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Cystatin-C in chronic renal failure and its correlation with creatinine clearance

Vasudha Dhupper[1], Veena Singh Ghalaut[2], Kiran Dahiya[3], Sumit Dokwal[4], Monica Verma[5], Isha Malik[6], Umesh Yadav[7], Vaibhav Vishal[8]

[1-8] Pt BD Sharma University of health sciences, Rohtak, Haryana, India

Abstract
Chronic Kidney Disease (CKD) is a worldwide public health problem, both for the number of patients and cost of treatment involved. Creatinine is the most widely used biomarker of kidney function. It is inaccurate at detecting mild renal impairment. Cystatin C, a non-glycosylated 13 kDa protein, has the potential to improve estimates of glomerular filtration rate (GFR) because it is less influenced by muscle mass or age unlike creatinine.40 known patients of CKD attending nephrology unit of medicine at PGIMS, Rohtak were enrolled as cases for this hospital based cross- sectional study, 40 age and sex matched healthy subjects were taken as controls. Both the cases and controls were analyzed for serum creatinine, cystatin C and urine creatinine. Creatinine clearance was calculated using standard formula. Serum Cystatin C increased with stage wise progression of CKD with mean level of 2.31 ± 0.97mg/L (stage III), 2.80 ± 0.55 mg/L (stage IV), 3.01 ± 0.81 mg/L (stage V) in comparison to controls (0.68±0.17 mg/L). Serum creatinine was also increased with stage wise progression in CKD with mean level of 1.7 ± 0.19 mg/dL (stage III), 2.72± 0.58 mg/dL (stage IV), 7.66 ± 2.33 mg/dL (stage V) in comparison to controls (0.84± 0.15 mg/dL). Serum Cystatin C was significantly correlated with creatinine clearance (r= -0.864; $p<0.001$) and serum creatinine (r= 0.665; $p<0.001$). Cystatin C has small variability and is unaffected by preanalytic factors such as routine clinical storage conditions, freezing and thawing cycles or interfering substances, such as bilirubin or triglycerides. Thus, it may be better to use Cystatin C for staging of CKD than creatinine clearance.

Keywords: chronic kidney disease, cystatin C, eGFR, serum creatinine, creatinine clearance

Introduction
Chronic Kidney Disease (CKD) is a worldwide public health problem, both for the number of patients and cost of treatment involved. Globally, CKD is the12th cause of death and the 17th cause of disability. Most common cause of CKD is diabetes mellitus especially type-II because of its higher prevalence. Non Diabetic group comprises of diseases including hypertensive nephropathy, glomerulonephritis, cystic kidney diseases, vascular diseases and tubulointerstitial nephropathy [1].

The term chronic renal failure applies to the process of continuing significant irreversible reduction in nephron number. Chronic renal failure is defined by a reduced GFR (< 60 mL/min/1.73m2) and/or the presence of markers of renal injury for > or = 3 months. Chronic renal failure indicates increased risk of both end stage renal disease and cardiovascular mortality [2].

It is important to identify factors that increase the risk for CKD like hypertension, diabetes mellitus, autoimmune disease, older age, African ancestry, a family history of renal disease, a previous episode of acute kidney injury, and the presence of proteinuria, abnormal urinary sediment, or structural abnormalities of the urinary tract [3].

Creatinine is derived from creatine metabolism in skeletal muscle and released into circulation at a relatively constant rate. It is freely filtered, neither metabolized nor reabsorbed and inexpensive to measure. The serum creatinine production (i.e. GFR x serum creatine), serum creatinine and urinary output, do not vary throughout the day. Thus, creatinine clearance is the most widely used measurement for glomerular filteration rate (GFR).

Its normal range is being 90-140ml/min in men and 80-125ml/min in women [4].

Analysis of the effect of dietary protein on creatinine clearance, independent of its effect on GFR, requires an understanding of the determinants of creatinine clearance other than GFR and of the methods for its measurement [5].

Cystatin C is a 13 KD a basic protein having 122 amino acids and belongs to cystiene protease inhibitor family. It is expressed by nucleated cells. It is produced at a constant rate and is freely filtered by the glomerulus. It is not secreted but reabsorbed by the tubular cells. Hence, it can be used as an ideal endogenous marker for estimation of GFR. The low molecular weight of cystatin C in combination with its stable production rate strongly indicates that the blood serum concentration of this protein is mainly determined by the GFR of the individual [6].

