Original Article

Model For Assessment of Cystatin C as predictive Cardiovascular Risk Marker in Patients with Chronic Kidney Disease.

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ABSTRACT

Background: Cystatin C, a protease inhibitor synthesized in all nucleated cells, has been proposed as a replacement for serum creatinine for the assessment of renal function, particularly to detect small reductions in glomerular filtration rate. In this study we determine a novel model to access cystatin C level of patients having cardiovascular risks with chronic kidney disease if other parameters are known.

Methods: Blood samples were collected (total 397 subjects) from patients with chronic kidney disease (CKD), cardio vascular disease (CVD) and both CKD with CVD, along with the normal healthy controls. Lipid profile, urea, creatinine, hs-CRP, physiological parameters and cystatin C were analyzed.

Result: We found that proportion of diabetics were significantly higher among diseased persons as compared to control subjects (Chi-square=53.61; p=0.0001). Mean values of total cholesterol, triglyceride, VLDL, urea, creatinine and cystatin C among CKD group as well as in CVD group of patients were significantly higher ($p<0.05$), while total protein, albumin and haemoglobin were significantly lower ($p<0.05$) as compared to healthy controls. In CKD patients with CVD, the mean values of potassium, glucose, urea, creatinine, cystatin C and total leucocyte count were significantly higher ($p<0.05$), while total protein, albumin and hemoglobin were significantly lower ($p<0.05$) as compared to healthy controls.

Conclusion: This study has demonstrated a novel predictive model which is cost effective than the gold standard for the assessment of serum cystatin C which is an endogenous risk marker in the patients of cardiovascular with Chronic Kidney Disease.

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Introduction

Chronic kidney disease (CKD) is an important public health problem worldwide, with an estimated prevalence of 13% in the Western world.[1] Epidemiological data from the US indicate that roughly 10% of the adult population shows some form of CKD; studies from Europe, Australia and Asia confirm this high prevalence. Patients with kidney disease are far more likely to die from CVD, than to develop kidney failure.[2] Chronic kidney disease does not cause pain; this is why CKD usually remains undetected for a longer period of time, until a screening test identifies the silent problems. Patients with CKD as well as with mild renal dysfunctions are known to be at an increased risk of developing CVD and cardiovascular events.[3,4] It is not clear if the risk is mediated by mechanisms secondary to renal dysfunction or by risk factors common to both CVD and decreased GFR.

Cystatin C, produced by a majority of nuclear cells is a nonglycosylated protein of 120 residue polypeptide chain with a molecular mass of 13 kDa.[5] It has been identified in a wide range of organs using both immunocytochemistry and in-situ hybridization, and has also been measured in a range of biological fluids i.e. cerebrospinal fluid, seminal fluid, plasma, saliva, urine, milk, amniotic fluid, synovial fluid, serum and tear.[6] Many studies have confirmed the high sensitivity and specificity of cystatin C for glomerular filtration rate (GFR) estimation; in most studies it was clearly superior to creatinine with regard to renal function assessment.[7] One of the key criteria that cystatin C needs to meet to be a potential replacement for creatinine is that its production rate should be constant or at least less variable than that of creatinine.[8] Recent studies, however, have shown that plasma cystatin C concentration is influenced by factors such as age,[9] body mass index (BMI),[10] sex,[11] smoking status,[9] and high concentrations of C-reactive protein (CRP).[9,11]

In prior studies on general population and in the elderly, cystatin C has been shown to be a better predictor of mortality and adverse cardiovascular events than serum creatinine.[12] Several recent studies demonstrated that cystatin C is superior to serum creatinine or creatinine based estimation equation for prediction of all causes mortality and incidence of congestive heart failure.[13] It appears to be a marker of cardiovascular risk, and high concentrations of circulating cystatin C have been shown to be consistently and strongly associated with cardiovascular outcomes in different clinical scenarios. Moreover, cystatin C seems to offer more complete prognostic information than other markers of renal function. This study was designed to explore the correlation of cystatin C with cardiovascular markers in CKD patients.

