Original Article

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Biochemistry Section

Reference Interval of Serum Cystatin C in Dravidian Population Subset of South Asian Ethnic Groups: An Observational Cross-sectional Study

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ABSTRACT

Introduction: Serum Cystatin C (Cys C) facilitates to detect mild renal dysfunction and overall risk of death in older patients with chronic renal diseases. The requirement for creating a particular reference interval is mandatory for such renal diseases in South Asian population since the value of serum creatinine varies largely with age, gender and population/ethnicity and data of same is short for South Asian population.

Aim: To determine the reference interval of serum Cystatin C amongst the Dravidian population subset of South Asian ethnic groups.

Materials and Methods: This observational cross-sectional study was conducted in Department of Biochemistry and Transfusion Medicine at Government Medical College, Thrissur, Kerala, India, from July 2020 to January 2021 among on 235 healthy adults (20-60 years). A 4 mL of blood was drawn by aseptic precautions and serum was separated within three hours of blood collection. Serum Cys C was assayed by latex enhanced immunoturbidimetric cystatin C assay using a calibrator traceable to international standard European Specific Protein Reference Material (ERM DA-471) developed by the International Federation of Clinical Chemistry (IFCC) and Laboratory Medicine. Serum Creatinine (sCr) by Jaffe method in a fully automatic clinical chemistry analyser was assessed. Categorical variables are presented using frequency (percentage) while continuous variables are summarised with an interpercentile range. The Kolmogorov-Smirnov test was used to assess the normality of the distribution of continuous variables.

Results: Total of 235 subjects (20-60 years of age with 17 males and 218 females) were included in the study. The non parametric reference intervals for Cys C were found to be from 0.39 to 0.79 mg% and that of sCr ranged from 0.79 to 1.2 mg%. The relationship between Cystatin C with age showed an increase in Cystatin C levels as age advances; Spearman's rank correlation coefficient (rho=0.197). The weak correlation between Cystatin C and sCr was also observed (rho=0.37; p-value <0.0001).

Conclusion: The level of serum cystatin C (0.39 to 0.79 mg%) can be used as a diagnostic concentration reference interval of the protein that helps to recognise, standardise and establish it as a potential biomarker to detect renal disorders in South Asian ethnic population.

Keywords: Biomarkers, Contrast-induced nephropathy, Glomerular filtration rate, Renal diseases, Serum creatinine

INTRODUCTION

It has been estimated by the World Health Organisation (WHO), that approximately 85,000 patients suffer from Chronic Kidney Disease (CKD) every year across the globe, and around 17.2% of such cases are seen to be prevalent in India [1,2]. Studies have proven that the progression of kidney disease can be restrained or even prevented by early detection and establishment of appropriate treatment.

The use of estimated Glomerular Filtration Rate (eGFR) is recommended in current clinical practice guidelines for the assessment of specific renal diseases [3,4]. The most commonly used formulae comprises of the Cockcroft-Gault formula, the modification of diet in renal disease, and the Chronic Kidney Disease Epidemiology Collaboration formulae, which uses serum Creatinine (sCr) for estimating GFR [5]. Such measurements of eGFR consists of numerous drawbacks, such as the incapability to distinguish early renal dysfunction owing to its low sensitivity [6]. Owing to such restrictions, serum Cystatin C (Cys C) has been anticipated as an ancillary endogenous marker for renal diseases [7]. Cys C is easily filtered and is entirely catabolised at the proximal tubule without the help of tubular secretion. Cystatin C being produced by all nucleated cells, is a member of the cystatin superfamily of cysteine protease inhibitors. It is a non glycosylated, low molecular weight, basic protein, consisting of 120 amino acids [8-10]. There are numerous mechanised procedures that have been adopted to measure the protein like the Particle Enhanced Turbidimetric Immunoassay (PETIA), an in-house latex PETIA and a latex Particle Enhanced Nephelometric Immunoassay (PENIA) [11-13].

