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SERUM CYSTATIN C AS A MARKER TO IDENTIFY PATIENTS WITH MODERATELY IMPAIRED RENAL FUNCTION

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ABSTRACT

The low molecular weight protein cystatin C produced by all nucleated cells and eliminated by glomerular filtration is of special benefit as a marker of renal function. A study was therefore undertaken to investigate whether serum cystatin C could be used as a marker to identify patients with moderately impaired renal function. A cross-sectional descriptive hospital based study was carried out and serum cystatin C was measured in fifty subjects aged 12 to 74 years with a 24 hr creatinine clearance estimation done at the same time. The gold standard creatinine clearance was used to compare the predicted glomerular filtration rate measured using serum cystatin C. Predicted glomerular filtration rate gave a sensitivity of 82% and specificity of 68% with a diagnostic cut-off value of 1.25mg/L cystatin C for identification of patients with moderately impaired renal function with a single random blood sample.

KEY WORDS

Cystatin C, Creatinine, Glomerular Filtration Rate, Moderately Impaired Renal Function.

INTRODUCTION

The gold standard procedures for measurement of gromerular filtration rate (GFR) are based upon plasma clearance of creatinine, ^{51}Cr-EDTA or iohexol. However this method requires timed urine collection which is imprecise and inconvenient. On the other hand, the clinical use of other gold standard measure of GFR requires administration of exogenous radioactive or contrast agents is also time consuming, expensive and not readily available in many hospitals in Sri Lanka. As creatinine based test results could be affected by age, sex, nutritional status, diet and tubular creatinine secretion particularly when GFR is reduced, attempts have been made to improve the clinical measurement of GFR. Hence interest in cystatin C as a marker of renal function has increased tremendously over the last few years due to its steady performance characteristics over serum creatinine (1).

Cystatin C is a 13-kDa basic protein belonging to the cystatin super family of cysteine protinases inhibitor with widespread distribution in biological fluids. It is produced by virtually all nucleated cells, and the production rate is unaffected by inflammatory processes, age, sex, and nutritional status, and is freely filtrated through the gromerular membrane and nearly completely reabsorbed and degraded by the proximal tubular cells (2). Therefore, the plasma concentration of cyctatin C is exclusively determined by the GFR, indicating a possible sensitive marker for GFR.

Serum creatinine is ubiquitously used as an indicator for GFR despite the knowledge that a substantial proportion of patients with reduced GFR display serum creatinine levels within the normal range. Even a 50% reduction of GFR not infrequently associated with a normal concentration of serum creatinine (3). Subjects with moderately impaired renal function are most likely to be asymptomatic and if such patients are identified early, the progress of the disease can be retarded thus improving the progress. The present study was therefore

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undertaken to identify patients with moderately impaired renal function using serum cystatin C as a maker of GFR and to establish the cut-off value for these patients with the use of a random blood sample.

MATERIALS AND METHODS

Study sample and setting : Fifty subjects (25 males and 25 females) ranging in age from 12 to 74 years were selected through clinics at Nawaloka Hospital, Colombo, Sri Lanka. A systematic sampling technique was adopted in recruiting participants in the study. Ethical clearance for this study was obtained from the Ethical Review Committee of Nawaloka, Hospitals and informed consent of parents and patients was obtained before the study. Patients on dialysis, glucocorticoid therapy and with clinical history of thyroid dysfunction and cardiovascular diseases were excluded from the study.

Study design : A cross-sectional hospital based descriptive study was performed and the sample number was decided based on the prevalence rate of patients presenting to the clinics of this hospital with kidney disease.

Sample collection and laboratory analysis : 3 ml. venous whole blood was collected in vials without anti-coagulant and serum was separated and aliquots of 0.5 ml were stored at -20°C pending analysis for cystatin C and creatinine. Twenty four hour collection of urine obtained from each subject was used to measure the rate of creatinine clearance adjusted to mean surface area of 1.732m^2 using Dubois-Dubois formula (4).

Laboratory assay of cystatin C and creatinine : Serum cystatin concentration was measured by particle enhanced immunoturbidimetry using the Dakocytomation assay kit (DAKO Ltd, Code No. LX002, Denmark) which showed a higher precision (2.1% CV) over the entire measuring range. Concentration of serum creatinine was measured by kinetic colorimetric assay using Randox commercial assay kit on a Konea Lab 20 chemistry analyzer.

The following formula proposed by the Dakocytomation, Denmark was used to derive the prediction of GFR from serum cystatin C (5).

