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Indian Journal of Forensic and Community Medicine

Journal homepage: www.ijfcm.org

Original Research Article

Serum cystatin C as a biomarker for early kidney disease detection among type 2 diabetes patients: A cross-sectional study

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Abstract

Background: Diabetic kidney disease is a critical microvascular complication of diabetes, accounting for 33.6% of the total cases. Diabetic nephropathy occurs due to /chronic hyperglycaemia, causing kidney damage, which is a significant concern. Early detection of diabetic kidney disease can significantly improve morbidity and mortality.

Material and Methods: This study was done on 159 already known cases of type 2 diabetes mellitus with no previously documented impaired renal function. Serum Creatinine and Serum Cystatin C was done and eGFR was calculated.

Results: In the present study the sensitivity of serum creatinine was 52% and specificity was 91%. The sensitivity of serum Cystatin C 72% and specificity was 100%. Area under curve was 65% for Serum Creatinine and 80% for Serum Cystatin C. When compared to Serum Creatinine and eGFR (rho = -0.6931), there was a significant negative correlation (rho = -0.9794) between serum cystatin C and eGFR. This demonstrates that a decline in eGFR can be detected by a significant increase in Serum Cystatin C levels.

Conclusion: With higher Sensitivity and Specificity of Serum Cystatin C, as well as a strong negative correlation between eGFR and Serum Cystatin C, it can serve as a biomarker for early diagnosis of kidney disease among type 2 diabetic patients.

Keywords: Serum cystatin C, Serum creatinine, Kidney disease, Biomarker.

Received: 13-08-2025; **Accepted:** 15-10-2025; **Available Online**: 09-12-2025

1. Introduction

The occurrence of diabetes worldwide is becoming more prevalent as a result of various factors such as the growing population, aging, urbanization, increasing prevalence of obesity, and lack of physical activity.[1] Diabetic kidney disease is a critical microvascular complication of diabetes, accounting for 33.6% of the total cases. According to the estimates in 2023, 529 million people were suffering from diabetes, which is expected to rise to 1.3 billion by 2050.[1,2] The World Health Organisation reports that 1.6 million deaths were attributed to diabetes and 2.2 million deaths were caused by diabetic complications such as chronic kidney disease.[3] Diabetic nephropathy occurs due to chronic hyperglycaemia, causing kidney damage, which is a significant concern.[4] Early detection of diabetic kidney disease can significantly improve morbidity and mortality.[5]

Diabetic nephropathy (DN) is a medical condition clinically diagnosed by estimating glomerular filtration rate (GFR) with the help of Creatinine clearance and assessing 24-h urine albumin levels.[4] However, studies have found that abnormal albuminuria and raised creatinine don't necessarily indicate DN using kidney biopsy findings.[5] This leads to

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https://doi.org/10.18231/j.ijfcm.12854.1761281555

difficulty in the early diagnosis of kidney damage as Serum Creatinine rises only after 50% of renal function is lost.[6] Many studies have shown that serum Creatinine values vary based on age, sex, race, muscle mass, diet and medication use.[6,7] As the renal tubules secrete Creatinine, the GFR is overestimated which leads to a significant delay in diagnosis.[5] Urine albumin also poses certain limitations, as it is measured by collecting urine over 24 hours and cannot be used as a screening tool. Secondly, some studies have shown that in 30% of patients with renal failure, albuminuria is not specific to diabetic kidney disease as it is influenced by multiple factors, including urinary tract infections, menstrual cycles, urine retention, and exercise. The "gold standard" for measuring GFR involves calculating the clearance of exogenous substances such as Inulin, 51Cr-EDTA, and Iohexol, which are solely excreted via glomerular filtration.[5,8] Nevertheless, these tests present certain drawbacks, including high costs, prolonged testing time, and a requirement for specialized expertise.

