Cystatin C, A Better Predictor of Renal Impairment in Essential Hypertensive Patients

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ABSTRACT:
Background: *Due to the increased relevance of renal impairment in hypertension in developing countries like India,this study was undertaken to evaluate the serum levels of cystatin C in essential hypertensive patients. A correlation between the degree of hypertension, serum cystatin C and Glomerular Filtration Rate(GFR) was also assessed to prove the superiority of cystatin Cover creatinine to detect renal impairment at an early stage.*
Materials and Methods:*160 subjects were enrolled in the present study.80 hypertensive patients with 36 in stage I and 44 in stage 2 were diagnosed according to JNC VII criteria.80 age and sex matched healthy normotensive controls were included. The levels of cystatin C, urea, creatinine, uric acid, and serum electrolytes were measured.*
Results: *The mean serum cystatin C in hypertensive patients(1.11±O.25 mg/l) was found to be significantly raised($p<0.0001$) as compared to controls(0.82±0.12 mg/l) whereas creatinine was increased significantly only in stage II hypertensives. Also a stronger negative correlation was observed serum cystatin C and GFR when compared with creatinine and GFR. A significant positive correlation was found beween cystatin C with urea, both in stage I and stage II hypertensive patients. However a significant positive correlation was found between cystatin C and creatinine and uric acid only in stage II hypertensives.*
Conclusion: *Cystatin C shows stronger negative correlation with GFR than creatinine shows with GFR. So it can serve as more reliable and sensitive marker for identifying small changes in GFR in essential hypertensive patients.*

Keywords: *Essential hypertension, Renal impairment, CKD, Cystatin C*

INTRODUCTION

Hypertension is one of the leading causes of the global burden of the diseases.[1] It is the most important public health problem of the developed and developing countries, with over 50 million(29%) adults having hypertension in USA alone.[2] In India, hypertension is the leading non-communicable disease risk & estimated to be attributable for nearly 10perentof all deaths.[3] Adult Hypertension prevalence has risen drastically over the past three decades from 5 percent to 20-40 percent in urban areas & 12-17 percent in rural areas. Presence of hypertension doubles the risk of cardiovascular diseases, ischemic & hemorrhagic stroke, renal failure, and peripheral arterial disease.[1] Hypertension is an independent risk factor for renal injury & End Stage Renal Disease. When renal function decreases, the serum concentration of many low-molecular weight proteins increases. As a consequence, the blood levels of some small proteins, such as lysozyme, β2-microglobulin and Cystatin C, have been proposed as indices of renal function.[4,5] Some studies suggest that serum cystatin C is superior to serum creatinine as a marker of GFR, particularly in identifying small decreases in GFR[6].Studies have shown that serial measures of cystatin C accurately detected changes in renal function in patients with normal or elevated GFR that creatinine-based methods could not identify[7,8,9,10].

Owing to the increased relevance of early detection and intervention in hypertensive patients this study was done to find a correlation between the degree of hypertension, the serum cystatin C and GFR estimated from cystatin C(e-GFR) to prove the superiority of cystatin C over serum creatinine in early stages of decline in GFR in essential hypertensive patients.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry, S.C.B. Medical College and Hospital, Cuttack from September 2013 to December 2014. 80 patients of age group 25-65 years, attending OPD and indoor in the Department of Medicine, S.C.B. Medical College and Hospital, Cuttack were included in the study. Patients with Essential Hypertension were selected and diagnosed on the basis of their history, physical examination, biochemical investigations and according to the JNC 7 (Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure) Criteria for the diagnosis of hypertension[11].

The control group includes 80 age and sex matched healthy adults with normal serum lipid profile, no symptoms and signs suggestive of hypertension and no family history of the disease.

Three ml of blood was collected after overnight fasting of 8 hours from all enrolled patients and healthy controls for the assessment of Cystatin-C levels and other biochemical parameters like, serum urea, creatinine, uric acid, Electrolytes(Na+ and K+), Demographic characteristics (name, age, sex), history of risk factors (smoking, family history, medications , alcohol intake etc.), systolic and diastolic blood pressures, were recorded in detail.

