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ORIGINAL ARTICLE

Evaluation of serum Cystatin C as a predictor of eGFR in type 2 diabetic patients with nephropathy

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ABSTRACT

Background: Diabetic nephropathy (DN) is the leading cause of chronic kidney disease (CKD) worldwide.
Objective: To evaluate serum Cystatin C as a predictor of eGFR in type 2 diabetic patients with nephropathy.
Methods: A cross-sectional analytical study was conducted at the Department of Nephrology, Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan, from 10th March to 9th September 2023. Patients with type 2 diabetes (T2D) for more than five years, both males and females, 30 to 65 years of age, and with nephropathy for the last 2 years were included in the study. Serum creatinine, serum Cystatin C (Cys-C), fasting blood sugar (FBS), glycated hemoglobin A1c (HbA1c), total protein, and albumin were measured. A spot urine sample was collected to analyze total urinary protein, albumin, and creatinine levels. The estimated glomerular filtration rate (eGFR) was calculated using the CKD Epidemiology Collaboration (CKD-EPI) equation. One-way ANOVA, Pearson correlation test, and Linear regression analysis were done to analyze the data.
Results: A total of 113 patients were analyzed, with a mean age of 55.5±6.1 years. The mean duration of T2D was 12.0±5.3 years. The mean HbA1c level was 9.1±1.3%. Based on Cys-C levels, the mean eGFR was 71.33±24.8 mL/min/1.73m². Among the participants, 43(38.1%), were suffering from Stage II, 32(28.3%) from Stage I, 32(28.3%) from Stage III, and 6(5.3%) from Stage IV CKD. A majority of 50(44.2%) of study participants had microalbuminuria. A statistically significant ($p<0.001$) negative correlation between eGFR and Cys-C level was observed among the study participants. Serum Cystatin C is a significant (<0.05) predictor of eGFR.
Conclusion: Serum Cystatin C was a significant predictor of eGFR in type 2 diabetic patients with nephropathy. The strong negative relationship between Cystatin C and eGFR supports its potential role as a valuable marker for assessing renal function in diabetic patients.

Key Words: Cystatin C, Glomerular Filtration Rate, Diabetic Nephropathy, Type 2 Diabetes

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INTRODUCTION

Diabetic nephropathy (DN) is the single most common cause of end-stage renal disease (ESRD) worldwide, which is a significant long-term consequence of untreated type 2 diabetes (T2D).[1] Projections indicate that nearly 380 million adults globally will be affected by diabetes by the year 2025.[2] With the prompt rise in the global incidence of diabetes mellitus (DM) and prolonged survival time of diabetic patients, the prevalence ratio of diabetes associated micro- and macro-vascular complications, including DN, is on the rise worldwide.[3] Diabetic patients with reduced renal function are at increased risk of cardiovascular events.[4]

Early diagnosis and timely management of patients are crucial for limiting the progression of the disease.[5] Moreover, the choice of appropriate drugs for managing patients with T2D is influenced by the estimated glomerular filtration rate (eGFR). Therefore, the routine Cystatin C (Cys-C) based eGFR measurement is strongly recommended for patients with T2D.[6] Detection of the renal dysfunction in T2D patients at early stages is vital, as suitable and effective interventions have been shown to impede the advancement to ESRD.[7]

It is reported that serum Cys-C levels rise early enough in DN, before the serum creatinine levels rise. Cys-C can detect aberrant eGFR in patients in early stages of DN that creatinine-based GFR cannot. A study by D. Singh and colleagues found that serum Cys-C is a highly effective and early indicator of DN, demonstrating strong sensitivity and specificity in predicting kidney dysfunction.[8] Similarly, Chernyaeva et al. also concluded that Cys-C provides a more accurate method for assessing renal function than serum creatinine, especially in patients with DN.[9]

As a result, the growing awareness of the necessity of early recognition and treatment of chronic kidney disease (CKD) necessitates the development of more reliable methods of determining eGFR.[10] Considering the rising prevalence of CKD resulting from diabetes in our setting, in addition to the high rate of mortality due to the complications of nephropathy, indicates the necessity for an improved eGFR marker. There has been a growing interest in using Cys-C as a surrogate marker for eGFR, particularly for its potential to accurately estimate an early decline in GFR in individuals with diabetes.

