ORIGINAL ARTICLE

Evaluation of serum Cystatin C as an early diagnostic marker in non-dialysis CKD patients as compared to serum creatinine.

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ABSTRACT... Objective: To test the hypothesis that Serum cystatin C is an early diagnostic marker in non-dialysis CKD patients as compared to serum creatinine. **Study Design:** Descriptive, Cross Sectional OPD/ Hospital Based study. **Setting:** Department of Biochemistry and Nephrology, Jinnah Postgraduate Medical Centre, Karachi. **Period:** January 2018 to December 2018. **Material & Methods:** Study subjects included the diagnosed cases of Chronic Kidney Disease (CKD) up to 4th stage with exclusion of patients on dialysis. A total 90 subjects of age above 18 years who were presenting to the Nephrology clinic of JPMC for screening and evaluation of chronic kidney disease were recruited after applying inclusion and exclusion criteria. **Results:** There was significant mean difference in all eGFR across studied groups with p-value lower than 0.05. Cystatin C based calculation of GFR is lower in all three groups (86.33±13.08, 18.73±8.44, 73.30±12.23) as compared to GFR calculated based on serum creatinine (102.33±17.84, 30.00±11.59,. Our study was also suggestive that the sensitivity of serum Cystatin C is higher as compared to creatinine for detection of reduced GFR. **Conclusion:** Our study shows that cystatin C is a reliable indicator of estimating kidney functions as compared to serum creatinine. A possible advantage of cystatin C is that being a large molecule, its blood levels might rise sooner than that of creatinine.

Key words: Chronic Kidney Disease, Cystatin C, Creatinine.

INTODUCTION

CKD is a significant global health problem. As reported by the Global Burden of Disease Study conducted in the year 2015, kidney disorders were the 12th most common causes of death which account for nearly 1.1million deaths throughout the world.[1] Mortality due to chronic kidney disease has raised upto 31.7% in the past ten years, making it a major cause of death alongside diabetes and dementia.[2] Asia with almost 60% population of the world has the highest prevalence of CKD in the world.[3] Studies from the south (India, Pakistan, and Bangladesh) have reported CKD prevalence near or > 20% in some communities.[4]

Pakistan ranks eight in the prevalence of CKD, every year 20,000 deaths occur due to CKD, and disease is rapidly growing in Pakistan.[5] In Pakistan, consumption of junk food, abuse of medications, hypertension and renal stones are few common causes of kidney disease in Pakistan.[6]

The risk factors for primary CKD are; hypertension, diabetes, and dyslipidemia all of which are significantly associated with obesity.[7]

Kidney damage is referred to abnormalities in kidney structure and function that are detected by imaging, biopsy, and alteration in urinary sediments or proteinuria (Proteinuria /creatinuria >200mg/g, albuminuria/creatinuria>30mg/g.[8]

Since CKD is a significant global health problem, detection of kidney function that is both convenient and is, especially in patients having some degree of renal dysfunction. Early detection and initiation of treatment in chronic kidney disease patients

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shown that it is possible to delay or even prevent frequency and sensitivity of adverse effects outcomes.[9]

Glomerular filtration rate (GFR) is considered a reliable indicator and is considered as a gold standard for evaluation of renal disease.[9] Low GFR values are correlated with increased mortality and cardiovascular events, hence GFR is an important tool in CKD diagnosis as well as management of disease.[9]

Serum creatinine is used in clinical practice to estimate the bedside GFR.[10] The GFR calculated from serum creatinine may give erratic results because serum creatinine is dependent upon GFR along with muscle mass which varies with gender, age, and weight.[11] Cirrhosis and muscle wasting diseases lead to a reduction in plasma creatinine; conversely, ingestion of high amounts of protein can increase plasma creatinine levels of up to 10%.[12] Furthermore, a marked reduction in GFR can be present before it shows in the concentration of serum creatinine beyond the upper limit of the reference range.[12]

