Scholars Journal of Applied Medical Sciences
Abbreviated Key Title: Sch J App Med Sci
ISSN 2347-954X (Print) | ISSN 2320-6691 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Pathology

Serum Cystatin C as an Early Predictive Marker of Nephropathy in Type 2 Diabetic Patients

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DOI: 10.36347/sjams.2024.v12i05.020 | **Received:** 13.04.2024 | **Accepted:** 18.05.2024 | **Published:** 24.05.2024

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Abstract **Original Research Article**

Background: Diabetic nephropathy, the most common complications of diabetes mellitus, is the chronic loss of kidney function occurring in diabetic patients. Usually, diabetic nephropathy manifestation grows after 10 years duration in case of type 1 diabetes but in case of type 2, it may manifest even at the time of diagnosis. Microalbuminuria is the earliest manifestation of diabetic nephropathy. Microalbuminuria is the persistent elevation of albumin in urine between 30-300 mg/day, below 30 mg/day is normoalbuminuric and above 300 mg/day is macroalbuminuria. So, screening for microalbuminuria should be initiated from the time of diagnosis of T2DM which is sometimes avoided by the physician, overlooked by patient and both. Moreover, urine for albumin may show even normal when nephropathy has already been started. For this, some other early detective markers need much more attention. Serum cystatin C can be that predictive marker for early recognition of diabetic nephropathy in case of T2DM patients. Serum cystatin C reflects increased urinary albumin concentration and reduced glomerular filtration rate both at the very early stages when microalbuminuria not even developed and GFR is not reduced. ***Objective:*** To evaluate serum cystatin C level as an early predictive marker of nephropathy in type 2 diabetic patients. ***Materials and Methods:*** This was a cross sectional study done in the Department of Biochemistry and Molecular Biology, BSMMU. Subjects (total one hundred sixty-six in number) from Endocrinology & Metabolism OPD of BSMMU who matched the inclusion and exclusion criterias were enrolled in the study by non-probability purposive sampling technique and an informed written consent was taken from all who agreed to participate in the study. Data were collected after IRB approval using a data collection sheet in Bengali. This sheet contained information about socio-demographic characteristics**,** information about any previous or co-existing disease, anthropometric measurements, data of physical examination and all the biochemical reports of the patient. Blood was collected from each individual to estimate serum cystatin C, serum creatinine, eGFR and spot urine was collected for micro albumin (urine). One hundred sixty-six total study subjects were enrolled then categorized into two groups. Group 1 consists of normo-albuminuric diabetic patients and Group 2 consists of albuminuric (including both micro and macro albuminuric) diabetic patients. For statistical analysis, SPSS for Windows (version 26.0) was used to test Spearman's rank correlation coefficient, Mann Whitney U test, Kruskal Wallis test followed by Dunn-Bonferroni, ROC analysis and Z test. All results considered statistically significant if P value was less than 0.05. ***Results:*** Serum cystatin C, serum creatinine and micro albumin (urine) level were found significantly higher in albuminuric patients (group 2) than normoalbuminuric patients (group 1). Whereas eGFR level found significantly lower in albuminuric patients (group 2) than normoalbuminuric patients (group 1). Serum cystatin C showed significant positive correlation with micro albumin (urine) and negative correlation with eGFR of total study subjects. AUC from ROC analysis revealed, serum cystatin C can be used as a good predictor and may give better performance than serum creatinine. ***Conclusion:*** Serum cystatin C may be used as an early marker to predict nephropathy in diabetic patients.

Keywords: Diabetes Mellitus, Diabetic nephropathy, Micro-albuminuria, Serum cystatin C, Serum creatinine.

INTRODUCTION

Diabetes mellitus has reached an unflagging state worldwide, 1 in 11 adults have DM of which 90% with type 2 diabetes mellitus [1]. An estimated 415 million adults aged 20-79 years were suffering from DM in the year 2015 and the number is projecting above 642 million in 2040 with the prevalence increasing from

8.8% to 10.4% (International Diabetic Federation, 2021). The prevalence of diabetes mellitus is 8% (12.88 million) of total population and 3% of total death of all ages in Bangladesh (WHO, 2016).

