Original Article

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Assessment of eGFR, using Cystatin-C and Creatinine Based Equations for the Early Detection of Renal Injury in Diabetic and Non Diabetic Patients

Biochemistry Section

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ABSTRACT

Introduction: Assessment of renal function in individuals with Type-2 diabetes is very important as diabetic nephropathy is the major cause of Chronic Kidney Disease (CKD) which leads to the most frequent cause of End Stage Renal Disease (ESRD) in diabetic patients. Glomerular Filtration Rate (GFR) can be considered as best index for assessment of renal function.

Aim: To assess the eGFR using Serum Cystatin-C and compare with Serum creatinine based equations for the early detection of renal injury in Diabetic and Non Diabetic patients.

Materials and Methods: The present cross-sectional study was carried out after getting approval by institutional human ethical committee. A total of 150 participants were part of the study after obtaining the informed consent. Group-I included 50 Normal healthy controls, Group-II included 50 Chronic Kidney Disease Patients without Diabetes Mellitus (CKD-ND) and Group-III included 50 patients of Chronic Kidney Disease with Diabetes Mellitus (CKD-DM). Serum Cystatin-C, Creatinine, Urea and Glucose were estimated in the serum sample. eGFR was calculated by using Creatinine and Cystatin C based CKD-EPI equation. Data was analysed by SPSS 20.0. Correlation analysis was done using Karl Pearson's correlation coefficient. A p-value less than 0.05 were considered as significant.

Results: Serum Cystatin-C and serum creatinine were significantly increased in Non diabetic patients with CKD, a considerable decrease in eGFR was observed in Group-II compared to Group-III. Serum Cystatin-C showed a significant negative correlation with eGFR among the groups. There was a strong correlation of serum Cystatin-C with eGFR in Group-II and Group-III compared to Controls.

Conclusion: Serum Cystatin-C can be used as an alternative marker to creatinine in CKD patients without diabetes.

Keywords: Chronic kidney disease, Chronic kidney disease epidemiology collaboration, Diabetic nephropathy

INTRODUCTION

Chronic Kidney Disease (CKD) is an emerging global health problem due to the increasing prevalence of conditions like diabetes mellitus, hypertension and cardiovascular diseases. It is a global threat to health in general and for developing countries like India, because of the dietary habits, socio economic status and life style. CKD initially is without specific symptoms and is detected by an increase in serum creatinine or protein levels in urine. In developing countries like India, diabetes and hypertension accounts for 40-60% cases of CKD [1] which is associated with significant morbidity, mortality and economic burden. It is expected by 2025 that nearly 380 million adults will become diabetic patients worldwide. Indian statistics reveal that there were 41 million diabetics in 2012 and this number is expected to rise to 70 million by 2025 [2]. The metabolic derangements, which are related to diabetes mellitus causes many physiological and pathological changes, thereby affecting various organ systems which may lead to certain complications [3]. The diagnosis of CKD at an early detectable stage is important to delay the renal complications. The "gold standard" for determining GFR was inulin clearance. Since it is an exogenous marker, which has to be administered intravenously, this is a laborious process and hence it is not being practiced for routine monitoring [4]. Serum creatinine is the most routinely used marker for the assessment of renal function. The values of serum creatinine does not show an increase until GFR levels are moderately decreased (40 mL/min/1.73 m^2). The levels of serum creatinine are affected by factors such as age, diet, muscle mass etc. Hence, it is insensitive and late marker for changes in GFR [5]. Hence, there is a need for identifying an alternative filtration marker for unbiased estimation of GFR. Among several biomarkers, Cystatin-C has been proposed to be a promising marker which can help to detect early kidney injury. Cystatin-C is a low molecular weight non glycosylated protein, which is produced by all nucleated cells in the body. It is removed from the blood stream and freely filtered by the glomerular membrane in the kidneys. It serves as an index of renal function. The serum levels of Cystatin-C are not influenced by infections, inflammation or neoplastic states, and also by body mass, diet, or drugs [6]. Therefore, on the basis of these key factors Cystatin-C has been proposed and attempted to be proved as a superior marker for predicting GFR than Creatinine [7]. Cystatin-C is also superior to creatinine as a marker of kidney function and it correlates better with eGFR than serum creatinine. Hence, the present study was undertaken to assess and compare the eGFR using Serum Cystatin-C and Serum creatinine based equations for the early detection of renal injury in Diabetic and Non Diabetic patients which will help in early identification of renal impairment there by reducing the risk of further complications.