The inclusion criteria were as follows: Adults aged 25 years and above diagnosed with hypertension according to JNC 7 (Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure).

Blood Pressure Classification	Systolic (mmHg)	Diastolic (mmHg)	Normal
<120	<80 Pre-hypertension	120-139	80-89
Stage 1 Hypertension	140-159	90-99	
Stage 2 Hypertension	≧160	≧100	

The exclusion criteria included Secondary hypertension categories (Endocrine hypertension, renal or renovascular, related to collagen vascular disease, aortic coarctation. etc), Diabetes mellitus, Hypertensive retinopathy and/or nephropathy, Chronic kidney disease, Previous history of corticosteroid/cyclosporine use.

Following parameters were measured both in control and cases. Serum urea (GLDH kinetic method), creatinine (Modified Jaffe Method), uric acid (Uricase-POD Method), electrolytes (Ion selective electrode method) and cystatin C. Cystatin C was measured by turbidimetric immunoassay method. Statistical analysis was done using SPSS and Microsoft Excel 10 software.

RESULTS

Present study included total 160 subjects which comprised of 80 hypertensive patients included in study group , out of which 36 were stage 1 hypertension cases and 44 were stage 2 hypertensives. The control group consisted of 80 age and sex matched healthy individuals.(Table 1).

DISCUSSION

Hypertension is one of the major public health problems now a days because of its high morbidity and mortality arising from the risks of cardiovascular and renal involvement. The present study was carried out to evaluate the serum levels of cystatin C in stage I and stage II hypertensive patients. Given the wide range of end organ damage associated with hypertension, it would be useful to quantitate the impact of hypertension with renal complications. Renal disease is symptomless in its early phases and hence laboratory diagnosis by measuring GFR is essential. Serum creatinine and urea are dependent upon variables like age, muscle mass and hence estimated GFR(eGFR)is used for assessing the renal function. Estimated GFR can't be used as gold standard as it is only a calculated parameter based on variables especially serum creatinine which shows a blind area even when the GFR falls up to 50% of normal.

In recent years the role of cystatin C in predicting early decline in GFR in comparison to serum creatinine and creatinine based GFR equations has been much debated. The present study has tried to explore the usefulness of cystatin C over serum creatinine for identification of a better marker of early renal involvement in essential hypertension patients which in turn can help in their timely intervention and management.

Present study included total 160 subjects which comprised of 80 hypertensive patients, out of which 36 were stage 1 hypertensive cases and 44 were stage 2 hypertensives. The control group consisted of 80 age and sex matched healthy individuals.(**Table 1**)

The age of patients and healthy controls ranged from 25yrs to 65yrs (**Table 1**). In the study group, maximum number of hypertensive patients were within the age group of 51-60 yrs. This finding was similar to that of Pramila devi R et al[12].In both the subgroup of stage 1 and stage 2 hypertension cases, maximum number of patients were within the same age group (51-60 yrs).

Out of the 80 hypertensive patients, 41 were males and 39 were females. Out of the 41 male patients, 22 were stage 2 hypertensives and out of the 39 female patients, 22 were stage 2 hypertensive cases.

Most of the patients having hypertension (both male and females) belonged to higher age group. It is due to decrease in arterial compliance in major arteries due to atherosclerosis. In females this may be also due to the hormonal changes that occur during menopause[13,14]. The decline in the oestrogen/ androgen ratio dilutes the vasorelaxant effects of oestrogens on the vessel wall and promotes the production of vasoconstrictive factors such as endothelin[15] and also causes an upregulation of the Renin Angiotensin System with an increase in plasma-renin activity[16].

Our study shows mean serum urea is significantly raised in stage 1 Hypertensives and stage 2 Hypertensives in comparison to controls.

Mean serum creatinine is significantly raised in only stage 2 Hypertensives in comparison to controls.In stage 1 hypertensives no significant increase is found. This is because creatinine is freely filtered by the glomerulus and not secreted by the tubules, but about(40-70%)is passively reabsorbed from the renal tubules. Serum creatinine does not increase significantly until GFR is reduced to less than 50% of its normal value because of increased tubular secretion of creatinine.