Diabetes mellitus, a chronic metabolic disorder has led to an increase in microvascular (nephropathy, retinopathy and neuropathy) and macrovascular (coronary artery disease, peripheral arterial disease and stroke) complications [2].

Diabetic kidney disease is referred to as diabetic nephropathy, the diagnosis is usually based on the presence of albuminuria (increased urinary albumin excretion) and/or reduced estimated glomerular filtration rate (eGFR) in the absence of other renal diseases in early cases of diabetes mellitus [3].

Micro-albuminuria is the first detectable functional abnormality seen in DM patients with diabetic nephropathy [4]. A proportion (nearly 40%) of micro-albuminuric patients may progress to overt nephropathy, characterized by the presence of overt proteinuria.

Urinary albumin excretion can be affected by several factors including plasma concentrations of atrial natriuretic peptide, arginine vasopressin, angiotensin II, aldosterone and fasting blood glucose, glycated hemoglobin, and mean arterial blood pressure [5].

Serum creatinine and creatinine clearance rate are the most widely used markers for the routine non-invasive estimation of GFR. Serum creatinine is considered relatively specific, but not very sensitive since its level significantly increase when more than 50% of the GFR is reduced [6].

Serum creatinine concentration may be significantly influenced by several extra-renal factors like muscle mass, changes in tubular secretion, dietary intake, analytical interferences etc. In elderly female patients with reduced muscle mass, measurement of serum creatinine may grossly underestimate the reduction in the GFR. Finally, numerous drugs and endogenous substances also interfere with the measurement of creatinine, leading to falsely high or low creatinine values [7].

Moreover, tubular involvement may precede glomerular involvement. For this, several tubular proteins and enzymes are detectable in blood even before the appearance of microalbuminuria and a rise in serum creatinine [8].

So, early detection of abnormal renal function is essential to slow down the progression to nephropathy and further to end-stage renal disease (ESRD). Many markers such as cystatin C, alpha 1-microglobulin, immunoglobulin G or M, angiotensinogen, liver type fatty acid-binding protein, urinary transferrin, serum osteopontin, urinary retinol-binding protein, and interleukin-18 have been screened as early indicators of diabetic nephropathy [9].

Cystatin C, a cysteine protease inhibitor, is freely filtered by the renal glomeruli, identified as a predictive marker of renal failure [10].

Cystatin C is cleared from the circulation by only renal filtration and is freely filtered at the glomerular filtration. Its serum concentration is not influenced by non-renal factors. Its small size and high perfusion index enable it to be freely filtered at the glomerulus.

Previous studies have suggested that serum cystatin C concentration is a better indicator of GFR than serum creatinine concentration in patients with diabetes, spine injury, liver cirrhosis, mild to moderate impaired kidney function, and also in elderly patients. As alternatives to serum creatinine-based equations, several serum cystatin C-based equations (cystatin C formulas) have been developed and proposed to estimate the GFR also [11].

RESEARCH OBJECTIVES

General objective:

To evaluate serum cystatin C level as an early predictive marker of nephropathy in type 2 diabetic patients.

Specific objectives:

- To measure serum cystatin C levels of the study subjects.
- To measure serum creatinine & to calculate eGFR of the study subjects.
- To measure micro albumin (urine) concentration of the study subjects.

MATERIALS AND METHODS

Study Design: Cross sectional study.

Place of Study: Department of Biochemistry & Molecular Biology, BSMMU.

Study Period: One year (March 2022 – February 2023).

Study Population: The study population aged 25-70 years type 2 diabetic patients of both genders were enrolled attending at Endocrinology & Metabolism OPD of BSMMU.

Sampling Technique: Non-probability purposive sampling.

Grouping of the study subjects:

- Group 1: Normo-albuminuric patients
- Group 2: Albuminuric patients
  - 2A. Micro-albuminuric patients
  - 2B. Macro-albuminuric patients

Sample size: So, 166 (one hundred sixty-six study subjects) were selected

Enrollment of participants

Inclusion Criteria:

- Adults 25-70 years of both genders.
- Diagnosed case of type 2 Diabetes Mellitus with a history of <10 years duration.