Materials and Methods

The present study was carried out in renal dysfunction and cardio vascular disease patients admitted at Narinder Mohan Hospital and Heart Centre, Ghaziabad. The study was designed on total 397 subjects who were divided into four groups of CKD (71), CVD (127), CKD with CVD (37) and healthy control (162). All patients had consented for the use of their blood samples for clinical research and ethical norms were approved from Institutional Ethical Committee.

Sample Collection: Blood samples were collected from various groups and routine investigations of biochemical and physiological parameters were carried out. All biochemical assays were analysed on fully automated Biochemistry analyser (Olympus AU-400). For physiological assay, fully automated Haematology analyser (Beckman Coulter LH-500) was used.

Enzymatic methods used for the estimation of glucose, triglyceride, cholesterol and urea were hexokinase, glycerol phosphate oxidase-peroxidase, cholesterol oxidase-peroxidase and urease-glutamate dehydrogenase respectively (Aw, 1969). Jaffe's kinetic method was used for creatinine estimation.[14]

HDL cholesterol estimation was carried out by first precipitating non HDL using antihuman beta-lipoprotein antibody and estimating the rest enzymatically by cholesterol oxidase-peroxidase method. LDL Cholesterol estimation was also done by the same enzymatic method but by first selectively protecting LDL, estimating the remainder, then releasing LDL and determining it selectively.[15]

Na^+ and K^+ estimation was carried out using direct ion selective electrode module of Olympus AU analyser.

Total serum protein and albumin were estimated by Biruet and bromocresol green method respectively.[16,17]

Hs-CRP was estimated using latex enhanced turbidimetric immunoassay.

Estimation of cystatin C was carried out by immune turbidimetric method. Cystatin C concentration was determined by measuring change in absorbance that resulted from the aggregation of cystatin C with anti-cystatin C antibody.[18]

Estimation of Heamoglobin and Total Leucocyte Count was done on LH -500 Beckman Coulter analyser. The analyser accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture.

Reagents for cystatin C estimation were procured from Accurex; all other estimations were carried out using reagents from Beckman.

Statistical Analysis: It was performed using statistical software Stata (version 10, Stata Corp, USA). Data were checked for the completeness, accuracy and normality condition for continuous data. Chi-square (χ^2) test was applied for the assessment of independence of association and goodness-of-fit tests. Analysis of variance was applied to test the equality of means of various biochemical parameters among four groups using f-statistic. T-test was applied for testing difference of means between two groups after checking for equality of variance. Multinomial logistic regression model was used for modelling the effect of various co-factors taking each disease outcome as dependent variable in comparison to healthy control. Linear regression analysis was performed to predict the level of cystatin C using important biochemical parameters.

RESULT

The CKD was more prevalent in young males (p=0.001) as compared to females.The comparison of mean age between males and females within each category of disease and healthy controls, and among four groups within each sex category, is presented in table-1. Two-way analysis of variance was performed to test the equality of age within each type and within sex. The mean age of subjects was significantly different among three types of diseased groups and healthy controls (F=26.90, p=0.0001), but not significantly different between male and female within each type of subjects (F-statistic=2.5, p=0.114). CKD patients were comparatively younger than CVD and both, but almost comparable to healthy controls in both sex category (p>0.05). In present study, proportion of diabetics were significantly higher among diseased persons as compared to control subjects (χ^2=53.61; p=0.0001). Also mean glucose level was significantly higher within each category of subjects (Table-2).

The mean value of biochemical parameters (with 95% CI) of the subjects within each category is depicted in table-3.

