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Original Research Article

Cystatin C: A Novel Marker for Early Renal Impairment in Diabetic Patients

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Abstract: Diabetes mellitus is a common cause of chronic kidney disease. Proteinuria in diabetics is associated with markedly reduced survival and increased risk of cardiovascular disease. The present study has been conducted to evaluate the role of serum Cystatin C in early detection of progression of chronic kidney disease in Diabetes mellitus patients. A total of one hundred and fifty type-2 diabetic patients were included in this hospital based case-control study and categorized into three groups of chronic kidney disease on the basis of estimated glomerular filtration rate and also grouped according to their urinary albumin creatinine ratio. Serum glucose, haemoglobin A1c, creatinine, cystatin C, ß-$_2$-microglobulin, urinary creatinine and micro albumin were evaluated. Glomerular filtration rate estimations were calculated using serum creatinine and serum cystatin C. Cystatin C was found to be statistically significant when estimated glomerular filtration rate was calculated using serum creatinine and by serum cystatin C. Cystatin C predicts earlier decline in glomerular filtration rate than creatinine, urinary albumin creatinine ratio and ß$_2$- microglobulin. Cystatin C indicates decline of glomerular filtration rate even when categorized according to chronic kidney disease staging and also exhibit early renal decline in normoalbuminuria subjects when categorized according to urinary albumin creatinine ratio. In conclusion, Cystatin C could be used as an early biomarker of diabetic nephropathy in normo albuminuric patients since antihypertensive and other medication render microalbuminuria detection unreliable. Further, early detection of decline in renal function can optimize detection of cardiovascular risk and help in clinical management to prevent complications. Hence, incorporation of Cystatin C into panel of renal function test is highly recommended.
Keywords: Chronic kidney disease, Diabetic nephropathy, Glomerular filtration rate, Proteinuria, Cystatin C

INTRODUCTION

Diabetes mellitus (DM) comprises of a group of metabolic disorders and metabolic deregulation associated with it cause secondary pathophysiologic changes in multiple organ system. Diabetes mellitus is one of the common cause of chronic kidney disease (CKD) [1] and is referred as diabetic nephropathy (DN) which is characterized by persistent albuminuria (>300 mg/24 hr), a relentless decline in glomerular filtration rate (GFR), raised blood pressure, and enhanced cardiovascular morbidity and mortality. Individuals with GFR 60 to 80 ml / min / 1.73 m^2 without kidney damage are classified as "decreased GFR". Chronic kidney disease (CKD) is defined as kidney damage or GFR < 60 ml / min / 1.73 m^2 for more than 3 months. Guideline 1 of Screening and Diagnosis of Diabetic kidney disease states that microalbuminuria is the best available test for screening of DKD, but it is imprecise [2]. When renal function decreases serum concentration of many low molecular proteins like cystatin-C and β_2-microglobulin increases in serum [3]. Proteinuria in diabetics is associated with markedly reduced survival and increased risk of cardiovascular disease. For this reason, additional research on the use of new biomarkers or better use of already available markers may lead to the important advances in this field. Hence, the present study has been conducted to evaluate the role of serum cystatin C in early detection of progression of CKD in diabetes mellitus. Studies on individual renal markers to detect early renal function decline have been conducted widely. However, combined study to compare biochemical parameters viz serum creatinine, serum cystatin C, serum β_2-microglobulin and urinary ACR ratio are scarce.

MATERIAL AND METHODS

A total of 150 diagnosed and clinically established patients of Diabetes mellitus (type-2) who attended OPD and wards of Medicine, J L N Medical College and Associated group of Hospitals, Ajmer were included in this Hospital based case-control study. The ethical clearance for the study was obtained from the ethical committee of the institute. The results were compared with age and sex matched 150 healthy subjects. Patients with thyroid dysfunction or taking medication due to thyroid disorder (in last 6 months), uncontrolled hypertension, polycystic kidney disease, renal malformation or agenesis, renal tumor, renal replacement therapy, presence of more than 10 leukocytes & erythrocytes / HPF in urine, cancer, patient on glucocorticoids therapy, terminally ill patients, HIV positive case and pregnant women were not included in the study.

