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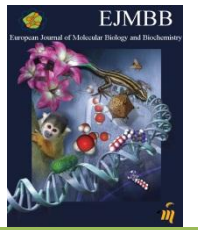

CYSTATIN C IN CHRONIC KIDNEY DISEASE

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ABSTRACT

Cystatin C has emerged as an alternative marker of kidney function. Because of its small size and basic isoelectric point, cystatin C is freely filtered by the glomerulus. It is not secreted, but is reabsorbed by tubular epithelial cells and subsequently catabolised so that it does not return to the blood flow. The use of serum cystatin C to estimate GFR is based on the same logic as the use of blood urea nitrogen and creatinine, but because it does not return to the blood stream and is not secreted by renal tubules, it has been suggested to be closer to the ideal endogenous marker. To determine the levels of serum cystatin C in patients with Chronic Kidney Disease (CKD). And to find out the correlation of serum cystatin C and serum creatinine with estimated Glomerular Filtration Rate in patients with CKD. A Cross-sectional study, carried out in the Department of Biochemistry in collaboration with Nephrology unit, Department of Medicine, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur between October 2013 to September 2015. Sixty nine (69) chronic kidney disease patients fulfilling criteria of definition of chronic kidney disease recommended by Kidney Disease Improving Global Outcomes (KDIGO). Estimation of serum cystatin C was done using Arbor Assays human cystatin C ELISA kit. Estimation of serum creatinine was done by the photometric colorimetric method. Serum cystatin C level was higher (mean = 1976.5 ± 540.3) than normal value (590-910 ng/dl) in all the CKD cases (100%)., while serum creatinine was increased in only 97.1 % of cases (mean=6.9 ± 4.4) as compared to normal value (0.6-1.6 mg/dl). Both cystatin C and creatinine had a good inverse correlation with GFR and statistically significant , (r_s=-0.676) and ($p<0.05$). (r_s=-0.973) and ($p<0.05$) respectively. This study suggest that both cystatin c and creatinine are good marker of renal function, these correlates well with GFR However, cystatin C was found to be a better marker as it was increased in 100% cases compared to creatinine which was increased only in 97.1% cases.

INTRODUCTION

Chronic diseases have become a major cause of global morbidity and mortality. In India the projected number of deaths due to chronic diseases will rise from 3.78 million in 1990 (40.4% of all deaths) to an expected 7.63 million in 2020 (66.7% of all deaths). The approximate prevalence of chronic kidney disease (CKD) is 800 per million population (pmp), and the incidence of

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end-stage renal disease (ESRD) is 150-200 pmp [1].

Renal function is essential for homeostasis. The kidneys play important pleiotropic roles including removal of metabolic waste products and maintenance of water-electrolyte balance and blood pressure. Early diagnosis of renal dysfunction and institution of appropriate therapy are vital to survival [2].

Glomerular filtration rate (GFR) is routinely assessed by measuring the concentration of serum markers such as blood urea nitrogen and serum creatinine. Although widely used these endogenous markers are not ideal and do not perform optimally in certain clinical settings. The other methods for determining GFR are to measure the clearance of exogenous substances such as inulin, iohexol, ^{51}Cr- EDTA, ^{99m} Tc-DTPA, ^{125}I-iothalamate. These techniques are time consuming, expensive, labor-intensive and require administration of substances that make them incompatible with routine monitoring [3].

Moreover, all creatinine-based estimating equations have limitations due to non-GFR determinants of serum creatinine, mainly the muscle mass, which cannot be accounted entirely by age, sex and race. Therefore, the clinician's reliance on creatinine-based equation for estimating the GFR could cause misclassification of CKD patients who may be at high risk of CKD and its complications [4]. There is thus a practical need for alternative marker to plasma creatinine which would be more specific, sensitive and reliable from the analytic and clinical point of view.

Recently, cystatin C has emerged as an alternative marker of kidney function that is independent of muscle mass, height, age and gender [5-8]. Cystatin C, because of its small size and basic isoelectric point, it is freely filtered by the glomerulus. It is not secreted, but is reabsorbed by tubular epithelial cells and subsequently catabolised so that it does not return to the blood flow [9]. The use of serum cystatin C to estimate GFR is based on the same logic as the use of blood urea nitrogen and creatinine, but because it does not return to the blood stream and is not secreted by renal tubules, it has been suggested to be closer to the ideal endogenous marker [3].