The assay of serum Cystatin C has several uses in clinical medicine wherein it proves to be a better marker than sCr to detect early stages of Contrast-induced Nephropathy (CIN) [14,15]. Serum Cystatin C can detect mild renal dysfunction and overall risk of death in older patients and higher mortality in cases of acute coronary syndrome [16-18]. Hence, the present study was planned to find the reference interval of serum Cystatin C amongst the Dravidian population subset of South Asian ethnic groups.

MATERIALS AND METHODS

This observational cross-sectional study was conducted in Department of Biochemistry and Transfusion Medicine at Government Medical College, Thrissur, Kerala, India, from July 2020 to January 2021. The approval was obtained from the Institutional Ethical Committee {letter no. B6-155/2019/MCTCR(28); dated- 02/03/2019}. A total of 252 healthy adults between the age group 20 to 60 years were selected after getting informed consent from volunteers of blood donation registered in the Transfusion Medicine Department.

Inclusion criteria: Healthy male and female patients aged between 20-60 years non smokers and not on steroids therapy, or any medications were included in the study.

Exclusion criteria: Subjects with Thyroid Stimulating Hormone (TSH) values outside the range of 0.4 to 4.5 mIU/L and with C-Reactive

Protein (CRP) levels more than 10 mg% [19] were excluded from the study.

Sample size: For the present study, sample size was set as 120 as per National Committee for Clinical Laboratory Standards (NCCLS) for finding out the reference interval of a biomarker [20]. An additional 132 subjects were included to accommodate the outliers and those with abnormal TSH and CRP values. Hence, total of 252 subjects were included in the study. After exclusion of 17 subjects on basis of serum TSH and CRP values, total subjects analysed were finally 235.

Procedure

All the included study participants were evaluated for their health status by history and clinical examination. A proforma was used for this purpose. A 4 mL of venous blood was drawn by aseptic precautions and serum was separated within 3 hours of blood collection. The estimation of serum Cystatin C and sCr along with other tests to establish exclusion criteria i.e., TSH and CRP was completed within 12 hours of blood collection.

- Serum Cystatin C was evaluated by latex enhanced immunoturbidimetric cystatin C assay using a calibrator traceable to international standard ERM-DA471 developed by the International Federation of Clinical Chemistry (IFCC) and Laboratory Medicine [21].
- Serum creatinine by Jaffe method in a fully automatic clinical chemistry analyser.
- TSH by electro chemiluminometer (Roch).
- CRP by immunoturbidimetry.

Cystatin C values of euthyroid and CRP negative (<10 mg%) subjects were used to determine the reference interval.

STATISTICAL ANALYSIS

MedCalc free trial version 18.9 was used for data analysis. There were no outliers identified by Turkey's method using MedCalc statistical software. Categorical variables are presented using frequency (percentage) while continuous variables are summarised with an inter-percentile range. The Kolmogorov-Smirnov test was used to assess the normality of the distribution of continuous variables. Reference Intervals (RIs) were determined by the non parametric method as described in the IFCC and Clinical and Laboratory Standard Institute (CLSI) guidelines [20,21]. This method was used to determine the 2.5 and 97.5 percentiles and the respective 90% Confidence Intervals (CI) around these estimates.

RESULTS

The data obtained from 235 subjects were analysed. The age of the study subjects ranged from 20 to 60 years and the majority fall in the age group of 20 to 25 years. The body weight of the study subjects were in the range of 39 to 125 kg. Out of 235 subjects, 17 were males and 218 were females [Table/Fig-1].

Parameters		Median (minimum, maximum)
A. Demographic details		
Sex (n,%)	Male	17 (7.2%)
	Female	218 (92.8%)
Age (years) Median (minimum and maximum)		20 (20,58)
Body weight (Kg)		69 (39,125)
B. Clinical details		
Thyroid stimulating hormone (mIU/L)		1.6 (0.45,4.36)
C-reactive protein (mg/L)		1.1 (0.04,9.4)
Serum creatinine		1.0 (0.6,1.3)