MATERIALS AND METHODS

Laboratory setting: The present cross-sectional study was conducted in the Department of Biochemistry in collaboration with Medicine and Nephrology at Central laboratory of Prathima Institute of Medical Sciences during the period of June 2015 to September 2016. Random blood glucose, HbA1c, Urea, Creatinine and Cystatin-C were estimated in selection of patients for CKD without DM.

The sample size (45 per group) was calculated using type one error (α) as 5%, power of the test as 90% with anticipated mean difference of Cystatin-C between groups was 0.5 and anticipated SD 0.7.

A total of 50 patients with CKD without Diabetes (CKD-ND), 50 CKD with Diabetes patients (CKD-DM) and 50 age and sex matched healthy controls were a part for this research. The study was approved by Institutional Ethics committee of Prathima Institute of Medical Sciences (IEC/PIMS: 2015/01). After obtaining written informed consent from all the participants, clinical history and demographic details of the patients were collected using structured questionnaire. The following criteria was used for recruiting the participants.

Inclusion Criteria

- Age ≥40 years, and ≤70 years
- Clinically confirmed diabetic patients with duration of diabetes >5 years and <10 years
- Clinically confirmed patients of CKD.

Exclusion Criteria

- Not willing to give consent
- Duration of diabetes <5 years and >10 years
- The patients with thyroid disease and those who are on treatment for thyroid disorders were excluded. Because thyroid function could affect the levels of Cystatin-C [8].
- Age and sex matched healthy controls were selected from the staff of college and hospital.

Sample Collection

Under aseptic conditions 5 mL random venous blood sample was collected from each participant, by anticubital vein puncture in a sterile disposable syringe of which 2 mL was collected in fluoride bulb and remaining was transferred in a plain bulb. After centrifugation, serum was separated and used for the estimation of biochemical parameters like Urea, Creatinine, Cystatin-C (renal profile).

From the Creatinine and Cystatin-C values obtained eGFR was calculated using CKD-EPI equation [9]. Serum Cystatin-C levels were measured by immuno-turbidimetric method using Accurex kits [10]. The eGFR level can be calculated using the Modification of Diet in Renal Disease (MDRD) formula, and CKD-EPI equation.

MDRD=186×{serum creatinine (mg/Dl)}$^{-1.154}$×age$^{-0.203}$×(0.742 if female)×(1.212 if black) [11].

A correction factor of 0.742 was used for women. (Based on body surface area)

CKD-EPI creatinine equation (2009) eGFR=141×min (S_{Cr}/κ,1)^{α}×max (S_{Cr}/κ,1)$^{-1.209}$×0.993^{Age}×1.018 (if female)×1.159 (if Black) [12];

The $eGFR_{cys}$ level was calculated by the Chronic Kidney Disease Epidemiology (CKD-EPI)

CKD-EPI equation: eGFR=127.7×(cystatin C in mg/L)$^{-1.17}$×(age in years)$^{-0.13}$×(0.91 if female) [9].

CKD-EPI Creatinine Cystatin Equation [2012] eGFR=135×min (S_{Cr}/κ,1)^{α}×max (S_{Cr}/κ,1)$^{-0.601}$×min (S_{cys}/0.8,1)$^{-0.375}$×max (S_{cys}/0.8,1)$^{-0.711}$×0.995^{Age}×0.969 (if female)×1.08 (if black) [13].

CKD-EPI Cystatin C equation eGFR=133×min (S_{cys}/0.8, 1)$^{-0.499}$×max (S_{cys}/0.8, 1)$^{-1.328}$×0.996^{Age}×0.932 (if female) [14].

Creatinine was estimated by Jaffe's Method [15]. Urea was estimated by Berthelot method [11]. Glucose was estimated by Glucose oxidase-Peroxidase method [16].

STATISTICAL ANALYSIS

Data was analysed by using SPSS 20.0 software. The results of three groups were expressed as Mean±SD. One-way ANOVA was applied to observe the significance of difference between groups. Karl Pearson's correlation coefficient was used to observe correlation. A p-value less than 0.05 were considered as significant.

RESULTS

We have focused on clinical and biochemical parameters and compared among the control and Test groups. The participants included in the study from all the groups were maximum in the age group of 46-55 years. [Table/Fig-1] shows the comparison of Group-I (Normal healthy subjects) with Group-II (CKD-ND) and Group-III (CKD-DM). Serum Cystatin-C, Urea and Creatinine levels were significantly increased (p-value<0.001) in Group-II and Group-III compared to values among controls (Group-I). The eGFR levels were significantly decreased in Group-II and in Group-III compared to Group-I. The correlation between eGFR and serum Cystatin-C and Creatinine was found to be statistically significant among Group II and Group III as compared to controls i.e., Group I [Table/Fig-2].