Biochemically Cystatin C is a non-glycosylated protein having a low molecular weight (13kDa). It belongs to the cysteine proteases family, which is formed generally by nucleated cells and is found in several body fluids. Due to its smaller MW, it is freely filtered by the glomerulus and is completely reabsorbed and degraded by proximal tubules.[13] It is neither affected by the muscle mass nor sex of an individual. Moreover it is not affected by states of inflammation or malignant conditions.[14] Nevertheless, large doses of glucocorticoids may increase the production of Cystatin C, and thyroid dysfunction may affect its plasma levels, which is lower in hypothyroidism and higher in hyperthyroidism.[15]

Several studies have indicated that Cystatin C is more precise than creatinine in estimating glomerular filtration rate, especially in older populations, and that it improves CKD detection (defined as estimated GFR).[16] Smaller volume of distribution and short half life are the factors which favor the improved accuracy of serum Cystatin C

as a marker of rapidly changing GFR compared to creatinine. Many studies have evaluated the effectiveness of Cystatin C as a marker of GFR estimation, mostly in comparison to creatinine.[17] Mainly because of strong association of muscle mass to creatinine concentrations, Cystatin C is considered to be a more reliable marker in subjects with extremes of muscle mass per se, including children, the elderly and in clinical conditions that negatively affect muscle mass.[18]

The present study was designed to evaluate the role of serum cystatin C as an early diagnostic marker in non-dialysis CKD patients. Limited data is available for this novel marker Cystatin C, in early detection of CKD. The results of this study will help clinicians in early detection of CKD and management of progression of CKD patients.

MATERIAL & METHODS
The research was conducted as a Descriptive, Cross sectional hospital / OPD based study. The research was performed in Biochemistry Department of Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre Karachi, in collaboration with Nephrology Department of JPMC. Karachi. The study was conducted during the period of January 2018 to December 2018. Study population includes the diagnosed cases of Chronic Kidney Disease (CKD) up to 4th stage with exclusion of patients on dialysis. An ethical approval for the study was obtained from Institutional Review Board of Jinnah Postgraduate Medical Centre (IRB/2018-GEN/4496/JPMC). Data obtained during the study is kept highly confidential.

Sample size was calculated by open Epi web site calculator using a reference Study.[19] The sample size was calculated n = 90.

A total 90 subjects of age above 18 years were selected in the present study presenting to the Nephrology clinic, JPMC for evaluation and screening of chronic kidney disease on the basis of inclusion and exclusion criteria. The subjects were interviewed in detail regarding their general information, history regarding their general and surgical ailments, data was collected regarding

patient's age, gender, duration of disease, height, weight, BMI, blood pressure (BP), pulse, temperature on the performed questionnaire. Verbal and written consent in Urdu/English was taken from every subjects duly signed or thumb printed. The inclusion criteria included Age 19 – 70 years, both genders (male and female), Consent to participate in study, high risk kidney disease patients (family history of diabetes / hypertension), patients taking anti-inflammatory drugs. The exclusion criteria was CKD patients on maintenance hemodialysis, patients having any chronic systemic disease, patients suffering from acute renal failure, patients with Thyroid dysfunction, patients on steroids or immunosuppressant including asthmatics and patients with malignancy.

Total 60 patients from Nephrology Department of JPMC and 30 healthy subjects from healthy population were included in the study after fulfilling the inclusion criteria.
The recruited study subjects were divided into 3 groups.

- **Group A:** Control group n = 30 healthy subjects.
- **Group B:** Test group n = 30 newly diagnosed CKD patients with GFR $\geq$ 60 mL/min/1.73m^2
- **Group C:** Test group n = 30 diagnosed CKD patients with GFR $\leq$ 60 mL/min/1.73m^2

For estimation of GFR using cystatin C and creatinine, Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI) and 4v Modification of Diet in Renal Disease (MDRD) equation were used.[20]

A predefined Performa was used to collect data. IBM SPSS version 23.0 was used for statistical analysis.

Data was presented as Mean and standard deviations. The means of three studied groups were compared by using One way Analysis of variance. All p-values of less than 0.05 were considered statistically significant. Data was represented graphically by using bar charts.