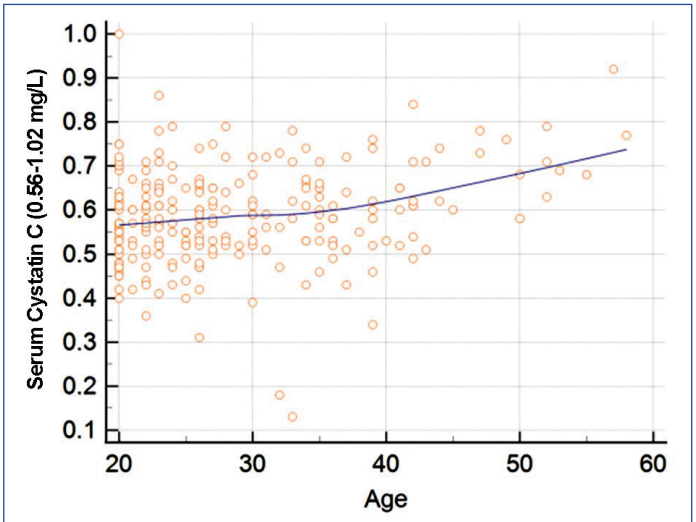
[Table/Fig-1]: Demographic and baseline clinical characteristics of study subjects (N=235).

The non parametric reference intervals for serum Cystatin C are found to be from 0.39 to 0.79 mg% and that of sCr ranged from 0.79 to 1.2 mg%. The 90% confidence intervals for the upper and lower reference limits are shown in [Table/Fig-2].

Parameters	Reference Interval (N=235)	90% Confidence Interval		Median
		Lower limit	Upper limit	
Serum Cystatin C (mg%)	0.39-0.79	0.18 to 0.42	0.77 to 0.92	0.59
Serum Creatinine (sCr) (mg%)	0.79-1.2	0.70 to 0.80	1.20 to 1.30	1.0

[Table/Fig-2]: Reference intervals {RIs for sCys C nd sCr (mg%)} as per Non parametric percentile (2.5 and 97.5) method (CLSI C28-A3).
CLSI: Clinical and Laboratory Standards Institute

The frequency distribution of serum Cystatin C levels for the reference individuals is shown in [Table/Fig-2]. The relationship between Cystatin C with age is displayed in [Table/Fig-3], which shows an increase in Cystatin C levels as age advances; Spearman's rank correlation coefficient (rho=0.197). Similarly, the weak correlation between Cystatin C and sCr was observed (rho=0.3701) [Table/Fig-4].



[Table/Fig-3]: Relationship between age and plasma cystatin C levels aged between 20-60 years.

Variables	Values
Sample size	235
Spearman's rank correlation coefficient (rho)	0.3701
p-value	<0.0001
95% Confidence interval for r	0.2542 to 0.4756

[Table/Fig-4]: Relationship between Cystatin C and Serum creatinine.
Cystatin C (Cys C): 0.39-0.79 mg%
serum Creatinine (sCr): 0.79 to 1.2 mg%

DISCUSSION

Cystatin C refers to an alkaline, non glycosylated protein,13 KD (Kilo Dalton), protease inhibitor, that is expressed continuously in the nuclei of all cells [5]. They can cross the glomerular filter membrane in the physiological environment, and is completely reabsorbed and degraded in the renal tubules without recirculation and the renal tubules does not secrete the protein-Cystatin [11,14]. It has been seen that under physiological conditions, there is very low concentration of cystatin C in urine and blood. However, this concentration increases when the renal tubules are damaged and are dysfunctional [22,23].

Cystatin C has proven to be a good indicator of renal diseases with help of rapid, sensitive and precise immunoassays, it can be used for diagnostic purposes in such cases [24]. In this study, reference intervals for plasma Cystatin C in adults were evaluated using latex-enhanced immunoturbidimetry based standardised Cystatin C assay

enabling traceability to the international standard ERM-DA471 [21]. The reference interval of Cystatin C among the reference individuals enrolled in this study was found to be 0.39 to 0.79 mg%.

In a similar study by Norlund L et al., the authors observed that, the mean value was 1.08 mg% in male patients and 1.03 mg% for female patients. They showed a gender difference (0.05 mg%); not statistically significant, owing to the difference in population [25]. In the present study, no separate reference interval for males and females could be found due to an inadequate sample size for male patients.