Exclusion Criteria:

- Patients with thyroid disease or taking medication due to thyroid disease in 6 months.
- Patients with uncontrolled hypertension with or without complications (stroke, heart failure etc.).
- Pregnant women and lactating mother.
- Patients with diagnosed case of cancer.

Variables studied

- Age
- Gender
- Serum cystatin C
- Serum creatinine
- eGFR
- Micro albumin (urine)

Data collection instruments

A pretested data collection sheet formatted first in English then translated in Bangla used as a data collection tool. The sheet included three sections (A, B, C).

Section A: Different information about socio-demographic characteristics.

Section B: Information about any previous or co-existing disease, anthropometric measurements, data of physical examination.

Section C: About all the biochemical reports of the patient.

Sample Collection

Five (05) ml blood samples were collected from study subjects with all aseptic precautions from the ante-cubital vein by a disposable plastic syringe and collected immediately into a dry clean test tube. The serum was then separated from the blood collected in the plain tube after clot formation and by centrifuging at 3000 rpm for 15 minutes. Serum was used for the estimation of cystatin C and creatinine and GFR was calculated by using MDRD formula that was serum creatinine based. Five (05) ml spot midstream urine sample collected from study subjects as per instruction given by with all aseptic precautions and preserved immediately into a dry container supplied by the OPD.

Data Processing and Analysis

In statistical analysis, SPSS for Windows (version 26.0) was used. Data were found as skewed distribution and presented as median (IQR). Spearman's rank correlation coefficient was employed to test the correlations between serum cystatin C and micro albumin(urine). AUC of both serum cystatin C and serum creatinine was compared by Z test of paired sample design to find out best predictor of nephropathy in diabetic patients. All results considered statistically significant if P value was less than 0.05.

RESULTS

This cross-sectional study was carried out in the Department of Biochemistry and Molecular Biology, BSMMU. One hundred and sixty-six (166) subjects were enrolled in this study from Endocrinology & Metabolism OPD. The blood and urine samples were collected with full septic precaution for estimation of serum cystatin C, serum creatinine, eGFR and micro albumin (urine) in the Department of Biochemistry and Molecular Biology, BSMMU.

Table I: Distribution of study subjects according to gender (n=166)

Gender	Group 1 (Normo-albuminuric) n=94	Group 2 (Albuminuric) n=72	Percentages (%)
Male (n=87)	47	40	53
Female (n=79)	47	32	47

Table I shows there were 87 male (47 in Group 1 and 40 in Group 2) and 79 females (47 in Group 1 and 32 in Group 2) out of one hundred sixty-six total study subjects.

Table II: Comparison of study subjects according to age (n=166)

Variable	Group 1 (Normo-albuminuric) n=94 Median (IQR)	Group 2 (Albuminuric) n=72 Median (IQR)	p value
Age	50 (43-60)	51 (40-60)	0.948

Mann Whitney U test was done to reach the level of significance.

Table II shows comparison of study subjects according to age. There was no significant difference of median (IQR) age between two groups (p value>0.05).

Table III: Comparison of all parameters between Group 1 and Group 2 individuals (n=166)

Parameters	Group 1 (n=94) Median (IQR)	Group 2 (n=72) Median (IQR)	p values
Serum cystatin C (mg/L)	0.78 (0.67-0.85)	0.98 (0.79-1.12)	0.000
Serum creatinine (mg/dL)	0.85 (0.76-1.08)	1.04 (0.84-1.32)	0.002
Micro albumin (urine) (mg/L)	8.25 (4.59-17.54)	132.50 (45.50-294.90)	0.000
eGFR (ml/min/1.73m^2)	91.95(77.70-109.20)	83.70 (65.85-103.10)	0.028

Mann Whitney U test was done to reach the level of significance.

Table IV shows comparison of all parameters done with study subjects (n=166) with p values. There was significant difference of median (IQR) of serum cystatin C, serum creatinine, micro albumin (urine) and eGFR between two groups (p< 0.05).