Mean serum uric acid is not significantly increased in stage 1 Hypertensives but significantly increased in stage 2 Hypertensives as compared to controls.It has been found that essential hypertension may be associated with hyperuricaemia with normal

renal functions[17,18]. Hyperuricaemia is predictive for the development of both hypertension and coronary artery disease; it is increased in patients with hypertension, and when present in hypertension, an elevated serum uric acid is associated with increased cardiovascular morbidity and mortality[19,20].

Our study shows serum cystatin C levels were significantly increased in both stage I hypertensives as well as stage II hypertensives when compared with controls.

Our study shows correlation of serum cystatin C and serum creatinine with C-G GFR in essential hypertensive patients. Serum creatinine showed a negative correlation which is statistically significant. Serum cystatin C showed a more negative and statistically more significant correlation with C-G GFR.

As several physiological factors influence creatinine secretion, C-G GFR tends to overestimate the GFR.But unlike creatinine cystatin C is not influenced by gender, muscle mass or diet, thereby giving more accurate GFR. The present study indicates that both cysC and cysC based GFR are more sensitive and reliable marker for assessing renal function, particularly in identifying small changes in GFR than any other parameter at present in use.

Table 1: Age and Sex Ditribution of Control and Study Group

Age Group (In Yrs.)	Control Group (n =80)			Study Group (n = 80)					
				Stage I Hypertension			Stage II Hypertension		
	M	F	Total	M	F	Total	M	F	Total
<30 yrs	11	3	14	2	2	4	1	0	1
31-40 yrs	10	20	30	2	2	4	0	2	2
41-50 yrs	10	9	19	6	4	10	5	9	14
51-60 yrs	7	6	13	6	7	13	14	10	24
>60 yrs	3	1	4	3	2	5	2	1	3
TOTAL	41	39	80	19	17	36	22	22	44

Table 2: Characteristics of Control Group and Study Group

Sl No.	Parameter	Control Group (n = 80)	Study Group (n = 80)	
			Stage I Hypertension (n=36)	**Stage II Hypertension (n=44)**
		Mean ± SD	Mean ± SD	Mean ± SD
1.	Age (in yrs.)	41.27±10.86	48.97±10.91*	53.86±7.43*
2.	BMI (Kg/m2)	24.39±2.02	24.63±2.00Ω	25.11±2.46Ω
3.	Duration of hypertension (in yrs)	-	6.13±3.40	7.93±2.92

Ω Statistically non-significant as compared to controls.
* Statistically significant (p<0.001) as compared to controls.

Table 3: Systolic and Diastolic Blood Pressure in Control and Cases

SL. No.	Parameter (mmHg)	Control group (n = 80)	Study group (n = 80)	
			Stage 1 hypertensive (n=36)	Stage 2 hypertensive (n=44)
		Mean ± SD	Mean ± SD	Mean ± SD
1.	SBP	116.2±5.80	150.27±3.82**	168.90±6.77**
2.	DBP	75.57±4.96	93.88±2.66**	108.45±5.92**

**Statistically significant (p <0.0001) as compared to control group

Table 4: Biochemical Parameters in Control Group and Study Group

Sl. No.	Parameter (mg/dl)	Control group (n =80)	Study group(n = 80)	
			Stage 1 hypertensive (n=36)	Stage 2 hypertensive (n=44)
		Mean ± SD	Mean ± SD	Mean ± SD
1.	Serum Urea	19.85±7.69	38.30±9.75**	49.52±11.70**
2.	Serum Creatinine	1.01±0.19	1.02±0.15	1.24±0.31*
3.	Serum Uric acid	3.71±1.26	3.70±1.66	6.36±2.25**
4.	Serum Na+(meq/l)	136.73±4.80	135.02±7.38	138.56±9.56
5.	Serum K+(meq/l)	4.16±0.70	4.10±0.96	4.18±0.99

*Statistically significant (p< 0.001) as compared to control & stage I hypertensives.
**statistically significant (p< 0.0001) as compared to control.