Material and Methods
The present study was conducted in the Department of Biochemistry in collaboration with Department of Medicine (Nephrology unit), Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, and Rohtak. A total of 40 known patients of chronic renal failure (CRF) attending nephrology unit of medicine were enrolled for this hospital based cross-sectional study. 40 age and sex matched healthy subjects were taken as controls. An informed written consent was taken from the patients for study. 40 patients known to be suffering with renal disease of more than 3 months duration were enrolled as cases of chronic renal failure (CRF) from nephrology unit of medicine for this hospital based cross-sectional study. The patient must be having eGFR <60 ml/minute/1.73m^2 (i.e. Stage III, IV and V). The eGFR was calculated by standard MDRD method [7]. Patients on

haemodialysis were excluded.

5 ml venous blood sample was collected in a plain evacuated blood collection tube under all aseptic precautions. Samples were processed within one hour of collection. Serum was separated by centrifugation at 3000 rpm for 10 minutes after clotting. Separated serum was analysed for serum creatinine on same day and rest serum was stored at -20^{0}C (maximum 3 months) for cystatin C analysis.

24 hr urine sample was taken for estimation of creatinine clearance. Serum and urine creatinine estimated by modified jaffe's method using commercial kit from Randox on Randox Rx Suzuka auto analyzer [8] while cystatin C analysis was done by immunoturbidimetric assay using kit from Accurax on Erba-XL 300 autoanalyzer [9].

$$\text{Creatinine Clearance (ml/min): } = \frac{\text{24 hour urine volume} \times \text{urine creatinine}}{1440 \times \text{serum creatinine}}$$

The eGFR was calculated by MDRD equation.

Estimated GFR (mL/min per 1.73 m2) = 1.86 x (PCr)–1.154 x (age)–0. 203

Multiply by 0.742 for women. The data was analysed by SPSS 20 (Statistical package for social sciences) using appropriate statistical methods.

Results

All the routine renal function parameters i.e serum urea, creatinine, uric acid, phosphate, alkaline phosphatase (ALP) in patients of CKD with mean level of 151.23 ± 71.85mg /dL,4.11 ± 3.00mg/dL,9.06 ±1.86 mg/dL, 6.70±1.32mg/dL, 141.00 ± 54.53U/L respectively were significantly raised (p < 0.001) in cases as compared to controls with mean of 37.30±4.82mg/dL,0.84±0.15mg/dL, 4.35±0.85mg/dL,3.47±0.71mg/dL,55.7±12.63U/L respectively. The serum calcium was significantly decreased in cases (p<0.001) with mean level of 7.11 ± 0.47mg/dL in comparison to controls with mean level of 9.33 ± 0.66mg/dL. (Table 1) Serum Cystatin C increased with stage wise progression of CKD with mean level of 2.31±0.97mg/L in stage III, 2.80±0.55 mg/L in stage IV, 3.01±0.81 mg/L in stage V in comparison to controls (0.68±0.17 mg/L). Serum creatinine was also increased with stage wise progression in CKD with mean level of 1.7±0.19 mg/dL in stage III, 2.72±0.58 mg/dL in stage IV, 7.66 ±2.33 mg/dL in stage V in comparison to controls (0.84± 0.15mg/dL). Creatinine clearance decreased with mean level of 41.96±8.30 ml/min in stage III, 36.73± 6.25ml/min in stage IV, 13.14±9.60 ml/min in stage V of CKD in comparison to controls (121.79 ±13.6ml/min)(Table 2, Figure 1 and 2).

Table 1: Comparison of demographic and renal parameters between cases and controls

	Control (n=40)	Case (n=40)	p value
Age (years)	47.68 ±8.65	50.63±14.45	0.272
Sex (Males: Females)	19:21	21:19	0.823
S. Urea (mg/dL)	37.30 ±4.82	151.23±71.85	<0.001
S. Creatinine (mg/dL)	0.84±0.15	4.11 ± 3.00	<0.001
S. Uric Acid (mg/dL)	4.35 ±0.85	9.06 ± 1.86	<0.001
S. Calcium (mg/dL)	9.33 ± 0.66	7.11 ± 0.47	<0.001
S. Phosphate (mg/dL)	3.47 ± 0.71	6.70 ± 1.32	<0.001
S.ALP (U/L)	55.7 ± 12.63	141.00 ± 54.53	<0.001
Cystatin C (mg/L)	0.69±0.17	2.72±0.83	<0.001
Creatinine Clearance(ml/mt)	121.79±13.6	30.17±15.12	<0.001