Table IV: Status of serum cystatin C level among normoalbuminuric patients (Group 1) with normal serum creatinine level and eGFR (n=94)

Total study subjects (Group 1) n	High serum cystatin C level n (%)	Normal serum cystatin C level n (%)
94	13 (13.8)	81 (86.2)

n= Frequency, %=percentage

Table IV shows that among 94 total normo-albuminuric patients (Group 1), 13 patients were found having high serum cystatin C (>1.03 mg/L) with normal serum creatinine level and eGFR.

Table V: Correlation of serum cystatin C level with Micro albumin (urine) and eGFR (n=166)

Variables		r value	p values
Serum cystatin C	Micro albumin (urine)	$^{+}$0.277	0.000
Serum cystatin C	eGFR	-0.111	0.156

Spearman's rank correlation coefficient test was done to reach the level of significance.

Table V shows Correlation of serum cystatin C level with micro albumin (urine) and eGFR. Serum Cystatin C shows highly significant positive correlation with micro albumin (urine) of the study subjects (p< 0.05). Serum cystatin c shows negative correlation with eGFR of the study subjects (p> 0.05).

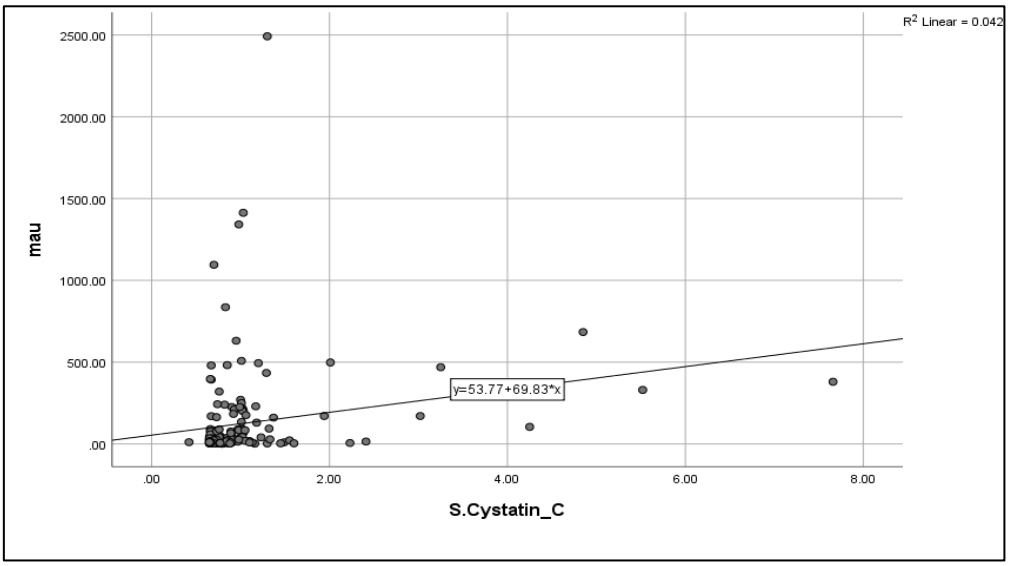

Figure 1: Scatter diagram of correlation of serum cystatin C with micro albumin (urine) of study subjects (n=166)

Figure 1 shows a highly significant positive correlation between serum cystatin C with micro albumin (urine) with r value +0.277 ($p<0.05$).