Comparison of CKD cases with healthy controls: mean values of total cholesterol, triglyceride, VLDL, potassium, urea, creatinine and cystatin C among CKD patients were significantly higher (p<0.05), and total protein, albumin and haemoglobin were significantly lower (p<0.05) as compared to health controls. Logistic regression analysis showed that after adjusting for confounders like age, sex, diabetic status and other biochemical parameters, it was observed that low albumin (p=0.0001), high urea (p=0.0001), high creatinine (p=0.0001) and high cystatin C (p=0.03) were significantly different compared to healthy individuals. Glucose level was also found to be significantly different (p=0.005) in CKD individuals after adjusting for other confounders (Table-4).

Likelihood ratio test using chi-square statistic was used for the model selection (log likelihood= - 157.023, Chi-square = 683.60; p=0.0001). Adjusted R-square for the logistic regression analysis was 0.685, indicating a high multiple correlation.

Comparison of CVD cases with healthy controls: Mean values of total cholesterol, triglyceride, LDL, VLDL, glucose, urea, creatinine, cystatin C and total leucocyte count among CVD patients were significantly higher (p<0.05), while total protein, albumin and haemoglobin were significantly lower (p<0.05) as compared to healthy controls. Logistic regression analysis showed high LDL (p=0.003), high glucose level (p=0.001), low albumin (p=0.001), high urea (p=0.001), and high creatinine (p=0.001) were significantly different compared to healthy individuals. Diabetic status was also found to be significantly associated (p=0.005) compared to healthy individuals after adjusting for other confounders (Table-4). Likelihood ratio test using chi-square statistic was used for the model selection (log likelihood = -151.023, Chi-square = 660.45; p=0.0001). Adjusted R-square for the logistic regression analysis was 0.69, indicating a high multiple correlation.

Comparison of both CKD and CVD cases with healthy controls: Mean values of potassium, glucose, urea, creatinine, cystatin C and total count among both CKD and CVD patients were significantly higher (p<0.05) and total protein, albumin and haemoglobin were significantly

Table 1: Comparison of age by groups and sex of subjects

Type	Male Mean (95%CI)	Female Mean (95%CI)	p-value
CKD	48.62 (44.12 - 53.13)	49.83 (45.23 - 54.44)	0.7071
CVD	58.66 (56.11 - 61.21)	58.41 (51.45 - 65.36)	0.938
Both CKD with CVD	65.89 (60.26 - 71.52)	57.44 (47.83 - 67.05)	0.1265
Healthy normal	49.63 (47.88 - 51.39)	46.05 (43.64 - 48.46)	0.07

F-statistic: For Type; 26.90 (0.0001); for sex 2.5 (0.114)

Table 2: Distribution of Mean Glucose Level (95%CI) among the cases and control subjects.

Type	Diabetic	Non-Diabetic	t-statistic (p-value)
CKD	159 (137 -181)	87.5 (84 - 91)	6.84 (0.000)
CVD	179 (165 - 193)	91 (87.5 - 94)	12.54 (0.000)
Both CKD with CVD	163 (142 - 185)	92 (83 -101)	6.38 (0.000)
Healthy normal	141(114.4 - 167.59)	90.61 (89.09 - 92.13)	4.16 (0.001)

Table 3: Mean Level (95% CI) of Biochemical Profile among four groups.