Table IV: shows the biochemical parameters in control group and study group.The mean serum urea was 19.85±7.69 mg/dl in control; 38.30±9.75 mg/dl in stage 1 hypertensives; 49.52±11.70 mg/dl in stage 2 hypertensives. Serum urea was significantly raised (p<0.0001) in stage 1 and stage 2 hypertensives as compared to control. The mean serum creatinine was 1.01±0.19 mg/dl in control group;1.02±0.15mg/dl in stage 1 hypertensives; 1.24±0.31mg/dl in stage 2 hypertensives. Serumcreatinine was significantly raised (p<0.001) in stage 2 hypertensives as compared to control & stage I hypertensives. The mean serum uric acid was 3.71±1.26 mg/dl in control group; 3.70±1.66 mg/dl in stage 1 hypertensives; 6.36±2.25 mg/dl in stage 2 hypertensives. It was found to be significantly higher (p<0.0001) in stage 2 hypertensives as compared to control.

Serum Na+ and serum K+ did not show any correlation when compared with controls.

Table 5: Serum Cystatin C (Mg/L) Concentrations in Between the Study Group

CATEGORY		MEAN±SD	CI (MEAN±2SE)
CONTROL GROUP(n=80)		0.82±0.12	0.80-0.84
STUDY GROUP(n=80)	STAGE I HYPERTENSIVES(n=36)	1.02±0.23**	0.96-1.08
	STAGE II HYPERTENSIVES(n=44)	1.18±0.25**	1.12-1.24

** Statistically significant (p<0.0001) as compared to control group.

TABLE V: Patients with stage 1 hypertension had a mean serum cystatin C of 1.02±0.23 (mg/L)and 95% confidence interval of mean 0.96-1.08 mg/L. In patients with stage 2 hypertension the mean serum cystatin C level was found to be 1.18±0.25 (mg/L) and 95%confidence interval of mean was 1.12-1.24 mg/L.

The serum cystatin C concentrations were significantly higher in stage 1 and stage 2 hypertension cases (compared to controls)(p<0.0001).

Table 6: Comparision of C-G(Cockcroaft-Gault) and Cystatin C Based Estimated Gfr(E-Gfr) between Study and Control Group

PARAMETER	CONTROL GROUP (n=80) MEAN±SD	STUDY GROUP (n=80)	
		Stage 1 hypertensive (n=36)	Stage 2 hypertensive (n=44)
Cockcroaft-Gault (e-GFR) (ml/min/1.73m²)	102.4±19.8	84.65±13.58**	72.65±15.51**
(Cystatin C) e-GFR (ml/min/1.73m²)	101.2±16.8	82.40±18.45**	69.90±17.08**

** Statistically significant (p<0.0001) as compared to control group

Table VI -Shows that the mean e-GFR calculated by C-G formula in the control group was 102.4±19.8 (ml/min/1.73m²) and e-GFR calculated by cystatin C based formula was 101.2±16.8 (ml/min/1.73m²).Similarly in the stage I hypertension cases mean C-G e-GFR is 84.65±13.58 (ml/min/1.73m²) and CYS C e-GFR is 82.40±18.45 (ml/min/1.73m²)and in the stage II hypertension cases the mean C-G e-GFR is 72.65±15.51 (ml/min/1.73m²) and CYS C e-GFR is 69.90±17.08 (ml/min/1.73m²).

CYS C e-GFR was found to be significantly reduced (p value <0.0001) in both stage I and stage II hypertensive patients as compared to the healthy controls. Whereas C-G e-GFR is also significantly reduced(p value<0.0001 in both stage I and stage II hypertensive cases as compared to controls).

Table-7: Correlation of Cystatin C with Other Renal Function test Parameters in Stage I Hypertensive Patients

PARAMETERS	r Value	p Value
UREA	0.74	<0.00001
CREATININE	0.14	0.41
URIC ACID	0.10	0.56

Table VII shows correlation study of cystatin C with other renal function test parameters (urea, creatinine, uric acid) in stage I hypertensive patients. With urea it shows a significant positive correlation(p value <0.00001 and r value 0.74).With creatinine it shows a positive correlation which is not statistically significant(p value 0.41 and r value 0.14).With uric acid it also shows a positive correlation which is not significant(p value 0.56 and r value 0.10).