Studies have shown cystatin C to be a better marker than creatinine in determination of GFR. Hence this study attempts to determine the utility of serum cystatin C in predicting decline in renal function in CKD patients, so that appropriate and timely intervention can be instituted to delay or arrest the progression to renal failure in these pateients.

AIMS AND OBJECTS

To determine the levels of serum cystatin C in patients with Chronic Kidney Disease (CKD). And to find out the correlation of serum cystatin C and serum creatinine with estimated Glomerular Filtration Rate in patients with CKD.

MATERIALS AND METHODS

A Cross-sectional study, carried out in the Department of Biochemistry in collaboration with Nephrology unit, Department of Medicine, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur between October 2013 to September 2015. Sixty nine (69) chronic kidney disease patients fulfilling criteria of definition of chronic kidney disease recommended by Kidney Disease Improving Global Outcomes (KDIGO) [10]. attending Nephrology clinic or admitted in the Medicine ward (Nephrology unit) during the study period were selected as study group irrespective of sex and socioeconomic status. Patients with thyroid dysfunction, on steroids or immunosuppressants, patients with malignancy, with acute infection and patient who refused to participate were excluded from the study.

Informed written consent and detail clinical history were taken from the patients before taking blood sample. Estimation of serum cystatin C was done by ELISA technique, using Arbor Assays human cystatin C ELISA kit as described by Pergande M and Jung K [11]. Estimation of serum creatinine was done by the photometric colorimetric method (Jaffe-reaction) as described by Bartels H [12]. All the investigation were recorded in the performa designed for the study. Ethical clearance was obtained from the Institutional Ethical Committee RIMS and confidentiality was maintained.

SPSS version16 was used for statistical analysis**.** Classical statistical parameters like mean, standard deviation and percentages were used. Correlation co-efficient 'r' was employed for test of significance.

RESULTS AND OBSERVATION

Table 1. Age distribution of the respondents

Age in years	Number	Percentage
≤40	21	30.4
41-60	36	52.2
>60	12	17.4
Total	**69**	**100.0**
Mean ± SD	**49.64 ± 1.4**	

Table 1 this table shows that majority of the CKD patients were from the age group 41-60 years which accounts for 52.2% of cases. The mean age was 49.64 years with standard deviation of 1.4 years.

Table 2. Sex distribution of the respondents

Sex	Number	Percentage
Male	40	58.0
Female	29	42.0
Total	**69**	**100.0**

Table 2 shows that more than half (58%) of the CKD patients were males.

Table 3. Distribution of the respondents by causes of CKD

Causes of CKD	Number	Percentage
Diabetes mellitus(DM)	29	42.0
Hypertension (HTN)	19	27.5
DM+HTN	12	17.3
Others	9	13.2
Total	**69**	**100.0**

Table 3 This table shows that the commonest cause for CKD was Diabetes mellitus (42.0%) followed by hypertension (27.5%) and combination of both in 17.3%.

Table 4. Distribution of the respondents by serum cystatin C level

Cystatin C level	Number	Percentage
Normal (590-910 ng/dl)	0	0.0
High (>910 ng/dl)	69	100.0
Total	**69**	**100.0**
Mean ± SD	**1976.5 ± 540.3**	

Table 4 shows that in all the CKD cases, serum cystatin C level was higher than normal and the mean value ± S.D is found to be 1976.5±540.3.

Table 5. Distribution of the respondents by serum creatinine level

Creatinine level	Number	Percentage
Normal (0.6-1.6 mg/dl)	2	2.9
High (>1.6 mg/dl)	67	97.1
Total	**69**	**100.0**
Mean ± SD	**6.9 ± 4.4**	

Table 5 shows that serum Creatinine level was high in 97.1% of CKD cases. The mean ± SD is found to be 6.9±4.4

Table 6. Correlation between cystatin C level and GFR

Cystatin C	GFR
Spearman Correlation Coefficient	**-0.676**
p-value	**0.000**
N	**69**

Table 6 shows that cystatin C has good inverse correlation with GFR (r_s=-0.676) and this finding was found to be statistically significant ($p<0.05$).

Table 7. Relation between creatinine level and GFR

Creatinine	GFR
Spearman Correlation Coefficient	**-0.973**
p-value	**0.000**
N	**69**

Table 7 shows that creatinine had very good inverse correlation with GFR (r_s=-0.973) which is found to be statistically significant ($p<0.05$).