Variable	CKD (n=71)	CVD (n=127)	Both (n=37)	Control (n=162)	F-statistic (p-value)
Total Cholesterol	189 (181 - 197)	196 (187 - 205)	190 (177 - 203)	174 (168 - 180)	7.38 (0.001)
Triglyceride	169 (158 - 179)	168 (160 - 177)	152 (134 - 171)	138(128 - 147)	10.35 (0.000)
HDL	40.4(39.2-41.6)	40.8 (39.5 - 42.0)	38.2 (35.7-40.7)	43.14(41.72 - 44.55)	5.48 (0.001)
LDL	114.9 (108 - 122)	128.8 (120.6 - 136.9)	121(109- 134)	106(101 - 111)	8.79 (0.001)
VLDL	33.74 (32 - 36)	33.68 (32.0 - 35.3)	30(27 - 34)	27.5 (25.68 - 29.32)	10.40 (0.001)
Sodium	138.6(137-140)	138.6(137.3-140.0)	139 (137-141)	140(139-140.47)	1.34 (0.26)
Potassium	4.97 (4.76 - 5.18)	4.38 (4.26 - 4.50)	5.02 (4.65 -5.41)	4.37 (4.31 - 4.44)	20.86 (0.000)
Glucose	103.7 (94.9 - 112.5)	109.5(102.1 - 116.8)	134 (114 - 152)	94.34 (91 - 97)	14.55 (0.000)
Total Protein	6.74 (6.55 - 6.92)	6.68 (6.55 - 6.82)	6.39 (6.08 - 6.69)	7.11 (7.04 - 7.20)	16.92 (0.000)
Albumin	3.39 (3.25 - 3.54)	3.48 (3.4 - 3.58)	3.27 (3.09 - 3.46)	4.16 (4.09 - 4.26)	72.52 (0.0001)
Urea	123 (109 - 137)	67 (61 - 73)	133 (114 - 152)	24.61 (23.52 - 25.71)	170.76 (0.000)
Creatinine	6.13 (5.32 - 6.95)	1.88 (1.77 - 2.00)	4.10 (3.23 - 4.98)	0.94 (0.86 - 1.00)	167.41 (0.0001)
Cystatin C	2.99 (2.61 - 3.37)	0.98 (0.90 - 1.05)	2.71 (2.20 - 3.22)	0.64 (0.61 - 0.67)	151.68 (0.000)
Haemoglobin	9.49 (8.93 - 10.05)	12.25 (11.85 -12.60)	11 (10 - 12)	13.10 (12.86 - 13.35)	59.66(0.000)
Total Count	**8115(7415 - 8815)**	**10200(9452 - 10950)**	**10843(9313-12374)**	**7667(6963 -8371)**	**10.87 (0.001)**

Table 4: Logistic Regression to model Chronic Kidney Disease (CKD), Chronic Vascular Disease (CVD) and CKD with CVD using biochemical indicators.

	Variables	Adjusted Beta (□)	SE (□)	z-statistic	p-value
CKD	Glucose	0.033	0.012	2.8	0.005
	Albumin	-4.47	0.93	4.8	0.0001
	Urea	0.214	0.043	4.91	0.0001
	Creatinine	1.96	0.453	4.32	0.0001
	Cystatin C	5.02	2.43	2.07	0.039
CVD	Low Density Lipid	0.034	0.0116	2.98	0.003
	Glucose	0.038	0.0105	3.62	0.001
	Albumin	-3.74	0.84	4.46	0.001
	Urea	0.21	0.043	4.82	0.001
	Creatinine	1.05	0.43	2.46	0.014
	DM	18.8	1.05	17.92	0.0001
CKD with CVD	Low Density Lipid	0.0245	0.13	1.89	0.059
	Glucose	0.044	0.011	3.81	0.001
	Albumin	-4.83	0.95	5.08	0.001
	Urea	0.223	0.043	5.13	0.001
	Creatinine	1.47	0.468	3.15	0.002
	Cystatin C	5.44	2.43	2.24	0.025
	DM	21.41	0.813	26.34	0.0001

lower ($p<0.05$) as compared to health controls. Logistic regression analysis showed high glucose level (0.001), low albumin (p=0.001), high urea (P=0.001), high creatinine (P=0.002) and cystatin C (0.025) were significantly different compared to healthy individuals. Diabetic status was also found to be significantly associated (p=0.0001) compared to healthy individuals after adjusting for other confounders (Table-4). Likelihood ratio test using chi-square statistic was used for the model selection (log likelihood = -154.023, Chi-square = 623.45; p=0.0001). Adjusted R-square for the logistic regression analysis was 0.685, indicating a high multiple correlation.

