ScysC for males and females in 30- 50years were 0.81±0.1 and 0.71±0.07 mg/L respectively. In 50- 60years group ScysC for males and females were 0.9±0.14 and 0.81±0.1mg/L respectively. There were significant differences ($p<0.05$) between male and female values .

Discussion:. Serum cystatin C level was higher in males and in adults above 50 years of age. Thus SCysC reference values adjusted for gender and age is needed for Sri Lankan adults. We suggest ScysC reference

intervals for less than 50years of age as 0.81±0.2mg/L for males and 0.71±0.14mg/L for females while 0.9±0.28mg/L for males and 0.81±0.2mg/L for more than 50 years of age.

Presenting authours's contact details

- Email - nadeejawi@yahoo.com
- 98,Sri Rahula Mawatha, Katubedda, Moratuwa
- 0776640181,0112758596
- 0112605162 - fax

Authors

- N. Wijayatunga
- R. Perera
- H. Peiris
- K. Wanigasuriya

Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

Department of Medicine, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

Key words

- Cystatin C
- Reference ranges
- GFR

Grant Number -: RG/2011/HS/21
Statement of Funds Received & Expenditure
For the quarter ending -15th July 2013

	Total Funds Received RS.	Total Expenditure Rs.	Balance Rs.
Personnel - Research Student	-	-	-
Technical Assistant	-	-	-
Other			-
Equipment	-	-	-
Consumable	3,000.00	-	3,000.00
Travel & Subsistance	750.00	-	750.00
Miscellaneous	7,500.00	-	7,500.00
PG Registration fee			-
Lab Services	578,000.00	499,987.12	78,012.88
Other			-
Total	589,250.00	499,987.12	89,262.88

Prepared By -

Unspent balance of the funds received

Funds received	589,250.00
Actual Expenditure	499,987.12
Balance	89,262.88
Cash imprest/Cash advance	0
Balance	89,262.88

Prepared By - 7/15

Snr. Assist. Bursar /Accountant

C.c -: Dr.P.R.Perera - Dept.of Biochemistry 15/07/2013

National Digitization Project

National Science Foundation

Institute : National Science Foundation

1. Place of Scanning : Sanje (Private) Ltd. Hokandara

2. Date Scanned : ....2017./.04./.07.....

3. Name of Digitizing Company : Sanje (Private) Ltd, No 435/16, Kottawa Rd,
Hokandara North, Arangala, Hokandara

4. Scanning Officer

Name : ....Angelo Melvin.....

Signature : ....A. M. Lmd.....

Certification of Scanning

I hereby certify that the scanning of this document was carried out under my supervision, according to the norms and standards of digital scanning accurately, also keeping with the originality of the original document to be accepted in a court of law.

Certifying Officer

Designation : Information Officer

Name : Renuka Sugathadasa

Signature : R.P. Sugathadasa

Date : .....

"This document/publication was digitized under National Digitization Project of the National Science Foundation, Sri Lanka"