



Original Research Article

Usefulness of Serum Cystatin C as an Index of Renal Function in Diabetic Nephropathy

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ABSTRACT

Introduction: Diabetic nephropathy, which is characterized by microalbuminuria, subsequent macroalbuminuria, & declining glomerular filtration rate, is a single most frequent cause of end stage renal disease in the world. Reduced renal function is associated with increased incidence of cardiovascular morbidity and mortality. Microalbuminuria is the best available test for screening of Diabetic Kidney Disease, but it is imprecise. Cystatin C is a peptide of 122-amino acid and was first investigated as marker of GFR. It is the product of a "housekeeping" gene expressed in all nucleated cells and is produced at a constant rate. It is freely filtered at the glomerular membrane and is reabsorbed and catabolized by renal tubular cells. It has potential advantages over creatinine when estimating glomerular filtration rate in that its production is not dependent on muscle mass. In addition, it is unaffected by age, fever, or exogenous agents.

Material & method: The study has been conducted on 150 diagnosed & clinically established patients of Diabetes mellitus (type-I & type-II) who attended OPD and wards of Medicine, J.L.N. Medical College and Associated group of Hospitals, Ajmer. Subjects were categorized in various chronic kidney diseases (CKD) staging viz CKD-1, CKD-2 & CKD-3. Anthropometric measurements and biochemical estimations were performed.

Results and discussion: Serum Cystatin C values were found to be significantly increased in subjects of CKD stage 1 (0.80 ± 0.14 mg/l), and serum creatinine level found to be increased in patients of CKD stage 3 (1.30 ± 0.41 mg/l) as compared to control subjects.

Conclusion: Cystatin C discloses earlier decline of GFR in subjects of CKD. Early detection of renal function decline can optimize detection of cardiovascular risk & help in clinical management to prevent complications.

Keywords: Cystatin C, Estimated Glomerular Filtration Rate (eGFR), Microalbuminuria, Chronic kidney disease (CKD), Diabetic nephropathy.

INTRODUCTION

Diabetic nephropathy (DN) is a single most frequent cause of end stage renal disease (ESRD) in the world. It is

characterized by microalbuminuria, subsequent macroalbuminuria, & declining glomerular filtration rate (GFR). Screening for diabetic nephropathy is currently done

by monitoring patients for the development of microalbuminuria along with estimation of serum creatinine. Reduced renal function is associated with increased incidence of cardiovascular morbidity and mortality. When renal function decreases, serum concentration of many low molecular weight proteins increase such as lysozyme, alpha-1 microglobulin, beta-2 microglobulin and cystatin –C, which have been proposed as indices of renal function. Chronic kidney disease may occur due to various causes like high blood pressure, diabetes mellitus, glomerulonephritis etc. The most common recognized cause of chronic kidney disease is diabetes mellitus. Kidney disease or kidney damage that occurs in people with diabetes is called diabetic nephropathy. [1] Guideline 1 of Screening and Diagnosis of Diabetic Kidney Disease [2] states that Microalbuminuria is the best available test for screening of Diabetic Kidney Disease, but it is imprecise. Cystatin C is a 122-amino acid, low molecular mass protein that was initially known as inter alia γ -trace, post γ -globulin, and γ -CSF. Cystatin-C (Cys - C) was first investigated as marker of GFR in 1985. [3] It is member of the family of cysteine proteinase inhibitors and has a molecular mass of 13,343 Da. It is the product of a “housekeeping” gene expressed in all nucleated cells and is produced at a constant rate. [4] It is freely filtered by the glomerulus and has potential advantages over creatinine when estimating glomerular filtration rate (GFR) in that its production is not dependent on muscle mass. In addition, it is unaffected by age, fever, or exogenous agents. [5] A plasma marker less subject to influences such as height, weight & muscle mass would allow easier identification of individuals with an abnormal GFR. Therefore, it is of worth to develop a more sensitive or specific indicator for detecting early renal impairment in diabetic patients. The study has been done to evaluate the

clinical usefulness serum cystatin C by measuring its levels & comparing it with other parameters related to kidney dysfunction in diabetes mellitus, such as serum creatinine and urinary albumin creatinine ratio (uACR).