RESULTS
A total of ninety samples were subdivided into 3 groups. Then mean age of control group samples was 44.07±16.23 and mean body mass index was 22.72±1.44 kg/m^2, in group B samples mean age was 47.50±16.47 and mean Body Mass index was 22.95±4.16 kg/m^2, Whereas samples of group C had mean age 40.73±15.27 and mean body mass index 24.50±2.94 kg/m^2. No significant difference in mean for Age and Body mass index across groups was detected using one way analysis of variance. Mean SBP in control group samples was 112.33±8.17, mean DBP was 75.0±5.09. The mean SBP of group B samples was 135.63±20.45, mean DBP was 88.0±9.97. Whereas samples of group C had mean SBP 128.67±15.25, mean DBP 86.0±11.02. Significant mean difference for Systolic and diastolic blood pressure across three studied groups using one way analysis of variance, with p-value less than 0.01.

In control group the mean creatinine was 0.80±0.10, mean of serum cystatin C was 1.07±0.74 and the mean Albumin: creatinine was 17.23±5.50. In group B samples the mean creatinine was 2.61±0.93, mean cystatin C was 3.30±1.13 and mean Albumin: creatinine was 18.47±11.74. Whereas in group C samples mean of Creatinine was 0.92±0.16, mean of cystatin C was1.10±0.13 and mean Albumin: Creatinine was 27.65±10.96, results showed there was significant mean difference for creatinine, cystatin C and Albumin: creatinine across three studied groups. The mean and standard deviation of eGFR across studied groups was seen. In control group samples the mean of eGFR by MDRD was 96.27±21.66, mean eGFR by CKD EPI Creatinine was 102.33±17.84, mean eGFR by CKD EPI Cystatin was 86.33±13.80 and mean eGFR by CKD EPI Cr-cys was 93.47±14.51. In group B samples mean eGFR by MDRD was 27.37±10.98, mean eGFR by CKD EPI Creatinine was 30.0±11.59, mena eGFR by CKD EPI Cystatin was 18.73±8.44 and mean eGFR by CKD EPI Cr-Cys was 22.07±9.85. in group C samples the mean eGFR by MDRD was 85.37±15.23, mean eGFR by CKD EPI Cr was 94.73±15.94, mean eGFR by CKD EPI Cys was 73.30±12.23 and mean

eGFR by CKD EPI CR-Cys was 82.37±13.20. A statistically significant mean difference in all eGFR across studied groups was seen with p-value less than 0.05.

Characteristics	Group						P-Value
	Control (n=30)		Group B (n=30)		Group C (n=30)		
	Mean	S.D	Mean	S.D	Mean	S.D	
Age (years)	44.07	16.23	47.50	16.47	40.73	15.27	0.26
BMI (kg/m²)	22.72	1.44	22.95	4.16	24.50	2.94	0.055
*p<0.05 was considered significant using One way ANOVA							

Table-I. Baseline characteristics of studied samples (n=90)

Characteristics	Group						P-Value
	Control (n=30)		Group B (n=30)		Group C n=30)		
	Mean	S.D	Mean	S.D	Mean	S.D	
SBP	112.33	8.17	135.63	20.45	128.67	15.25	<0.01*
DBP	75.00	5.09	88.00	9.97	86.00	11.02	<0.01*
*p<0.05 was considered significant using One way ANOVA							

Table-II. Baseline characteristics of studied samples (n=90)

Characteristics	Group						P-Value
	Control (n=30)		Group B (n=30)		Group C (n=30)		
	Mean	S.D	Mean	S.D	Mean	S.D	
Creatinine (mg/dl)	0.80	0.10	2.61	0.93	0.92	0.16	<0.01*
Cystatin (mg/dl)	1.07	0.74	3.30	1.13	1.10	0.13	<0.01*
Albumin:Creatinine	17.23	5.50	18.47	11.74	27.65	10.96	<0.01*
*p<0.05 was considered significant using One way ANOVA							