Parameters	Group-I (Control) n=50	Group-II (CKD-NDM) n=50	Group-III (CKD-DM) n=50	p-value
Cystatin-C (mg/L)	0.74±0.45 (0.05-1.79)	3.59±2.20 (0.7-8.99)	3.17±2.36 (0.9-11.67)	<0.001*
eGFR (mL/ min/1.732 m^2)	92.60±18.85 (48-132)	15.40±12.21 (03-49)	19.14±13.51 (03-47)	<0.001*
Creatinine (mg/dL)	0.85±0.18 (0.6-1.3)	6.24±4.13 (1.5-21.4)	4.78±2.77 (1.5-13.3)	<0.001*
Urea (mg/dL)	22.88±4.48 (17-36)	84.86±40.76 (32-212)	81.08±41.79 (21-169)	<0.001*
Random Blood Glucose (mg/dL)	97.72±16.10 (74-132)	101.44±19.21 (70-138)	259.74±120.50 (158-532)	<0.001*

[Table/Fig-1]: Comparison of Control group with CKD-ND and CKD-DM. Data was presented as Mean±SD. *significant one-way ANOVA

Groups	Correlation coefficient	r	p-value
Control Group	eGFR and Cystatin-C	-0.317	0.025*
	eGFR and Creatinine	-0.788	<0.001*
	Cystatin C and Creatinine	0.242	0.090
Non-Diabetic With CKD	eGFR and Cystatin-C	-0.447	0.001*
	eGFR and Creatinine	-0.765	<0.001*
	Cystatin C and Creatinine	0.493	<0.001*
Diabetic With CKD	eGFR and Cystatin-C	-0.324	0.022*
	eGFR and Creatinine	-0.819	<0.001*
	Cystatin C and Creatinine	0.259	0.069

[Table/Fig-2]: Correlation analysis between Serum Cystatin-C, Serum Creatinine and eGFR.
*significant with 5% level of significance

There is a strong negative correlation between serum creatinine and eGFR in CKD-ND (Group-II) when compared to CKD-DM (Group-III).

[Table/Fig-3] shows the relative efficacy of eGFR equations in detecting CKD among the three groups. CKD-EPI Creatinine Cystatin Equation (2012) is showing 98% sensitivity and 96% accuracy among the various equations. Correlation analysis showed a negative correlation between Cystatin-C and eGFR among Group-II and Group-III when compared with controls. The correlation coefficient was -0.447 and p-value was <0.001 for Group-II and r was -0.324 and p-value was 0.022 in Group-III. This indicates that there was a significant negative correlation between Cystatin-C and eGFR in Group-II compared to controls.

Formula	Group	Sensitivity	Specificity	PPV	NPV	Accuracy
CKD-EPI Creatinine Equation (2009)	Control Group	0.0%	98.0%	0.0%	100.0%	98.0%
	ND with CKD Group	100.0%	0.0%	98.0%	0.0%	98.0%
	DM with CKD Group	100.0%	0.0%	92.0%	0.0%	92.0%
CKD-EPI Creatinine Cystatin Equation (2012)	Control Group	0.0%	94.0%	0.0%	100.0%	94.0%
	ND with CKD Group	98.0%	0.0%	98.0%	0.0%	96.0%
	DM with CKD Group	97.8%	25.0%	93.8%	50.0%	92.0%
CKD-EPI Cystatin C Equation (2012)	Control Group	0.0%	88.0%	0.0%	100.0%	88.0%
	ND with CKD Group	93.9%	0.0%	97.9%	0.0%	92.0%
	DM with CKD Group	91.3%	25.0%	93.3%	20.0%	86.0%
MDRD Study Equation	Control Group	0.0%	98.0%	0.0%	100.0%	98.0%
	ND with CKD Group	100.0%	0.0%	98.0%	0.0%	98.0%
	DM with CKD Group	100.0%	0.0%	92.0%	0.0%	92.0%

[Table/Fig-3]: Relative efficacy of eGFR equations in detecting CKD in all three groups.

DISCUSSION

The detection of decline in renal function at an early stage by using the diagnostic tools like Cystatin-C, can reduce and delay the complications there by improving the quality of life. Estimation of GFR is one of the important steps in the diagnosis of CKD. In this study, we aimed at evaluating the serum Cystatin-C, Serum Creatinine levels and calculation of eGFR using various equations in diabetic and non diabetic patients. The levels of serum Cystatin-C were significantly increased in non diabetic patients with CKD compared to controls. eGFR levels were significantly decreased in Non diabetic patients with CKD compared to controls. The levels of serum Cystatin-C were decreased in diabetic patients with CKD compared to Non diabetic patients with CKD. An eGFR levels were increased in diabetic patients with CKD compared to Non diabetic patients with CKD. This might be due to the selection of subjects based on duration of Diabetes mellitus. In Non diabetics with CKD the increment in serum Cystatin-C levels indicate the severity of renal dysfunction which is due to the manifestation of glomerular process before the tubular phase [9].