Table 8: **Correlation of Cystatin C with Other Renal Function Test Parameters in Stage Ii Hypertensive Patients**

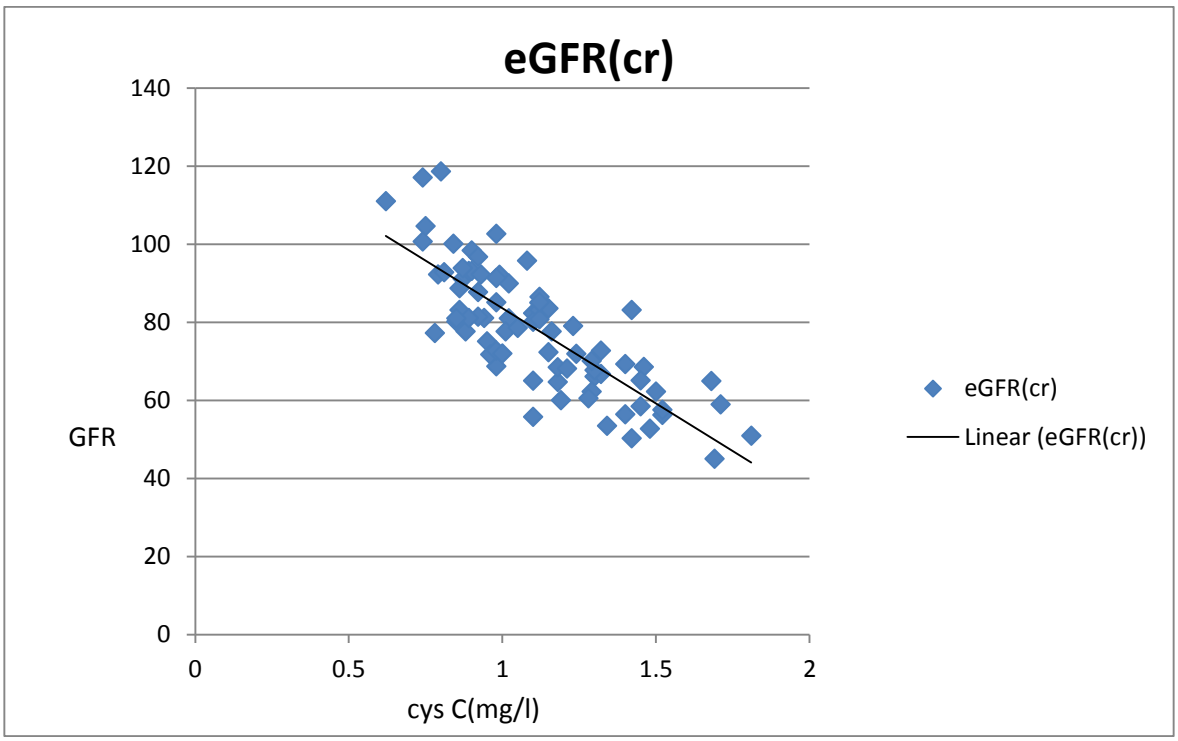
PARAMETERS	r Value	p Value
UREA	0.40	<0.01
CREATININE	0.42	<0.005
URIC ACID	0.33	<0.05

Table VIII shows correlation study of cystatin C with other renal function test parameters (urea, creatinine, uric acid) in stage II hypertensive patients.With urea it shows a significant positive correlation (p value <0.01 and r value 0.40).With creatinine it shows a positive correlation which is statistically significant(p value <0.005 and r value 0.42).With uric acid it shows a significant positive correlation (p value <0.05 and r value 0.33).