GFR ml/min = 89.12 x serum cystatin C $(mg/L)^{-1.675}$

Statistical analysis : Statistical analysis was performed using SPSS software version-10. Standard parametric and nonparametric tests were performed and p values below 0.05 were considered statistically significant. The correlation between the two endogenous markers of GFR was studied by linear regression analysis. Diagnostic sensitivity, specificity was calculated and identification of moderately impaired renal function was studied by receiver-operating characteristic (ROC) plot.

RESULTS

Serum cystatin C based estimated GFR for study population showed a statistically high correlation (r^2 = 0.76) with the creatinine clearance (Figure 1). However, serum cystatin C showed better correlation (r^2 = 0.63) against the gold standard when compared with the serum creatinine (r^2 = 0.38). There were no particular distinguishing clinical features such as drug history and skeletal lesion in patients with increased serum cystatin C. On average, creatinine clearance and estimated GFR based on cystatin C showed a positive relationship with a mean estimated GFR of 77.5 $ml.min^{-1}.(1.732\ m^2)^{-1}$.

Sensitivity and specificity values of estimated GFR against the gold standard calculated at 60 GFR $ml.min^{-1}.(1.732m^2)^{-1}$ is given in Table 1. The gold standard test was positive in 28 subjects of whom 23 tested positive for estimated GFR based cystatin C test giving a sensitivity of 82%. Of the 22 subjects tested negative by the gold standard test, 15 were correctly identified by the cystatin C test giving a specificity value of 68%. The cut-off point with regard to estimated GFR using serum cystatin C value for detecting moderate kidney disease was regarded as 60 $ml.min^{-1}.(1.732m^2)^{-1}$ (6). The cystatin C and creatinine in subject with moderate kidney disease ranged from 1.16 to 1.51 mg/L and 0.91 to 1.8 mg/dl respectively.

ROC plot for cystatin C based GFR of 60 $ml.min^{-1}.(1.732m^2)^{-1}$ is presented in Figure 2. From these data, cut-off

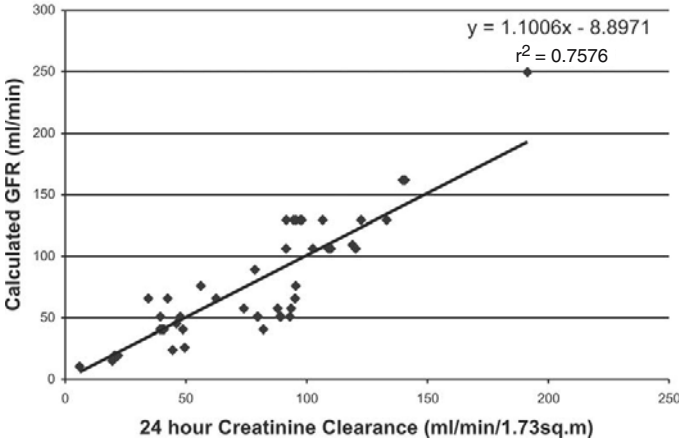

Figure 1. Regression Correlation of estimated GFR based on cystatin C versus 24 hour creatinine clearance

Table 1 : The mean concentrations of serum cystatin C, creatinine and their correlations of moderately impaired renal function subjects

Biochemical parameters	Glomerular Filtration Rate at 60 ml/min/1.732m^2 Mean ± SD
Serum cystatin C (mg/l)	1.33 ± 0.54
Serum Creatinine (mg/dl)	1.36 ± 0.62
Sensitivity (%)	82.1
Specificity (%)	68.2
Coefficient of correlation (r^2)	
Estimated GFR vs CC	0.76 ± 24.90
Serum creatinine vs CC	0.38 ± 1.39
Serum cystatin C vs CC	0.63 ± 0.35

CC = creatinine clearance; n = 50

value for serum cystatin C concentration of 1.25mg/L was established with a sensitivity of 82% and specificity of 68% for detecting moderate kidney disease. The ROC plot yielded significantly higher area under the curve of 0.89 ± 0.05 for moderately impaired renal function.