The present study evaluated serum Cystatin C as a predictor of eGFR in type 2 diabetic patients with nephropathy.

METHODS

An analytical cross-sectional study was conducted in the department of Nephrology, Liaquat University of Medical & Health Sciences, Jamshoro, Pakistan. Patients with T2D for more than 5 years, both male and female, 30 to 65 years of age, and with nephropathy for the last two years. DN was defined by an eGFR of less than 60 ml/min/1.73 m² and urinary albumin excretion greater than 30 mg/day. Pregnant women, history of corticosteroid use, thyroid disorder, renal disease due to other causes, and patients having a body mass index (BMI)>35kg/m^2 were excluded.

The sample size 118 was calculated using OpenEpi software based on a diabetes prevalence rate of 26.7% in Pakistan, with a margin of error of 8% and a 95% confidence interval.[11] However, during the defined study period, a total of 113 eligible participants were successfully recruited instead of 118. Written informed consent was obtained from all enrolled patients before data collection. Participants' socio-demographic, clinical, and anthropometric data were recorded. A total of 10 cc of venous blood sample was collected for the biochemical analysis of serum creatinine, serum Cys-C level, fasting blood sugar (FBS), glycated hemoglobin A1C (HbA1c), total protein, albumin, hematocrit, and serum urea. Moreover, a spot collection of a urine sample in a sterile container is used to analyze total urinary protein, urinary albumin, and urinary creatinine level. Serum Cys-C levels were estimated using a particle-enhanced immuno-turbidimetric test on an auto-analyzer, and eGFR was calculated using the CKD Epidemiology Collaboration (CKD-EPI) equation. All measurements were performed in a central biochemistry laboratory adhering to internal quality control standards, and instruments were calibrated regularly as per manufacturer protocols.

Ethical Approval

The study was conducted from 10th March to 9th September 2023 after obtaining the approval from the Research Ethical Committee of Liaquat University of Medical and Health Sciences, Jamshoro, Pakistan (LUMHS/REC/-27) on 9th March 2023.

Statistical Analysis

Data was analyzed using SPSS Version 22. Categorical variables were expressed as frequencies and percentages, while quantitative variables were expressed as mean ± SD. Group comparisons were done by one-way ANOVA and post-hoc analysis using the Games-Howell test due to unequal variance. A linear regression test was applied in this study to determine the predictive relationship between serum Cystatin C levels and eGFR in type 2 diabetic patients with nephropathy. Pearson correlation test was also used to determine the relationship between serum Cys-C and uACR.

RESULTS

A total of 113 patients with DN were included in the study. The mean age of the participants was 55.5±6.1 years. The socio-demographic details of the study participants are given in Table 1. The mean duration of T2D was 12.0±5.3, and the mean duration of nephropathy was 4.3±2.9 years. The mean and median of the clinical and biochemical parameters of the study participants (n=113) are given in Table 2. Among the participants, the majority, 43 (38.1%), were suffering from Stage II CKD, while 32 (28.3%) were in Stage I, 32 (28.3%) were in Stage III, and 6 (5.3%) were suffering from Stage IV CKD. The mean value of serum albumin among the study participants was 3.81±0.43 g/dL, while the mean albumin level in the urine sample was 127.8±112. For albuminuria, the American Diabetes Association (ADA) cut-off values were used. It defines albuminuria based on the urine albumin-to-creatinine ratio (UACR), with a value of less than 30 (mg/g creatinine) considered normal, 30-299 (mg/g creatinine) indicating moderately increased albuminuria, and 300 (mg/g creatinine) or higher signifying severely increased albuminuria. The participants were subdivided into normoalbuminuric, microalbuminuria, and macroalbuminuria (Table: 3).