Table 2: Distribution of serum Cystatin c, Serum creatinine, Creatinine Clearance in various stages of CKD (staging according to eGFR)

	Control (n=40)	Stage III (n=13)	Stage IV (n=13)	Stage V (n=14)
CystatinC(mg/L)	0.68 ±0.17	2.31±0.97**	2.80 ±0.55*	3.01 ± 0.81**
S.Creatinine(mg/dL)	0.84± 0.15	1.7 ± 0.19**	2.72± 0.58*	7.66 ± 2.33**
Creatinine Clearance(ml/mt)	121.79 ± 13.6	41.96 ± 8.30**	36.73 ± 6.25*	13.14 ± 9.60**

* p Value <0.05 (in comparison to previous stage)

** p Value <0.001(in comparison to previous stage)

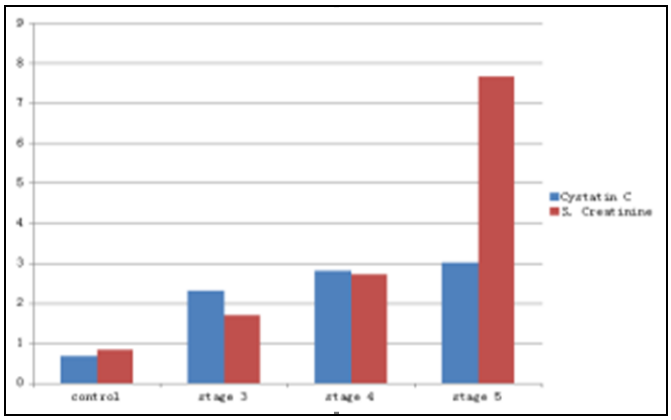

Fig 1: Graph showing stagewise increase in cystatin C and serum creatinine in CKD patients

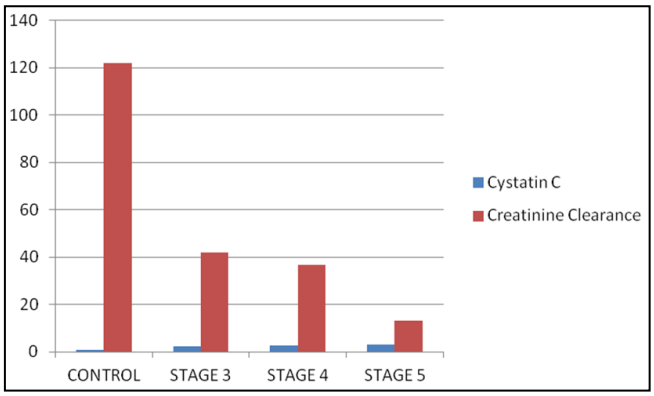

Fig 2: Graph showing stagewise progression of Cystatin C with decrease in creatinine clearance

Serum cystatin C was significantly correlated with creatinine clearance (r= -0.864; p<0.001) and serum creatinine (r= 0.665; p<0.001).

Table 3: Correlation of serum cystatin c with serum creatinine, creatinine clearance

	Cystatin C (n = 80)	
	r value	p Value
S. Creatinine	0.665	<0.001
Creatinine Clearance	-0.864	<0.001

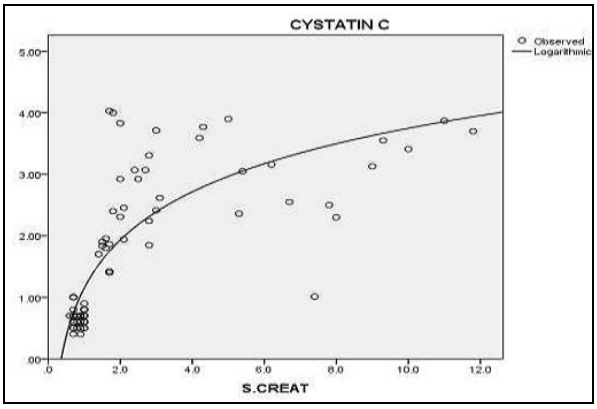

Fig 3: Graph showing logarithmic correlation between serum Cystatin C and serum creatinine in different stages of CKD (r=0.665; p<0.001)