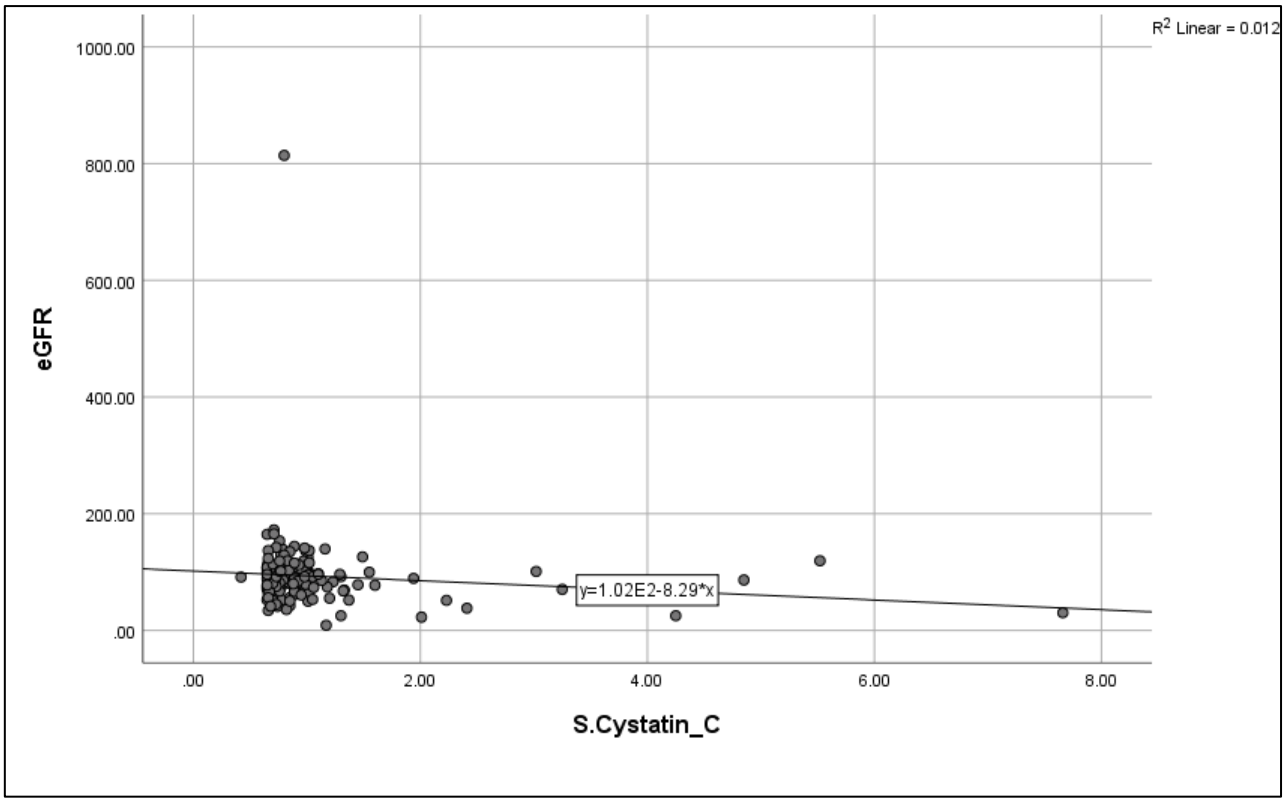

Figure 2: Scatter diagram of correlation of serum cystatin C with eGFR of study subjects (n=166)

Figure 2 shows a negative correlation between serum cystatin C with eGFR with r value -.111 ($p>0.05$).

Also, another study made by Huda, M.N *et al.*, (2020) showed that cystatin C is a better marker of impaired renal function and more sensitive marker compared to serum creatinine.

Sagheb *et al.*, (2014) showed, serum cystatin C is the most appropriate marker for calculating GFR, instead of serum creatinine, and for predicting kidney malfunction progression. Kumar and Kumar (2015) stated that serum cystatin C can be used instead of serum creatinine to measure GFR in type 2 diabetes mellitus.

Shimizu *et al.*, (2003) study, Receiver operator characteristic (ROC) plots demonstrated that the area under the curve (AUC) of cystatin C (0.76) was greater than that of creatinine (0.66). As an early prognostic marker of type 2 diabetic nephropathy, serum cystatin C was better than serum creatinine in terms of sensitivity and specificity. It appears that the levels of serum cystatin C may predict early prognostic stages of patients with type 2.

In this study, comparing AUC of both serum cystatin C (0.748) and serum creatinine (0.657) it is clear that cystatin C may give a better performance to predict nephropathy of diabetic individuals than serum creatinine.

Recent studies have shown that calculation of the eGFR with a combination of creatinine and cystatin C more accurately reflects measured GFR than either marker alone, findings that are probably due to the lesser overall effects of non-GFR determinants of either marker when both markers are included.