Serum Cys-C levels were significantly different between all groups of albuminuria. Serum Cys-C level was significantly lower in the normo-albuminuria group compared to the microalbuminuria ($p<0.001$) and macroalbuminuria groups ($p<0.001$). Serum Cys-C levels were also significantly higher in the macroalbuminuria group than in the microalbuminuria group ($p< 0.001$). There was no statistically significant difference ($p>0.05$) in the serum creatinine level between albuminuria groups. (Table: 4)

A statistically significant negative correlation between eGFR and Cys-C level was observed among the study participants ($p<0.001$). Linear regression analysis showed that serum Cyst-C is an important predictor ($p<0.001$) of eGFR. The model indicated that for each 1 mg/L increase in the serum Cystatin C, eGFR decreased by 59.26 units (B= - 59.26, SE = 1.98). The relationship was strong, as indicated by the standardized beta coefficient (β=-0.943), suggesting a strong inverse relationship. This indicates that every unit rise of serum Cys-C is due to a decline in eGFR (Table 5).

Table 1: Socio-demographic and anthropometric parameters of the study participants

Variables	n (%)
Age (years)	
≤ 50	37 (32.7%)
51-60	49 (43.3%)
> 60	27(24.0%)
Gender	
Male	40(35.4%)
Female	73(64.6%)
Resident	
Urban	65(57.5%)
Rural	48(42.5%)
BMI	
Normal	59(52.2%)
Overweight	41(36.3%)
Obese	13(11.5%)
Smoking	
Yes	49(43.4%)
No	64(56.6%)

Table 2: Clinical and biochemical parameters of study participants

Variables	mean ± SD
Duration of diabetes (years)	12.08 ± 5.3
Duration of DN (years)	4.30 ± 2.90
HbA1c (%)	9.170 ± 1.30
FBS (mg/dL)	217.1 ± 43.6
Serum Cystatin C (mg/L)	1.15 ± 0.39
Serum Creatinine (mg/dL)	1.23 ± 0.58

DN=Diabetic nephropathy; FBS=Fasting blood glucose.

Table 3: Distribution of albuminuria among study participants

Albuminuria	n (%)
Normoalbuminuria(<30mg/g creatinine)	43 (38%)
Microalbuminuria(30-299mg/g creatinine)	50 (44%)
Macroalbuminuria(>300mg/g creatinine)	20 (17%)

This study evaluated the characteristics based on the Cys-C test and urine albumin creatinine ratio (uACR). Patients' average Cys-C levels were 1.153± 0.394 mg/L, and their mean uACR levels were 165.2±147.06 mg/g. The results of the Pearson's correlation test showed a statistically significant positive correlation between Cys-C levels and the uACR ($p< 0.05$), as shown in Table 6.

Table 4: Comparison of serum Cystatin C and creatinine levels across albuminuria groups

Variables	Normo-albuminuria (mean ± SD)	Micro-albuminuria (mean ± SD)	Macro-albuminuria mean ±SD	p value
Serum Cystatin C (mg/L)	0.8286±0.01[a]	1.1354±0.01[b]	1.8995±0.04	<0.05*
Serum Creatinine (mg/dl)	1.1756±0.87	1.2463±0.86	1.3190±0.12	0.594

One-way ANOVA followed by a post hoc test was applied. [a,b]Statistically significant difference (p<0.05). [a]Compared to micro-albuminuria & macro-albuminuria groups. [b]Compared to macro-albuminuria.

Table 5: Relationship between serum Cystatin C and estimated Glomerular Filtration Rate (eGFR)

Coefficients [a]					
Model	Unstandardized Coefficients		Standardized Coefficients	T	p value
	B	Std. Error	Beta		
(Constant)	139.71	2.41		57.83	<0.001*
Serum Cystatin C (mg/L)	-59.26	1.98	-0.943	-29.90	<0.001*

*Linear regression test applied. *p<0.05 was taken as statistically significant.*

Table 6: Correlation between Cystatin C and urine Albumin/Creatinine (uACR) ratio