Diabetic subjects were categorized into three groups of chronic kidney disease (CKD) on the basis of estimated glomerular filtration rate (eGFR) [4]:

1. CKD – 1: (eGFR ≥ 90 ml/min/1.73 m^2)
2. CKD – 2: (eGFR between 60-89 ml/min/1.73 m^2)
3. CKD – 3: (eGFR between 30-59 ml/min/1.73 m^2)

Diabetic patients were also divided in three groups according to their urinary albumin creatinine ratio (uACR) viz. normo-, micro-, and macroalbuminuria (ACR < 30, 30-299, and ≥300 µg/ mg creatinine, respectively).

Detailed history of the disease including age of onset, duration, complication of diabetes and treatment of all the study subjects was taken. Height and weight were noted and body mass index (BMI) was calculated. After an overnight fast, morning blood samples were collected using aseptic technique and centrifugation done at 3000 rpm to separate serum, which was subjected to the biochemical estimations. Serum glucose, haemoglobin A1c (HbA1c), serum and urinary creatinine, urinary micro albumin estimations were performed on Randox imola fully automated clinical chemistry analyser. Serum Cystatin C and Serum β-$_2$-microglobulin (β_2-MG) were evaluated by Enzyme linked immunosorbent assay.

MDRD (Modification of Diet in Renal Disease Study) equation was used for Glomerular filtration rate estimation [5]: $eGFR_{creat}$ [mL/min/1.73 m^2] = 186 × serum creatinine (mg/dl)$^{-1.154}$ × age (years)$^{-0.203}$ × 0.742 (correction factor for women). Estimated GFR using Cystatin C ($eGFR_{cys}$) was calculated using the equation according to CKD-Epi Equation [6]: $eGFR_{cys}$ [mL/min/1.73 m^2] = 76.7 × [cystatin C (mg/L)]$^{-1.19}$

Statistical Analysis:

Microsoft Excel (Microsoft office - 2007 software), Graph Pad Prism software were used for analysis and interpretations of data. Significant association between two means was tested by student's t-test, while chi-square t-test was used to analyze group differences for categorical variables. Correlations analysis was done by using Pearson's correlation method. If $p < 0.05$, the results were considered significant.

RESULTS

The study was conducted on 150 type 2 diabetic subjects of age group (44.91 ± 12.22) and BMI of (25.04 ± 3.26). These were categorized into three groups according to estimated GFR using creatinine into various chronic kidney diseases staging viz: CKD stage 1, CKD stage 2 and CKD stage 3 and also grouped according to their urinary albumin creatinine ratio (uACR) into normoalbuminuria, microalbuminuria and macroalbuminuria groups. Values of biochemical parameters of control and diabetic kidney disease subjects are presented in table 1.

In the initial CKD stage i.e. CKD stage 1 (0.86 ± 0.18 mg/dl), values of serum cystatin C showed a significant increase as compared to control (0.69 ± 0.05 mg/dl). Further, on comparison of serum cystatin C levels between CKD stage 1, CKD stage 2 and CKD stage 3 groups, a highly significant increase in values of serum cystatin C in CKD stage 3 (1.18 ± 0.16 mg/dl) was observed as compared to CKD stage 1 and CKD stage 2 groups. Similarly, values of serum cystatin C of CKD stage 2 group were significantly higher as compared to CKD stage 1 group. uACR values in CKD stage 2 (165.59 ± 133.7 µg/mg creatinine) is significantly increased as compared to CKD stage (95.43 ± 19.11 µg/mg creatinine) and control subjects (21.5 ± 5.26 µg/mg creatinine). The increase is highly significant in stage 3 subjects.