DISCUSSION

Our study shows that majority of the CKD patients were from the age group 41-60 years which accounted for 52.2% of cases as shown in Table 1. This finding was consistent with that of Huda N *et al* [13], in whose study, the age group of more than 40 years were significantly prone to develop CKD compared to age less than 40 years. Similar findings were also observed by Chen J *et al* [14], Coresh J *et al* [15] and Zhang QL *et al* [16] Age represents one of the most important factor that effect kidney function. Generally kidney is stable after infancy until late adulthood [17] GFR declines by 1ml/min/1.73m^2

after age of 30 years in healthy adulthood [18]. The decrease in kidney function might be due to the changes in kidney structure associated with aging [19]. The increase in prevalence of CKD in elderly might be due to the related comorbidities of CKD, such as cardiovascular diseases or diabetes.

It is also seen that more than half (58%) of the chronic kidney disease patients were males and less females (42.0%) as shown in table 2. Thus the highest prevalence of chronic kidney disease patients were seen in males. This was consistent with the findings of Shankar a *et al* [20] and Singh *et al* [21] who also reported the occurrence of CKD more in males than in females.

The commonest cause for CKD in our study was diabetes mellitus (42.0%) followed by hypertension (27.5%) and combination of both in 17.3% and other causes (13.2%) as shown in table 3. This study was similar to that observed by Murphree DD and Thelen SM [22] , Rajapurkar MM *et al* [23].

In our study serum creatinine had very good inverse correlation with GFR (r_s=-0.973) and this finding is found to be statistically significant ($p<0.05$) and serum cystatin C also had a good inverse correlation with GFR (r_s=-0.676) and this finding observed by Zhang M et al [24] , Bevc S *et al* [25]

This study suggests that both cystatin C and creatinine are good markers of renal function and these correlates well with GFR as has been reported by Randers E et al [26,27] , Risch L *et al* [28] and Vinge E *et al* [29] . However, cystatin C was found to be a better marker as it was increased in 100% cases (table 4) compared to creatinine which was increased only in 97.1% cases (table 5). Combination of creatinine and cystatin C may provide more precise GFR estimates and could correctly reclassify patients.

CONCLUSION

Cystatin C seems to be a promising alternative to creatinine as an endogenous marker of GFR in CKD patients. However the combination of creatinine and cystatin C provides more precise GFR estimates and could correctly reclassify patients. It would result in more selective use of resources and better management of patients, this will help in timely intervention and delay the progression of renal CKD which will reduce the morbidity, however, cost may be a limiting factor.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

REFERENCES

1. Agarwal SK, Srivastava RK. (2009). Chronic kidney disease in India: challenges and solutions. *Nephron Clin Pract*, 111(3), 197-203.
2. Onopiuk A, Tokarzewicz A, Gorodkiewicz E. (2015). Cystatin C: a kidney function biomarker. *Adv Clin Chem*, 68(1), 57-69.
3. Sarker PD, Rajeshwari G, Shivaprakash TM. (2005). Cystatin C- a novel marker of glomerular filtration rate: a review. *Indian Journal of Clinical Biochemistry*, 20(1), 139-44.
4. Peralta AC, Katz R, Sarnak MJ, Joachim I, Fried FL, Boer ID, *et al.* (2011). Cystatin C identifies chronic kidney disease patients at higher risk for complication. *J Am Soc Nephrol*, 22(1), 147-55.
5. Bokenkamp A, Domanetzki M, Zinck R, Schumann G, Byrd D, Brodehl J. (1998). Cystatin C: a new marker of glomerular filtration rate in children independent of age and height. P*ediatrics*, 101(5), 875-81.
6. Perkins BA, Nelson RG, Ostrander BE, Blouch KL, Krolewski AS, Myers BD, *et al* (2005). Detection of renal function decline in patients with diabetes and normal or elevated GFR by serial measurement of serum cystatin C concentration: result of 4 years follow up study. *J Am Soc Nephrol*, 16(5), 1404-12.
7. Stevens LA, Coresh J, Greene T, Levey AS. (2006). Assessing kidney function: measured and estimated glomerular filtration rate. *N Engl J Med*, 354(23), 2473-83.
8. Dharnidharka VR, Kwon C, Stevens G. (2002). Serum Cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis*, 40(2), 221-6.
9. Grubb A. (1992) Diagnostic value of analysis of cystatin C and protein HC in biological fluids. *Clin Nephrol*, 38(1), 520-7.
10. Levley AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, et al. (2005). Definition and classification of chronic kidney disease: a position statement from Kidney Disease Improving Global Outcome [KDIGO]. *Kidney Int*, 67(6), 2089-100.
11. Pergande M, Jung K. (1993). Sandwich enzyme immunoassay of cystatin C in serum with commercially available anti bodies. *Clin Chem*, 39(9),1885-90.
12. Bartels H. (1971). Photometric colorimetric test for endpoint measurement of creatinine, method without deproteinisation. *Clin Chim Acta*, 32, 81-85.
13. Huda MN, Alam KS, Ur-Rashid H. (2012). Prevalence of chronic kidney disease and its association with risk factors in disadvantageous population. *Int J Nephrol*, 2012, 1-7.
14. Chen J, Wildman RP, Gu D, Kusek JW, Spruill M, Reynolds K, *et al.* (2005). Prevalence of decreased kidney function in Chinese adults aged 35 to 74 years. *Kidney Int*, 68(6), 2837-45.