Variables	mean ±SD	Rho	p value
Cystatin C (mg/L)	1.153±0.394	0.843	<0.001*
Urine Albumin / Creatinine ratio (mg/g)	165.2±147.06		

*Pearson's test applied for correlation. *p< 0.05 was taken as statistically significant.*

DISCUSSION

This study evaluated the utility of Cys-C-based eGFR in patients with T2D with nephropathy. It demonstrated that cystatin-based eGFR is a more accurate and earlier detection of renal dysfunction in this population compared to creatinine-based eGFR. In the present study, the majority of our participants had uncontrolled blood sugar with a mean HbA1c level of 9.1±1.3% and a mean FBS of 217.07±43.6. These findings are similar to what was reported in earlier research carried out by Williams R. et al., Vankwani R. et al., Spakota S. et al., and Asghar S et al, who also reported uncontrolled blood glucose among their diabetic patients with Nephropathy. [12-15]

DN is categorized by albuminuria or declined GFR, while uACR is a standard method for classifying the different grades of DN.[16] Based on it, microalbuminuria was observed in 44.2% of participants while normoalbuminuria and macroalbuminuria were seen in 38.1% and 17.7% of participants, respectively. These findings are consistent with the Saudi Arabian study by Al-Rubeaan K et al., who reported that 40% of patients had microalbuminuria and 17.6% of patients had macroalbuminuria.[17]

Since Cys-C is less influenced by muscle mass and diet compared to creatinine, it has been widely expected to offer a more accurate estimate of GFR than creatinine.[18-22] The findings of the study revealed a statistically significant, strong positive correlation between CKD in different stages and serum Cys-C levels. Moreover, present study showed that there was a statistically significant positive correlation (p<0.05) between Cys-C levels and the levels of ACR values among participants. A statistically significant negative correlation between eGFR and Cys-C level was observed among the study participants. It is revealed that every unit rise of serum Cys-C is due to the decline in eGFR. Khalid UB et al. also revealed that for the diagnosis and staging of chronic renal disease, the eGFR determined using the CKD-EPI demonstrated greater clinical correlation than the modification of diet in renal disease (MDRD) equation.[23] Similarly, Zou et al. supported our findings, reporting that the CKD-EPI equations based on cystatin C provided less biased and more accurate estimates of eGFR [24] Similarly, Dastidar R also reported that serum Cys-C is a more accurate predictor of renal function compared to serum creatinine.[25]

Based on the findings, the present study concludes that Cys-C is a more accurate predictor and accurate serum marker of DN compared with other markers like serum creatinine or urinary albumin levels in discriminating T2D patients with reduced GFR compared to minimally disturbed GFR.

CONCLUSION

Serum Cystatin C was found to be a significant predictor of eGFR and a reliable serum biomarker for the early detection of diabetic nephropathy in type 2 diabetic patients with nephropathy. The strong negative relationship between Cystatin C and eGFR supports its potential role as a useful marker for assessing renal function in diabetic patients.

Limitations of study and future recommendations

It was cross-sectional, and it was difficult to determine the causal association between risk variables and the natural course of normoalbuminuric renal insufficiency. Moreover, it was a single-centered study with a limited number of patients; only those with T2D were included, and due to a lack of non-diabetic renal disease controls, there will be an issue of generalizability of our findings. Additionally, there was no comparison with creatinine-based GFR, which could have provided further insights into the renal function in the study population.

Further research is necessary to directly compare its predictive accuracy with that of serum creatinine and other traditional markers.

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AUTHOR'S CONTRIBUTIONS:

- **AF:** Conception of study, study design, data acquisition & analysis, manuscript drafting
- **PM**: Design of work, interpretation of data, critical review
- **ZRM:** Conception of study, critical review, manuscript drafting
- **MK:** Conception of study, data acquisition & analysis, manuscript drafting, critical review
- **HJM:** Data collection, interpretation of data, manuscript drafting
- **MF:** Data collection and manuscript drafting

All authors approved the final version to be published and agreed to be accountable for all aspects of the work, ensuring that any questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved

CONFLICT OF INTEREST:

None

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