Many individuals with type 2 diabetes passes through a period of pre-diabetes and may experience renal dysfunction, detection of CKD at early stage is important as early intervention can slow the loss of kidney function, that improves survival and quality of life. The inability of creatinine to detect early decline in GFR is due to the fact that serum creatinine levels only begin to rise above the normal range when approximately 50% of renal function is already lost, suggesting that GFR can change before serum creatinine becomes abnormal [17].

Studies suggested that serum Cystatin-C is a more sensitive marker for detecting early changes in glomerular filtration in type 2 diabetic patients than creatinine-based measurements [18]. Cystatin-C produced by a majority of nuclear cells is a non-glycosylated protein of 120 residue polypeptide chain with a molecular mass of 13 kDa [19]. The level of serum Cystatin-C raises earlier than serum creatinine since it is freely filtered by the glomeruli and catabolized by the proximal tubules without secretion thus, it can be considered as a good marker for estimating glomerular filtration rate. Cystatin-c is not affected by nutritional status and inflammatory processor and dietary factors. It remains unaffected with age or muscle mass unlike creatinine. Based on the above key factors it can be considered as a potential substitute for creatinine [20-22].

This suggests that serum Cystatin-C levels are related to impairment in tubular function and can be suggested as a early marker of renal diseases. After glomerular filtration, it is fully catabolized in the proximal renal tubules and is not returned into blood circulation; only small amounts of Cystatin-C is excreted in urine, which indicates that it is produced at a relatively constant rate irrespective of muscle mass. Cystatin-C provides a better estimate of GFR than estimating equations based on serum creatinine. Cystatin-C is a good marker of renal function and correlates better to direct measurement of GFR. Cystatin-C can be replaced by creatinine, due to its constant production rate and less variability than that of creatinine [9].

In this study we found that concentration of serum Cystatin-C has negative correlation with eGFR which indicates that there is a possibility of renal damage in non-diabetic subjects with CKD. The correlation between eGFR and serum Cystatin-C and Creatinine was found to be statistically significant among Non Diabetic patients with CKD and Diabetic patients with CKD as compared to controls. Serum creatinine does not rise until there is a reduction in GFR (40 mL/min/1.73 m^2) this insensitivity is associated for small to moderate decreases in GFR in creatinine blind GFR area (40-70 mL/min/1.73 m^2) which ultimately results in a delay in detection of renal damage. So serum creatinine may not be a good parameter for estimation of GFR at reduced levels of glomerular function. Though serum creatinine levels are normal during the early stage of kidney disease, it does not necessarily indicate normal renal function. This is because the serum creatinine levels come into picture when 50% of the kidney function is deteriorated. The creatinine blind area is the range between 40 and 70 mL/min/1.73 m^2 where the actual decrease in GFR occurs initially. Cystatin-C does not have any blind area. Early reduction in GFR will not be detected with creatinine testing, whereas cystatin-C will show a true positive reduction in GFR. Cystatin-C helps to identify a “preclinical” state of kidney dysfunction that is not detected with serum creatinine or estimated GFR [23]. It was found that levels of serum creatinine were raised in over 96% of patients that had reduced renal function in comparison with serum Cystatin-C levels. Cystatin-C might offer an advantage to traditional CKD markers with respect to early detection of diabetic nephropathy and its progression which helps in timely intervention preventing further complications. Since creatinine estimation has limited value in CKD prognosis, hence the study focuses on Cystatin-C as it is extremely sensitive to minor changes in GFR.

LIMITATION

The reason for non-significant correlation may be due to non linearity and small sample size. Further studies are recommended by the inclusion of biochemical parameters like NGAL, Beta 2 microglobulin and urinary angiotensinogen for better outcome and quality of life in CKD patients.

CONCLUSION

Though serum Cystatin-C assays are quite expensive compared to conventional serum creatinine assays, estimation of serum cystatin-c can be used as a better diagnostic test to screen patient when serum creatinine level is inconclusive in certain individuals with long duration of diabetes, uncontrolled diabetes and hypertension. Diagnostic accuracy is favourable for serum Cystatin-C when

compared with serum creatinine in patients with decreased renal function. However, it is more sensitive for the estimation of eGFR than serum creatinine.

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