15. Coresh J, Selvin E, Stevens LA, Manzi J, Kusek JW, Eggers P, *et al*. (2007). Prevalence of chronic kidney disease in the United States. *JAMA*, 298(17), 2038-47.
16. Zhang QL, Rothenbacher D. (2008). Prevalence of chronic kidney disease in population-based studies: systematic review. *BMC Public Health*, 117(8), 1-7.
17. Price CP, Finney H. Developments in the assessment of glomerular filtration rate. (2000). *Clin Chim Acta*, 297(1), 55-66.
18. Snively CS, Gutierrez C. (2004). Chronic kidney disease: prevention and treatment of common complications. *Am Fam Physician*, 70(10), 1921-8.
19. Lamb EJ, O'Riordan SE, Delaney MP. (2003). Kidney function in older people: pathology, assessment and management. *Clin Chim Acta*, 334(1), 25-40.
20. Shankar A, Klein R, Klein BE. (2006). The association among smoking, heavy drinking, and chronic kidney disease. *Am J Epidemiol*, 164(3), 263-71.
21. Singh AK, Farag MK, Mittal BV, Subramanian KK, Reddy RK, Acharya VN, *et al.* (2013). Epidemiology and risk factors of chronic kidney disease in India – results from the SEEK. *BMC Nephrology*, 114(14), 1-10.
22. Murphree DD, Thelen SM. (2010). Chronic kidney disease in primary care. *J Am Board Fam Med*, 23(4), 542-50.
23. Rajapurkar MM, John GT, Kirpalani AL, Abraham G, Agarwal SK, Almeida AF, *et al.* (2012). What do we know about chronic kidney disease in India: first report of the Indian CKD registry. *BMC Nephrol*, 13(10), 1-8.
24. Zhang M, Cao X, Cai G, Wu D, Wei R, Yaun X, *et al.* (2013). Clinical evaluation of serum cystatin C and creatinine in patients with chronic kidney disease : a meta-analysis. *Journal of International Medical Research*, 41(4), 944-55.
25. Bevc S, Hojs R, Ekart R, Zavrsnik M, Gorenjak M, Puklavec L. (2012). Simple cystatin C formula of glomerular filtration rate in overweight patients with diabetes mellitus type II and chronic kidney disease. *Journal Of Diabetic Research*, 20, 1-7.
26. Randers E, Erlandsen EJ, Pedersen OL, Hasling C, Danielsen H. (2000). Serum cystatin C as an endogenous parameter of the renal function in patients with normal to moderately impaired kidney function. *Clin Nephrol*, 54(3), 203-9.
27. Randers E, Kristensen JH, Erlandsen EJ, Danielsen H. (1998). Serum cystatin C as a marker of the renal function. *Scand J Clin Lab Invest*, 58(7), 585-92.
28. Risch L, Blumberg A, Huber A. (1999). Rapid and accurate assessment of glomerular filtration rate in patients with renal transplants using serum cystatin C. *Nephrol Dial Transplant*, 14(8), 1991-6.
29. Vinge E, Lindergård B, Nilsson-Ehle P, Grubb A. (1999). Relationships among serum cystatin C, serum creatinine, lean tissue mass and glomerular filtration rate in healthy adults. *Scand J Clin Lab Invest*, 59(8), 587-92.