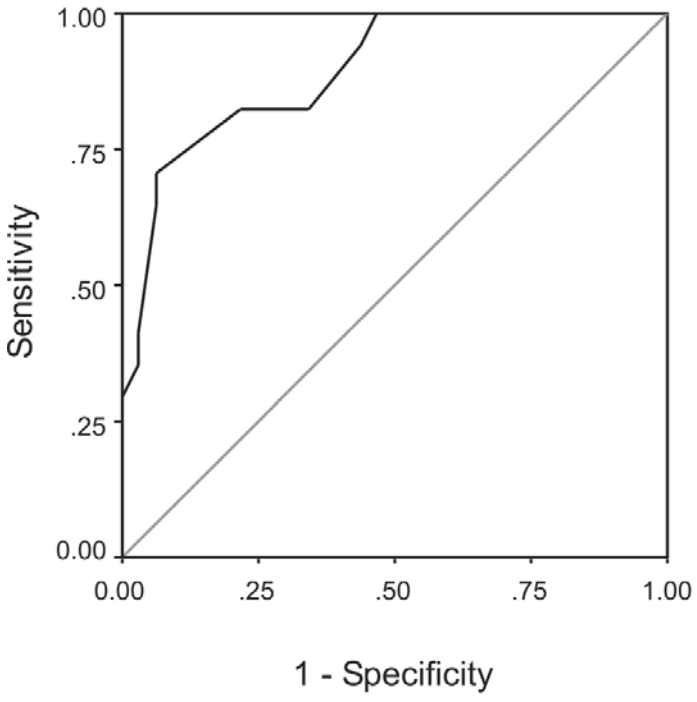

Figure 2 : Parametric ROC plot for the diagnostic accuracy of cystatin C to detect GFR at 60mL/min/1.732m^2

DISCUSSION

The proteins with low molecular weight eliminated by glomerular filtrate are generally considered to be potential markers of renal function. However, serum creatinine proved to be the superior renal function marker over other substances such as - α_1 and β_2 -microglobulin as their production rate varies considerably with immune reaction (5). The cystatin C has a constant rate of production and is completely filtered and metabolized by the glomerular tubules and totally independent of age, sex, diet, inflammation and chemotherapy (2,7,8,9).Cystatin C level remains to be constant after the first year of age and virtually no significant correlation between maternal and neonatal cystatin C levels. This is in contrast to creatinine, where the creatinine in an infant is largely derived from the mother. Furthermore, with the development of automated immunoturbidimetric method, serum cystatin C levels are measured with more accuracy using a micro volume of serum samples within a very short time (10,11).

Several studies conducted in USA and Europe have suggested that plasma/serum cystatin C may be used as an accurate GFR marker, citing several advantages over serum creatinine estimation. However, literature available on Asian population does not indicate the estimated GFR based on serum cystatin C concentration and comparing their values for diagnostic performance with the gold standard procedures to identify the cut-off value for subjects with moderately impaired renal function.

The present study revealed that there is a good correlation between the estimated GFR based on serum cystatin C and 24 hour creatinine clearance. Although serial values are necessary for assessing GFR trends in a given patient, only single value of serum Cystatin C and creatinine were used in this study as has been the case with recently published work on this subject (8). The equation of the Modification of Diet in Renal Disease study (MDRD) uses serum creatinine in combination with age, sex and race to estimate the GFR and to improve the limitations with the use of serum creatinine. Thus, MDRD equation is more accurate than measuring creatinine clearance from 24 hour urine collection (1). However, the estimated GFR based on MDRD equation has not been developed and validated for population in the South Asian countries. Therefore, patients with reduced GFR display normal serum creatinine levels even at 50% reduction of GFR and are likely to have a significant impact on the estimated GFR at the stage of moderately impaired renal function. This would explain the high standard deviation reported for the estimated GFR based on serum creatinine in the present study.

Previous studies on Sri lankan population indicated similar findings with the estimated GFR based on serum creatinine (12). Present study also revealed that introduction of a gender factor into the cystatin C based GFR prediction for all age groups did not show statistical differences on the diagnostic performance. The ROC analysis for serum cystatin C based GFR had a high diagnostic efficacy at identifying patients with normal and moderately impaired renal function. The cutoff concentration of 1.25 mg/L of serum cystatin C concentration seems to be an accurate estimate for identification of patients with moderately impaired renal function with a GFR of 60 $ml.min^{-1}.(1.732m^2)^{-1}$. Few studies have reported the use of cystatin C to identify patients with impaired renal function (13), on the cutoff value of cystatin C for detecting moderately impaired renal function in SARC region population. Therefore, the cut-off level reported by the present study may be of special benefit in identification of patients with moderately impaired renal function. As patients with reduced GFR show serum creatinine levels within the normal range even with a 50% reduction of GFR, the 1.25mg/L cutoff value reported in the study can be used as a good estimate for identification of patients with moderately impaired renal function using a single random blood sample.

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