Table-1: Biochemical parameters of control & diabetic kidney disease patients

S. No.	Parameters	CONTROL (n = 150)	Stages of chronic kidney disease (CKD)		
			CKD-1 (n = 62)	CKD-2 (n = 65)	CKD-3 (n = 23)
1	Age	45.50 ± 12.62	43.09 ± 11.48	44.06 ± 13.40	45.09 ± 13.5
2	Gender, male	95	42	43	10
3	BMI (kg/m2)	22.71 ± 1.67	23.04 ± 1.5	24.04 ± 1.21	25.04 ± 3.21
4	Duration of diabetes	--	4.5 ± 4.8	5.8 ± 5.3	7.7 ± 5.2
5	Serum glucose (fasting) (mg/dl)	88.09 ± 13.48	107.54 ± 12.94	112.98 ± 12.01	120.28 ± 11.85
6	Serum HbA1c (%)	6.26 ± 0.50	6.86 ± 0.46	7.05 ± 0.57	7.65 ± 0.68
7	Serum Creatinine (mg/dl)	0.72 ± 0.08	0.84 ± 0.99	1.05 ± 0.15	1.36 ± 0.32[@]
8	Serum Cystatin C (mg/l)	0.69 ± 0.05	0.86 ± 0.18 *	1.10 ± 0.22[$]	1.18 ± 0.16 [@#]
9	Serum β_{2-} microglobulin (mg/l)	1.08 ± 0.25	1.63 ± 1.28	2.28 ± 2.47	2.85 ± 2.71 *
10	Urinary ACR (µg /mg creatinine)	21.5 ± 5.26	95.43 ± 19.11	165.59 ±133.7[$]	209.67 ± 119.55 [#]
11	eGFR $-_{creatinine}$ (ml/min/1.73m2)	115.63± 12.69	105.32 ± 11.32	75.71 ± 7.97	50.26 ± 6.59
12	eGFR $-_{cystatin}$ (ml/min/1.73m2)	119.97± 11.84	95.42 ± 19.11	71.64 ± 18.38	54.63 ± 12.79

[@] Highly significant as compared to control (p value < 0.001)
* Significant as compared to control (p value < 0.05)
[$] significant with CKD-1 (p value < 0.05)
[#] highly significant as compared to CKD1,2 (p value < 0.001)

Table-2: Biochemical Parameters of Renal damage in diabetics categorized according to uACR.

Parameters	Healthy subjects (n=150)	Normoalbuminuria (n=31)	Microalbuminuria (n=83)	Macroalbuminuria (n=36)
Serum cystatin C (mg/l)	0.69 ± 0.05	0.82 ± 0.13 *	0.96 ± 0.19*	1.32 ± 0.10[@]
Serum creatinine (mg/dl)	0.72 ± 0.08	0.94 ± 0.14	0.98 ± 0.25	1.1 ± 0.03[#]
Serum microglobulin (mg/l)	1.08 ± 0.25	1.12 ± 0.24	1.25 ± 0.50	3.89 ± 1.55[@]

[@] highly significant as compared to control (p value < 0.001)
*significant as compared to control (p value < 0.05)
[#] significant as compared to microalbuminuria (p value < 0.05)

Table 2 depicts biochemical parameters of renal damage in diabetics viz serum β-$_2$ microglobulin and urinary ACR values in all the three stages. A significant increase in serum cystatin C was observed in normoalbuminuria subjects (n=31) as compared to control subjects. Serum cystatin C values of microalbuminuria (0.96 ± 0.19 mg/l) group were significantly higher than control (0.69 ± 0.05mg/l) and normoalbuminuria (0.82 ± 0.13 mg/l) group and there is also a highly significant increase in values between normoalbuminuria & macroalbuminuria (1.32 ± 0.10 mg/l) group for serum cystatin c. Further, a highly significant increase in the values of serum cystatin C (0.96 ± 0.19) and in serum β_{-} microglobulin (1.25 ± 0.50) was also observed in microalbuminuria group subjects (n=83) as compared to control (0.69 ± 0.05 mg/l, 1.08 ± 0.25 mg/l respectively). Serum creatinine was found to be significantly raised in macro albuminuric subjects (1.1 ± 0.03 mg/dl).