Table-III. Mean comparison of creatinine and cystatin and albumin: Creatinine across studied groups
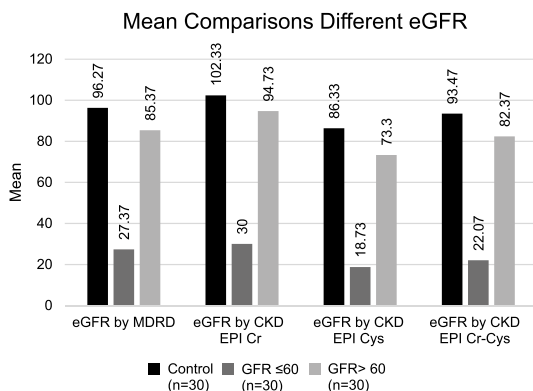

Figure-1. Presenting mean eGFR across studied groups

DISCUSSION

The study is about the evaluation of Serum Cystatin C as an early diagnostic marker in Non-dialysis chronic kidney disease patients in comparison to serum creatinine.

The mean of Age and Body mass index across groups showed no significant difference. Our study did not find any correlation between raised BMI and the occurrence of renal failure nor with the progression of CKD. Results of our study are similar to the published work of Stenvinkel et al.[21] Our study illustrated that among participants, those in Group A reflected an ideal blood pressure while both Group B and C are at the level of pre-high blood pressure. A slight difference between the two test groups is represented. Significantly, Group B has the highest risk as it reflects 135.63/88 SBP/DBP respectively. Our findings correspond to studies that blood pressure is associated with kidney disease by Ng et al.[22] Having this said, then it is more logical that hypertension can be associated with patients with CKD. Clinical trials show that a higher level of SBP and high DBP increased the risk of kidney disease of those who are with clinical CKD as it describes uncontrolled hypertension.[22] In our study the multiple comparisons of eGFR between studied groups, results showed, mean eGFR of control group samples by MDRD was significantly higher than group B and group C samples, whereas group C samples have significantly higher eGFR by MDRD as compared to group B Samples.

eGFR estimated by CKD EPI Cr of group B was significantly low as compared to control group and group C Samples, whereas control group Samples had significantly higher eGFR CKD EPI Cr as compare to group C samples.

eGFR by CKD EPI Cys of group B samples was substantially lower as compared to control group and group C samples whereas group C has a significantly low mean as compared to control group samples.

eGFR by CKD EPI Cr-Cys of group B samples was significantly low as compared to the control group and group C samples. The mean of control group samples was also considerably higher as compared to group C samples. The results of our study correspond to the work of Shlipak et al.[24]

Our study based on calculating GFR on Cystatin C was suggestive that Cystatin C based calculation of GFR is lower as compared to GFR calculated based on serum creatinine.

Our study was also suggestive that sensitivity of serum Cystatin C is high as compared to serum creatinine for the detection of reduced GFR. In the pursuit of a convenient and rapid method in assessing kidney functions, measurement of serum creatinine and Cystatin C are being optimized. A possible advantage of Cystatin c is that being a large molecule, its blood levels might rise sooner than that of creatinine. As compared to conventional serum creatinine assays, serum cystatin C levels detection can also be done in addition to screening patients with serum creatinine especially in individuals with a longer disease duration, or uncontrolled diabetes mellitus or hypertension.

The diagnostic accuracy of Cystatin C in detection of deranged kidney function it is more reliable for estimation of GFR as compared to serum creatinine when cut-off values are set to 60 ml/min/$1.73m^2$.[23,24]

Estimating GFR based on Serum Cystatin C seems to be promising methods for evaluating the renal function of CKD patients. This study will help nephrologists in early detection of deranged kidney function and thus improve the patients management.

One limitation of this study was that the sample size was small. Thus the results obtained cannot be applied to the general population at large. More studies are required to evaluate the effect of different treatment regimes (medications or dialysis) on the levels of serum creatinine and serum cystatin C in these patients during subsequent follow up.

CONCLUSION
From our study, we conclude that Cystatin C is a better marker than serum creatinine, as Cystatin C can detect changes in GFR earlier than serum creatinine, hence gives advanced information on deterioration of the kidney.
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