Figure-1 shows the multiple linear regression analysis in order to predict the value of cystatin C based on other biochemical variables. The result is indicating that age, potassium level, albumin, urea, creatinine and haemoglobin are significant predictor variables for the average cystatin C level. This model is highly significant (F-statistic at 6,388 degrees of freedom = 159.63; p=0.0001). Adjusted R-square for the regression analysis was 0.72, indicating a high multiple correlation of these variables with dependent variable i.e. cystatin C. The model for estimating cystatin C value based on these results is as follows:

Mean Cystatin C = -0.98 + 0.0075(Age) + 0.186(Potassium) + 0.168(Albumin) + 0.004(Urea) + 0.31(Creatinine) + (-0.05) (Haemoglobin).

Discussion

The aim of this study was to investigate the role of cystatin C as diagnostic marker in CKD patients and to elucidate its role in association with CVD. Previous studies have demonstrated that mild to moderate kidney disease independently predicts morbidity and mortality among patients suffering with cardiovascular disease and chronic heart disease (CHD).[12,19] In patients with acute coronary syndrome, an elevation of creatinine or reduction of e-GFR is related to a poor prognosis. Vanholder et al., 2005, reported that patients with chronic kidney disease are at a significant risk, for developing cardiovascular disease and latter may promote former, resulting in a vicious cycle.[20] The present study explores the role of cystatin C in CKD patients with cardiovascular disease in two different age grouped persons under diabetic and non-diabetic conditions. Further, since creatinine estimation has a limited value in CKD prognosis; hence, the study focusses on cystatin C as the marker of choice for CKD as well as CVD.

In this study, the mean age of subjects was significantly different among three types of diseased subjects and healthy controls, but not significantly different between male and female within each type of subjects. CKD patients were comparatively younger than CVD and both, but almost comparable in age to healthy controls. The

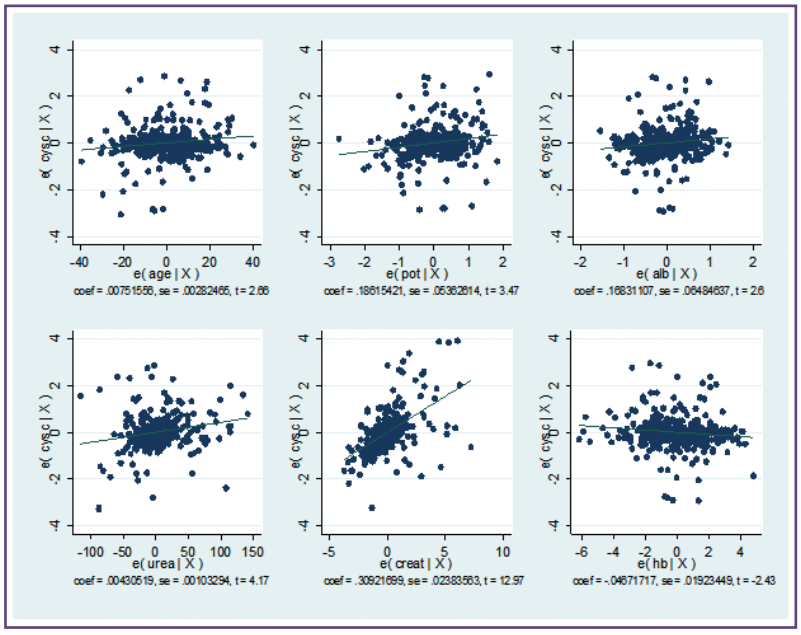

Fig. 1: The figure indicating the effect of independent variables like age, potassium, albumin urea, creatinine, hemoglobin, constant on dependent variable, i.e. cystatin C. There was significant effect of age (p=0.008), potassium (p=0.001), albumin (p=0.01), urea (p=0.001), creatinine (p=0.0001), hemoglobin (p=0.016), constant (p=0.022) on Cystatin level, after controlling for other independent variables.