DISCUSSION

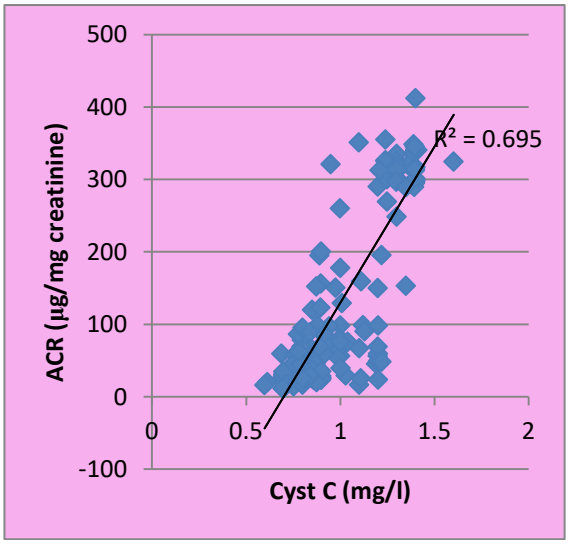
Renal function decline in various kidney diseases is usually judged by estimated GFR using serum creatinine levels and diseased subjects are categorized into various chronic kidney disease (CKD) staging viz: CKD stage 1, CKD stage 2, CKD stage 3, CKD stage 4 and CKD stage 5[4]. Creatinine was earlier studied and established as a marker, it revealed

significance, but only in CKD stage 2 to stage [7] and serum β_2-microglobulin [8] levels were found to be increased as chronic kidney disease progressed from Stage 1 to onwards.

The findings of present study depicts that serum creatinine values in CKD 3 group were highly significant as compared to values of control and these are not significantly increased in CKD stage 1 and 2. Creatinine can be cleared to a certain extent via tubular secretion so serum creatinine is insensitive to mild to moderate reduction in GFR (the "the creatinine blind" range). Creatinine is regularly secreted and at times reabsorbed by renal tubules. Hence, it provides only rough guide to the GFR. There is a significant increase in values of serum β_2-microglobulin in CKD stage 3 groups as compared to control. A similar trend was observed in CKD stage 2 groups when compared with CKD stage 1 group. Similar findings have been reported earlier [8]. It was found during early studies that it is readily filtered through the glomerulus and almost completely reabsorbed and destroyed by proximal tubular cells [9]. This hypothesis suggests the pathological involvement of glomerulus as well as renal tubular.

Urinary ACR values in CKD stage 3 subjects were highly significant as compared to CKD stage 1 and 2. Urinary albumin was found to be inadequate as an indicator of any level of GFR impairment[10] as it is influenced by urinary tract infection, high dietary protein intake, congestive heart failure, acute febrile illness, vaginal discharge, water loading and drugs (NASIDS, ACE inhibitors) etc[11]. Values of serum cystatin C in CKD stage 1 showed a significant increase as compared to control. Serum cystatin C values are subsequently increasing as the disease advances and there is a highly significant increase in advanced CKD stages i.e. CKD stage 2 and 3. However, creatinine and urinary ACR levels showed significance in later stages of CKD. This may be because glomerular pathology cause increased albumin excretion in urine and tubular pathology cause defective metabolism of cystatin C as it is completed metabolized by renal tubular cells.