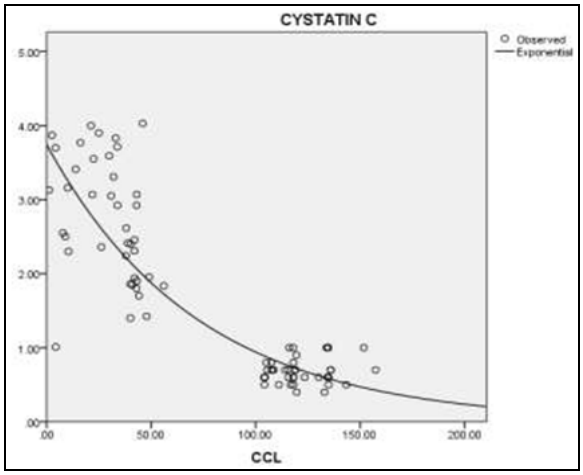

Fig 4: Graph showing exponential correlation between serum cystatin C and Creatinine Clearance in different stages of CKD (r=-0. 864; p<0.001)

The Creatinine clearance was significantly decreased (p<0.001) in cases in comparison to controls. Also, creatinine clearance was decreased significantly with progression of CKD (p<0.001) for each successive stage i.e. stage III, IV and V (p value for difference between controls and stage III <0.001, stage III and stage IV <0.001, stage IV and V <0.001).

Table 4: Stagewise correlation of renal markers in CKD (p value)

	Cystatin C	S. Creatinine	Creatinine Clearance
Controls and Stage III	<0.001	0.37	<0.001
Stage III and Stage IV	0.013	0.05	<0.001
Stage IV and Stage V	0.04	<0.001	<0.001

Discussion

Creatinine is the most widely used biomarker of kidney function. It is inaccurate at detecting mild renal impairment, and levels can vary with muscle mass and age [5].

Cystatin C, a non-glycosylated 13 kDa protein, has the potential to improve estimates of GFR, because it is thought to be less influenced by muscle mass or diet. Cystatin C is unique among cystatins as it seems to be produced by all human nucleated cells. The structure of the cystatin C gene is compatible with a stable production rate of Cystatin C by most nucleated cells. The low molecular weight of cystatin C in combination with its stable production rate strongly indicates that the blood serum concentration of this protein is mainly determined by the glomerular filtration rate of the individual. Several other low molecular weight proteins like β-2 micro globulin, Retinol Binding Protein (RBP), complement factor D, have been investigated for their utility in monitoring GFR but none have proven useful to the influence of non-renal factors on their circulating concentrations [6].

In patients with impaired renal function receiving corticosteroids, β-2 micro globulin concentration is decreased in a dose dependent manner reflecting the anti lympho proliferative effect of corticosteroids on mononuclear cells which are the principle source of β-2 micro globulin and this limits the use of β-2 micro globulin as a GFR marker [10].

Among the controls in our study, the mean cystatin C was 12.5% higher in males (0.72 ± 0.20 mg/L) as compared to females (0.64 ± 0.12 mg/L).

Studies with Northern-blot experiments have revealed that the cystatin C gene is expressed in every human tissue examined, including kidney, liver, pancreas, intestine, stomach, antrum, lung and placenta. The highest cystatin C expression was seen in seminal vesicles [11].

Hojs R, *et al*. had shown earlier that cystatin C were 9% lower for women than men [12]. The normal range Serum cystatin C reference values differ in many populations with sex and age across different studies. The average reference interval is 0.52-0.90 mg/L (mean 0.71 mg/L) in adult females while 0.56-0.98 mg/L (mean 0.77 mg/L) in adult male [13].

Our study evaluates the use of cystatin C as a marker for chronic kidney disease and its progression. Cystatin C and serum creatinine were found to be significantly high in CKD patients as compared to controls (p < 0.001 for all markers) while creatinine clearance were significantly decreased in

patients of CKD as compared to controls ($p<0.001$).

In the earlier research, concentrations of serum creatinine and cystatin C increased progressively with decreasing GFR and their diagnostic performance for the detection of even minor deterioration of renal function (GFR <90 mL min−1 (1.73 m^2) $^{-1}$) was similar. Patients experienced progression of CKD, defined as doubling of baseline creatinine and/or terminal renal failure during prospective follow-up. It was concluded that serum creatinine and cystatin C for detecting even minor degrees of deterioration of renal function is good and these markers provide reliable risk prediction for progression of kidney disease in patient with CKD [14].