MATERIALS AND METHODS

The study has been conducted on 150 diagnosed & clinically established patients of Diabetes mellitus (type-I & type-II) of either sex who attended OPD and wards of Medicine, J.L.N. Medical College and Associated group of Hospitals, Ajmer. The results were compared with 150 age and sex matched controls. Exclusion criteria included patients with thyroid dysfunction and taking medication due to thyroid disorder (in last 6 months), uncontrolled hypertension, renal tumor, renal replacement therapy, non-diabetic CKD, polycystic kidney disease, renal malformation or agenesis, presence of more than 10 leukocytes & erythrocytes / HPF in urine, cancer, patient on glucocorticoids therapy, HIV positive case and pregnant women.

Diabetic nephropathy subjects were categorized on the basis of estimated GFR (eGFR) into various chronic kidney diseases staging:

- I. CKD – 1 (eGFR \geq 90 ml/min/1.73 m²)
- II. CKD – 2 (eGFR between 60-89 ml/min/1.73 m²)
- III. CKD – 3 (eGFR between 30-59 ml/min/1.73 m²)

All the diabetic patients were also grouped and assessed on the basis of mild & moderate kidney functions (eGFR \geq 60 ml/min/1.73m² and eGFR < 60 ml/min/1.73m², i.e high GFR and low GFR groups respectively.

Detailed history of the disease including age of onset, duration, complications of diabetes and treatment was taken from all the subjects. All the

anthropometric measurements including height, weight body mass index (BMI) were performed. Blood sample collection was done by aseptic technique and subjected to the biochemical estimations. Serum Creatinine was estimated by Jaffe's colorimetric kinetic method. Serum Cystatin C was evaluated by Enzyme linked immunosorbent assay (ELISA) method. Clean catch spot urine collection was done and subjected to estimation of Urinary Microalbumin by Immunoturbidimetric method and urinary creatinine by Jaffe's colorimetric kinetic method.

Glomerular filtration rate (eGFR_{creat}) was estimated using the abbreviated MDRD

(Modification of Diet in Renal Disease Study) equation: ^[6] eGFR_{creat} [mL/min/1.73 m²] = 186 × serum creatinine (mg/dl)^{-1.154} × age (years)^{-0.203} × 0.742 (correction factor for women) and (eGFR_{cys}) based on serum cystatin C was estimated using the equation according to CKD-Epi Equation: ^[7,8] eGFR_{cys} [mL/min/1.73 m²] = 76.7 × [cystatin C (mg/L)]^{-1.19}

Statistical analysis: Data were grouped & analyzed using Microsoft Excel (Microsoft office - 2007 software). Students' t-test was applied to the data. Pearson's correlation analysis was performed. All results were considered significant if $p < 0.05$.

Table-1-Biochemical parameters of Control and Diabetic nephropathy subjects

S. No.	Parameters	CONTROL (n = 150)	CKD-1 (n = 62)	CKD-2 (n = 65)	CKD-3 (n = 23)
1	Serum Creatinine (mg/dl)	0.71 ± 0.08	0.84 ± 0.96	1.04 ± 0.25	1.30 ± 0.41 [⊗]
2	Serum Cystatin C (mg/l)	0.68 ± 0.05	0.80 ± 0.14 [⊗]	0.85 ± 0.29	1.19 ± 0.16
3	Urinary ACR (µg/mg creatinine)	14.2 ± 8.2	17.73 ± 21.6 [⊗]	25.03 ± 34.21	98.71 ± 87.21

[⊗] highly significant as compared to control

* significant as compared to control

Table-2- Biochemical parameters of Control and Diabetic nephropathy subjects.