When subjects are categorized according to urinary ACR viz. normoalbuminuria, microalbuminuria and macroalbuminuria, it was found that there is a significant increase in serum cystatin C values in normoalbuminuria subjects as compared to control subjects. Serum cystatin C values of microalbuminuria group were significantly higher than control and normoalbuminuria group and there is also a highly significant difference between normoalbuminuria and macroalbuminuria group for serum cystatin C. Serum B-$_2$ microglobulin was highly significant in macroalbuminuria group as compared to control which is in concurrence with previous study [12]. The trend of serum creatinine levels is not significantly different between the normo and micro albuminuria groups but there is a highly significant increased value of macroalbuminuria group as compared to values of normoalbuminuria group. Many patients with CKD maintain normal serum creatinine level despite having significantly impaired function as creatinine is influenced by so many other factors like muscle mass, diet, age and sex etc [13]. Increased serum cystatin C values in normoalbuminuric subjects suggest that the tubular pathology precede the glomerular pathology. As a result serum β-2 microglobulin and serum cystatin C values changes at early stages of diabetes and increased as the degree of renal injury advances. Serum β_2-microglobulin is found to be altered in proliferative, inflammatory syndrome, hepatic and autoimmune disorders [14, 15] and cystatin c is not found to be affected by such factors.



Graph -1: Cystatin C correlation with Albumin Creatinine Ratio in diabetic subjects.

The routine classical evaluation of diabetic nephropathy includes appearance of microalbuminuria, decreased creatinine clearance and increased serum creatinine [16]. It is also established that in both type 1 and type 2 diabetes mellitus, urinary excretion of small amounts of albumin (microalbuminuria) is predictive of morbidity & mortality due to renal complications and cardiovascular disease [17].

Table-3: Comparison of the estimated GFR

E**GFR**creat	e**GFR**cysC	
	> 60ml/min	<60ml/min
>60 m/min/1.73m^2	106	21
<60 m/min/1.73m^2	10	13

Chi square equals 15, p value is less than 0.0001

When estimated GFR using serum creatinine and serum cystatin C were compared, it was observed that p value found to be less than 0.0001 (chi-square *p* value <0.0001) which is statistically significant between both groups. This indicates that cystatin C predicts earlier decline in renal function in (table-3). Result analysis of the study shows that Cys C is positively correlated with β-2-microglobulin and creatinine (r=0.51 & 0.66) with the kidney function decline as studied earlier [18]**.** Pearson's correlation analysis shows that the serum level of cystatin C is related to ACR, creatinine, eGFR (graph-1) [19]**.** Separate studies have been conducted in type 1 and type 2 diabetics on serum cystatin C and serum creatinine levels for estimation of GFR [20, 21]. Literatures reporting serum cystatin C as less sensitive marker than creatinine for early detection of renal impairment in diabetics are available [22, 23]. However, results of our study clearly indicate that cystatin C is a novel marker for detection of early impairment in CKD in diabetics at an early stage. The other markers viz serum creatinine, serum β_2-MG, uACR, when compared with serum cystatin C were not found to be sensitive marker for detection of progression of CKD. Similar trend was observed in cystatin C levels when diabetics were categorized on the basis of uACR. Therefore, it clearly indicates that estimation of serum cystatin C levels is useful in diabetics to prevent earlier diabetic nephropathy and its complications.

CONCLUSION:

In conclusion, findings of the present study show that Cystatin C is a more sensitive marker of renal disease in diabetes mellitus when estimated GFR is unreported at >60 ml / min. Cystatin C indicates decline of GFR in early stages of CKD and albuminuria than creatinine and β_2-microglobulin. Cystatin C could be used as an early biomarker of diabetic nephropathy in normoalbuminuric patients since antihypertensive and other medication render microalbuminuria detection unreliable. Further, early detection of decline in renal function can optimize detection of cardiovascular risk and help in clinical management to prevent complications. Hence, incorporation of Cystatin C into panel of renal function test is highly recommended. However, limitation of the study was that the diabetic subjects who were hypertensive, were not asked to discontinue their antihypertensive medications. Therefore, albuminuria might be underestimated in these patients.

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