Another analogous study conducted by Finney H et al., 309 participants exhibited a noteworthy difference between values for males (mean 0.74 mg%) and females (mean 0.68 mg%), although the range for the distributions was identical (0.42 mg%) and hence discrete ranges were not required [24]. The reference interval obtained for Cystatin C latex-enhanced needs immunoturbidimetry in the present study was found to be 0.39-0.79 mg%, which was almost similar to the outcome of Finney H et al., with a value of 0.51-0.98 mg/L; done by latex particle enhanced immune nephelometric assay (latex PENIA) [24]. Another similar study by Groesbeck D et al., also showed that the mean serum Cystatin C level was 0.84 mg% and was higher among the males than the females [26].

On the contrary, a study that was done by Pergande M and Jung K, reported significantly lower levels of Cystatin C (1.78±0.26) in females than that in males (2.14±0.31) but since their sample size was small with 33 subjects in both females and males, their study results did not match the present study outcome [27]. The Cystatin C range in the present study was found to be different from other studies conducted by Andersen KJ et al., with a value of 0.61-1.21 mg% both in healthy and diseased conditions, Erlandsen EJ et al., with a value of 0.54-1.21mg/L in both male and female population, and Edinga-Melenge BE et al., with a value of 0.60-1.10 mg/L, wherein the particle-enhanced turbidimetric immune assay was used in camaroonian adult population [11,28,29].

Few other studies that recorded the reference range of Cystatin C were done by Kottgen A et al., with a value of 0.61-1.04 mg% done in United States population [30], Li DD et al., value of 0.60-1.08 mg% in a Chinese population [31] and Okonkwo IN et al., in a Nigerian population with a value of 0.64-1.12 mg% [32]. The present study consisted of subjects belonging to the Dravidian population subset of South Asian ethnic groups revealed a reference interval of 0.39-0.79 mg/L which was found to be lower than the other ethnic groups [30-33].

The study conducted by Edinga-Melenge BE et al., explained that the serum Cystatin C levels had a tendency to be slightly affected by certain distinguishing factors like age and gender [29]. However, the influence of gender on the various range levels of this specific protein still remains unclear. However, there are certain studies that have reported that the levels of Cystatin C were independent of gender variances while few other have stated their direct significant influence on the same [33-36]. Edinga-Melenge BE et al., found that gender formed one autonomous illustrative feature for the values of serum Cystatin C [29]. The present study showed that serum cystatin C levels were 11% higher in males than in females (0.90 mg/L vs 0.80 mg%; p-value <0.001). Similar results were also observed by Kottgen A et al., who showed a difference of 8% between males and females [30]. However, Al Wakeel JS et al., reported lower levels of serum cystatin C in males as compared to females (0.72 mg% vs 0.77 mg%; p-value <0.001) in a Saudi population [27]. Hence, it can be deduced that a single adult reference range for Cystatin C, with distinct clinically significant differences between the males and females was obtained [37]. Thus, through multicentre study of the baseline levels of serum cystatin C, it creates the diagnostic concentration reference interval of the protein in order to encourage the recognition, standardisation and establishment of specific serum Cystatin C levels.

Limitation(s)

Due to the non random sampling method used and single measurement of serum cystatin C levels, generalisation of the present findings to the entire Dravidian south Asian population cannot be done as the representatives of the study population would have been better obtained with randomisation. Secondly, a separate reference interval for males and females could not be found due to unequal division of study participants amongst both the genders.

CONCLUSION(S)

This study depicted serum Cystatin C in Dravidian population subset of South Asian ethnic population and showed that males had suggestively higher levels of Cystatin C as compared to the females. The reference limits of serum Cystatin C in the study population belonging to the South Asian ethnic groups was observed as 0.39-0.79 mg%. Hence, it is expected and assumed that the present study data would further instigate other researchers to conduct similar studies on a larger population that would represent the population of a whole country of nation with vivid diversity.

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