Table 9: Correlation of Serum Cystatin C(Mg/L) and Serum Creatinine(Mg/Dl) with C-G Gfr(Ml/Min/1.73 M^2) In Essential Hypertensive Patients

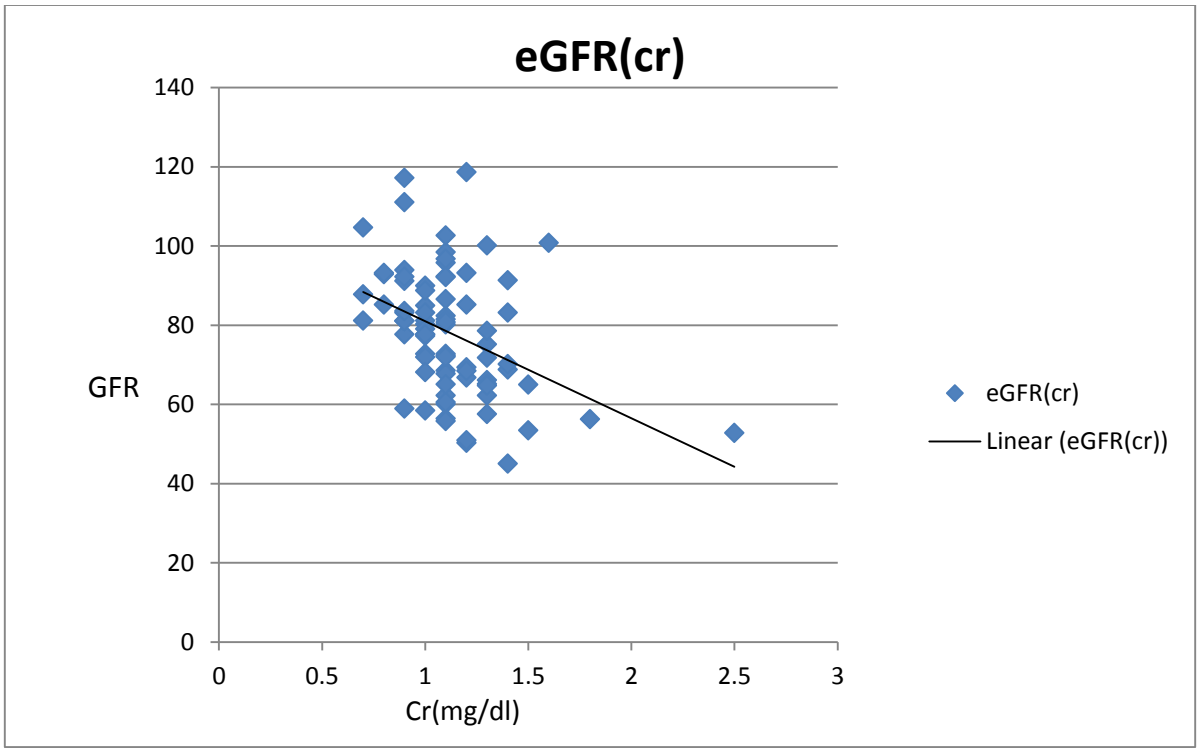
PARAMETERS	r Value	p Value
SERUM CYSTATIN C VS C-G GFR	**-0.80**	**<0.00001**
SERUM CREATININE VS C-G GFR	**-0.39**	**<0.001**

Table IX shows correlation study of serum cystatin C and serum creatinine with C-G GFR in essential hypertensive patients.When serum creatinine is compared with C-G GFR it shows a negative correlation which is statistically significant(p value <0.001 and r value -0.39). When serum cystatin C is compared with C-G GFR it shows a negative correlation which is statistically more significant(p value <0.00001 and r value -0.80).



Graph 1: eGFR vs cysC

Graph 1 shows with mild to moderate reduction of GFR, cystatin C levels also shows increased values(normal level 0.52-0.90 mg/l).



Graph 2: eGFR vs creatinine

Graph 2 shows even with mild to moderate reduction of GFR levels creatinine levels still being in normal range.

Graph 1 and Graph 2(comparison between creatinine & cystatin C with eGFR) shows while the eGFR showing mild to moderate reduction, creatinine values are well within normal limit, while cystatin C levels are elevated. This makes cystatin C as an early predictor of adverse renal outcome compared to conventional renal function test parameter like creatinine.

CONCLUSION

In summary when compared between creatinine and cystatin C for better marker for assessment of renal function, although serum creatinine and creatinine based GFR are well established marker for renal function, this study shows that cystatin C and cystatin C based GFR can serve as more reliable and more sensitive indicator for early detection of compromised renal function in essential hypertensive patients. The finding is in accordance with other related recent studies and can be established further by including more number of cases.

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