DISCUSSION

The prevalence of diabetes is globally growing rapidly, especially in developing countries. With the increasing prevalence of diabetes, the prevalence of Diabetic nephropathy (DN) is also predicted to increase. If there is no immediate improvement in clinical strategy of prevention of DN, T2DM is that summons in our upcoming days for sure.

The motive of this cross-sectional study was to evaluate serum cystatin C as a predictive marker of nephropathy in type 2 diabetic patients attending Endocrinology & Metabolism OPD of BSMMU. Those

who matched the inclusion and exclusion criteria were enrolled in the study by non-probability purposive sampling technique and an informed written consent was taken from all who agreed to participate in the study. All the study subjects then categorized into two group after doing their blood and urine test. Group 1 was selected with that patients having normo-albuminuria and Group 2 with that patients having albuminuria. Group 2 was included with patients having both micro-albuminuria and macro-albuminuria and named as albuminuria.

In this study most of the confounding variable were excluded during enrollment of the participants. Regarding age, there were no significant differences and regarding gender male were predominant in both groups.

Richard (2020) revealed that cystatin C is influenced less by age, gender, and muscle mass than creatinine.[12] Many studies have shown that levels decline from birth to age 1, then remain stable until about 50 years of age. Whereas cystatin C has been found by some other few studies to be influenced by age, gender, cigarette smoking, high CRP, steroid use, and thyroid disorders also. The early detection of DN mainly focuses on the urine albumin excretion rate. 42% of patients with type 2 diabetes have already suffered kidney damage even when their urine albumin excretion was either normal or high.

In this study, there were a few participants with high cystatin C level and normal micro albumin (urine), serum creatinine and eGFR. They have already some loss of kidney function but except cystatin C all markers were found within normal range. So, cystatin C values in the research were seen to increase even before clinical albuminuria began and, therefore, could act as an early marker of micro-albuminuria in detecting nephropathy.

Yadav B *et al.,* (2021) has shown serum cystatin C and microalbumin both could be considered as markers for early detection of nephropathy in T2DM patients [13]. The more prominent rise in serum cystatin C values provide an earlier diagnosis of diabetic nephropathy among T2DM patients. Micro-albuminuria screening is critical because it enables interventions aimed at preventing diabetic nephropathy and is included in daily treatment for diabetic patients to monitor the progression of kidney disease and to evaluate therapeutic effects (30–300 mg/day) to macro albuminuria (> 300 mg/day), which affects 25% of patients within 10 years of being diagnosed with diabetes.

In this study, a highly significant relationship was found between cystatin C and micro albumin (urine) and a negative correlation was also found between cystatin and eGFR.

Gupta *et al.,* (2017) showed there was a significant relationship between cystatin C and albuminuria level. In addition to serum creatinine, serum cystatin C also has a significant relationship with a decrease in glomerular filtration rate [14].

However, in the study, there was increased serum levels of cystatin C significantly in diabetic patients with micro-albuminuria and macro-albuminuria. Thus, patients with albuminuria had higher levels of cystatin C compared to normo-albuminuria patients.

Results can also be found in research conducted by Al-Hazmi *et al.,* (2020) that serum levels of cystatin C increased significantly in diabetic patients with moderate albuminuria and heavy albuminuria when compared to the control group [15].

In this study it was found that serum cystatin C is a good biomarker than serum creatinine and may be a better predictor of nephropathy for diabetic individuals after analyzing AUCs of ROC for serum cystatin C and serum creatinine.

Qiu, X *et al.,* (2017) have shown that Cystatin C has been identified as a better diagnostic ability than serum creatinine for assessing the functionality of the kidney, especially to identify the slight reductions in glomerular filtration level [16].

CONCLUSION

This study concludes that, there was a positive correlation between serum cystatin C & micro albumin (urine) and negative correlation between serum cystatin C & eGFR of total study subjects. Serum cystatin C level was found increasing with the increasing types of albuminuria. Serum cystatin C was also found significantly elevated in normoalbuminuric type 2 diabetic patients when other markers were found within normal range that is considered a very early stage before the development of micro-albuminuria. So, serum cystatin C can be used as a new early predictive marker for diabetic nephropathy and also may give better performance over the most used marker serum creatinine in type 2 diabetic patients.

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