S. No.	Parameter	CONTROL (n = 150)	eGFR ≥ 60 (ml/min/1.73m ²) High GFR group (n = 127)	eGFR < 60 (ml/min/1.73m ²) Low GFR group (n = 23)
1	Serum Creatinine (mg/dl)	0.71 ± 0.08	0.96 ± 0.61	1.30 ± 0.41 [⊗]
2	Serum Cystatin C (mg/l)	0.68 ± 0.05	0.83 ± 0.22 [⊗]	1.19 ± 0.16
3	Urinary ACR (µg /mg creatinine)	14.2 ± 8.2	21.38 ± 27.9 [⊗]	98.71 ± 87.21

[⊗] Highly significant as compared to control

* significant as compared to control

RESULTS AND DISCUSSION

As shown in table-1, it is evident that increase in levels of serum creatinine in subjects of CKD 2 stage (1.04 ± 0.25 mg/dl) is non-significant as compared to subjects of CKD 1 stage (0.84 ± 0.96 mg/dl). However, subjects of CKD 3 stage (1.30 ± 0.41 mg/dl) showed highly significant increase in creatinine value as compared to subjects of CKD stage 1 & CKD stage 2 respectively. Similar findings have been reported previously. ^[9]

The mean±SD of urinary ACR is significantly increased in subjects of CKD stage 2 (25.03 ± 34.21 µg/mg) as compared to subjects of CKD stage 1 (17.73 ± 21.6 µg/mg). Similarly highly significant

increased values ($p < 0.001$) in subjects of CKD stage 3 (209.67 ± 119.55 µg/mg) were observed as compared to subjects of CKD stage 2. Serum Cystatin C values are increased in subjects of CKD stage 2 (0.85 ± 0.29 mg/l), which further increases in CKD stage 3 (1.19 ± 0.16 mg/l). It is evident from the results of the study that Cystatin C concentration was significantly increased in subjects with stage 1 to 3 and stage 2 to 3 groups in CKD classification (all $p < 0.001$). (Table-1).

Subjects with lower e GFR group (< 60) have highly significant increased values of serum creatinine & serum cystatin C as compared to higher GFR subjects (≥ 60). Urinary ACR (µg /mg creatinine) values are

significantly lower ($21.38 \pm 27.9 \mu\text{g}/\text{mg}$) in high GFR group. This study is in accordance with the previous study [10] as spot u ACR & serum cystatin C were significantly higher in low GFR group, but serum creatinine values were not different in both groups which may be due to other factors affecting creatinine values. In the present study, it was observed that, in diabetic patients spot urine ACR was correlated well with urine albumin excretion. Cystatin C values increment in lower GFR group is highly significant than higher GFR group. There was a highly significant increase in the concentration of serum cystatin C in patients with lower GFR (less than $60\text{ml}/\text{min}/1.73\text{m}^2$) group. eGFR values were found to be correlated well in lower GFR group. A strong correlation was observed between eGFR based on CKD-Epi_{cystatin} and MDRD equation for creatinine when eGFR was below $60 \text{ mL}/\text{min}/1.73 \text{ m}^2$ (table-2). Cys C was positively correlated with creatinine and negatively with GFR values. [11] In the study the serum level of cystatin C was found to be correlated well with albuminuria and urinary ACR. In Pearson's correlation analysis, the serum level of cystatin C was related to ACR, creatinine, eGFR.

The routine classical evaluation of diabetic nephropathy includes appearance of microalbuminuria, decreased creatinine clearance and increased serum creatinine. [12] It is also established that in both type 1 & type 2 diabetes mellitus, urinary excretion of small amounts of albumin (microalbuminuria) is predictive of morbidity & mortality due to renal complications & cardiovascular disease. [13] Results of the study clearly indicate a principal drawback of plasma creatinine concentration as a measure of creatinine clearance is that, because its concentration is influenced by several co-variates. A significantly impaired GFR may be

compatible with a creatinine concentration within the normal population range.

When renal function decreases, the serum concentration of cystatin C increases. Cystatin C is a more sensitive marker of renal disease in diabetes mellitus where estimated GFR is unreported at $>60 \text{ ml} / \text{min}$ and antihypertensive medications render microalbuminuria detection unreliable. Contradictory findings have been reported earlier [14,15] that Cystatin C is not more sensitive than creatinine for detecting early renal impairment in patients with diabetes. Therefore study was undertaken to explore whether cystatin C is a better index of renal function than creatinine or not.

CONCLUSION

Although albumin excretion detects renal function decline but it is altered with certain medications. Therefore, it is concluded that Cystatin C is a more sensitive marker of renal disease in diabetes mellitus where estimated GFR is unreported at $>60 \text{ ml} / \text{min}$. Cystatin C discloses more early decline of GFR in early stages of Diabetic nephropathy. Early detection of renal function decline 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.

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