proportion of diabetics, as well as the mean glucose level was significantly higher among the cases as compared to control; reinforcing the fact that diabetes is a risk factor of CVD and CKD. Across all categories and age groups, we found higher mean levels of serum cystatin C under diabetic conditions as opposed to non-diabetic one. Xie Qing et al., 2012, have shown that serum cystatin C levels were significantly associated with the presence and severity of asymptomatic coronary artery disease (CAD) in metabolic syndrome patients with normal kidney function, suggesting that cystatin C is probably more than a marker of glomerular filtration rate.[21] Trilki et al., (2013), recently reported that serum cystatin C was a marker of cardiovascular disease in type-2 diabetes patients.[22] This was also supported by a study of Andrezel et al., 2013, who had reported that in young or middle aged diabetic patient in CKD, cystatin C gave a positive correlation with CVD patients.[23] It appears that estimation of cystatin C is necessary for the patient with CKD as well as CVD and is a useful tool to diagnose chronic kidney disease in high risk groups of diabetes mellitus. This supports Noora et al., 2011, who had reported that new cystatin C assay may offer an alternative to current commercial assays to detect and monitor impaired kidney function.[24]

In present study, relationship between cystatin C and cardiovascular risk factors have been linked, as our data shows that the mean values of total cholesterol, triglyceride, VLDL, urea, creatinine and cystatin C among CKD, CVD and CKD with CVD patients were significantly higher. Parikh et al., 2008, evaluated the association between cystatin C and conventional cardiovascular risk factors in 3241 predominantly white participants.[25] The authors showed that high concentrations of cystatin C were independently associated with cardiovascular risk factors such as age, female sex, BMI, low HDL, cholesterol and smoking, even in individuals without CKD or micro albuminuria. A cross-sectional analysis of data on individuals with CKD in the study disclosed a similar risk profile. Our study also shows high cystatin C levels in patients of CKD and CKD with CVD in comparison to CVD alone category, although the levels even in the latter category were significantly higher with respect to controls.

Our study shows no significant difference in serum levels of cystatin C and LDL-C in CKD and CKD with CVD patients although a significant relationship between these two parameters exists in CVD alone category. This clearly shows that in CKD patients progressing towards CVD, carrying out LDL-C estimation may not be of any help. This may have to do with the fact that additional factors are more important in the pathogenesis of CVD in CKD patients than disturbances of cholesterol metabolism (Massy et al., 2013; LDL Cholesterol in CKD- to treat or not to treat; Kidney International 2013).[26] Similarly we also did not find any difference in the relationship between cystatin C and HDL-Cholesterol in CKD and CKD with CVD patients. This is in accordance with an earlier study which also did not find any correlation between HDL-Cholesterol and cystatin C in CKD patients.[27]

It has also been conclusively shown earlier by Shlipak et al., (2005) that cystatin C levels in serum are strongly correlated with mortality.[12,28] Zethelius et al., 2008, assessed a combination of biomarkers and reported improved patients stratification with cystatin C compared to established cardiovascular risk factors.[29] Despite such strong evidence, cystatin C estimations have not found many takers probably due to its high cost assay. We therefore, attempted to predict cystatin C values using the other assayed biochemical parameters. On subjecting our data to multiple linear regression analysis and adjusting for confounders like age, sex and diabetic status, we found that age, potassium level, albumin, urea, creatinine and haemoglobin are significant predictor variables for predicting the average cystatin level. This model is highly significant (F-statistic at 6,388 degrees of freedom = 159.63; p=0.0001). Adjusted R-square for the regression analysis was 0.72, indicating a high multiple correlation of these variables with dependent variable i.e. cystatin C.

Conclusion

This study has demonstrated a novel predictive model which is cost effective than the gold standard for the assessment of serum cystatin C which is an endogenous marker in the patients of cardiovascular with Chronic Kidney Disease if other parameters i.e. Age, Potassium level, Albumin level, Urea level, Creatinine level and hemoglobin level are known. A limitation of our study is its small sample size. A larger prospective study may be done to further validate this equation.

Conflict Of Interest

All authors have no conflict of interest.

Funding

None

Competing Interests

None declared

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