In present study, serum cystatin C increased with stage-wise progression of CKD with mean level of 2.31 ± 0.97mg/L in stage III, 2.80 ± 0.55 mg/L in stage IV, 3.01 ± 0.81 mg/L in stage V in comparison to controls (0.68±0.17 mg/L). Serum creatinine was also increased with stage-wise progression in CKD with mean level of 1.7 ± 0.19 mg/dL in stage III, 2.72± 0.58 mg/dL in stage IV, 7.66 ± 2.33 mg/dL in stage V in comparison to controls (0.84± 0.15 mg/dL). Thus, the levels of both serum cystatin C and serum creatinine were significantly increased in each successive stage of CKD (stage III, p <0.001 and <0.001; stage IV, p = 0.013 and 0.05; and stage V, p = 0.04 and <0.001 for serum cystatin C and serum creatinine respectively in comparison to their previous stage). This was similar to earlier research by Zati Iwani *et al* in 2013 [16], which illustrated that serum cystatin C increased with the progression of CKD, and it was significantly higher in the subjects with mild to moderate eGFR (stages II and III.[15] In addition to this we also found that creatinine clearance was decreased significantly with progression of CKD for each successive stage i.e. stage III, IV and V. Lastly, in each successive stage both cystatin C and serum creatinine were significantly increased. These findings were in congruence with NKF-KDOQI guidelines (National kidney foundation Kidney Dialysis Outcomes Quality Initiative) classification and staging of CKD [4].

In our study, serum cystatin C did show highly significant negative correlation with creatinine clearance (r = -0.864). Cystatin C inversely related with creatinine clearance. Dharnnidharka VR *et al* calculated overall correlation coefficient for the reciprocal of serum cystatin C (r = 0.816) was superior to that of the reciprocal of serum creatinine (r = 0.742) and thus concluded that serum cystatin C is clearly superior to serum creatinine as a marker of GFR [16]. Newman *et al* in 1995 proved diagnostic sensitivity of cystatin C for abnormal GFR to be significantly ($p < 0.05$) more sensitive than creatinine (71.4 vs. 52.4%) [17]. Roos *et al* in 2007 [19] reported a systematic review by comparing the diagnostic accuracy of cystatin C with serum creatinine which included studies that assessed accuracy of cystatin C for all grades of renal function [18].

Certain longitudinal studies on cystatin C predominantly support our finding that serum cystatin C reflects GFR changes more rapidly compared to serum creatinine [19-21]. One explanation may be that cystatin C, unlike creatinine, resembles more closely an ideal endogenous marker of glomerular filtration except for a few, negligible exceptions [22-24]. This is in contrast to the numerous non-renal factors that influence the generation of creatinine, its tubular secretion, and back leak, which may result in inaccurate reflection of GFR by creatinine [25]. Another potential explanation emerges from recent observations that cystatin C and creatinine differ in regard to their glomerular filtration characteristics during pregnancy and diabetic nephropathy [26, 27]. It remains speculative whether this phenomenon occurs also in CKD but this still demands to be well described. Cystatin C has been identified as a superior marker to creatinine in chronic renal insufficiency with small variability. Serum cystatin C measurement is highly accurate and precise. The commercially available immunoturbidimetric assay provides rapid, automated measurement of cystatin C and requires few minutes until results are available. Additionally, it has been well established earlier that preanalytic factors such as routine clinical storage conditions, freezing and thawing cycles, or interfering substances, such as bilirubin or triglycerides, do not affect cystatin C measurement [17]. Thus, use of cystatin C for staging of CKD may be more accurate in comparision to creatinine clearance, which is a calculated parameter and require adjustment of variables which can significantly alter serum creatinine. Larger studies are required to evaluate use of cystatin C or both serum cystatin C and serum creatinine more specifically as suggested by recent studies as by Stevens *et al*, an equation that used both serum creatinine and cystatin C with age, sex, and race was better than the equations that use only one of these markers [28]. Besides use in CKD, Cystatin C has also been linked with other diseases. It has been used to evaluate ARF where no other factor was identified to modify serum cystatin C levels, which enhances its usefulness urine volume demonstrated any effect on the predictive value of serum cystatin C in ARF [29].

Conclusion

Serum cystatin C is another available tool to detect chronic kidney disease, an alternative biomarker of kidney function that is a better predictor of mortality than creatinine and is also less affected by age, race, or muscle mass. Now Cystatin C has opened the new avenues for research in the Indian context.

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