Cystatin C is a promising new biomarker for the early detection of diabetic kidney disease.[4] Cystatin C is a single-chain, non-glycosylated basic protein produced by all cells of the body at a constant rate. It is reabsorbed and almost completely catabolized in the proximal renal tubule.[6] Therefore, because of its constant rate of production, its serum concentration is determined by glomerular filtration.[4] It is unaffected by age, gender, muscle mass, protein consumption, and so on, which makes it a better option than serum creatinine for estimating the glomerular filtration rate.[7] Furthermore, even a slight rise in Cystatin C can indicate mild kidney damage long before it is detected by Serum Creatinine and albuminuria.[6-8] With these advantages there is a need to assess the clinical utility of Serum Cystatin C as a biomarker in renal function assessment. But many studies are done in clinical settings where most of them are already in end stage renal disease. Hence, the present study is planned to assess Serum Cystatin C as a biomarker for the detection of early kidney disease among type 2 diabetes patients especially in community settings.

2. Objective

To assess Serum Cystatin C as a biomarker for the detection of early kidney disease among type 2 diabetes patients.

3. Methodology

3.1. Source of data

Type 2 diabetic participants residing under the field practice area of Department of Community Medicine.

3.2. Study design

Cross sectional study

3.3. Study period

4 months (March –June 2025)

3.4. Sample size

The formula used for sample size calculation is,

$$n = \frac{\widehat{Sn}\left(1-\widehat{Sn}\right)Z_{\frac{\propto}{2}}{}^{2}}{Prev * d^{2}}$$

$\widehat{Sn}$ is the pre-determined values of sensitivity, d is the maximum marginal error required, $Z_{\frac{\propto}{2}}$ is the value corresponding to the level of confidence required and Prev is the prevalence. The sensitivity of Serum Cystatin C in detecting early kidney failure was observed to be 70.83% in a study conducted in Mumbai.[9] Considering similar results and assuming 60% prevalence of diabetic kidney disease,[7] at 95% confidence level and 10% maximum error, the sample size was

$$n = \frac{0.7083x\left(1-0.7083\right)\ x1.96^{2}}{0.6x0.1^{2}}$$

3.5. Study populations

Already known cases of type 2 diabetes mellitus with no previously documented impaired renal function.

3.6. Method of collection of data

250 type 2 diabetes patients were registered in the diabetic clinic of an urban health facility under the field practice area of department of Community Medicine. Out of these, 159 type 2 diabetic patients were selected using simple random sampling method. Information was collected using a predesigned pretested questionnaire. 5 ml venous blood sample from each study participant was taken by trained and certified laboratory technicians prior to breakfast (at least 8 hours of fasting) using aseptic precautions. Following centrifugation, the blood was moved to EDTA and a dry, clean serum separator tube. The serum was subsequently transferred to Nunc and kept at -20°C until laboratory testing was completed and the pathologist reported the results.

1. Fasting blood sugar was done for all participants.
2. Serum Creatinine (SCr) was done for all participants by using Jaffe's method.

Serum Creatinine levels[10] – 0.5-0.9 mg/dl (females)
0.6-1.3 mg/dl (males)

Serum Cystatin C was done for all participants by using nephelometry: Latex particle agglutination measured by immunoturbidimetry method.

Serum Cystatin C levels[10] –Reference range (0.6-1.0 mg/l)

The estimated glomerular filtration rate (eGFR) was calculated using the following formula[11]

eGFR = 127.7 x cystatin C–1.17 (mg/L) x age–0.13 (years) x 0.91(if female) or 1.06 (if male)

eGFR (mL/min/1.73 m²) = 141 × min (Scr/κ, 1) ^α × max (Scr/κ, 1) ^–1.209 × 0.993^Age × [1.018 if female]

3.7. Study variables

Information regarding sociodemographic variables, medical history, personal history & anthropometric measurements (such as Height, Weight, BMI, Waist-hip ratio), Serum Creatinine and Serum Cystatin C was collected from all participants.

3.8. Inclusion criteria

Known type-2 diabetic patients.

3.9. Exclusion criteria

Participants with urinary tract infections, malignancies, liver disease, thyroid gland dysfunction, Congestive heart failure, Hypertension, HIV, and women who are pregnant were excluded. Participants not willing to participate in the study were excluded.

3.10. Informed Consent

Written informed consent was taken from every study participant.

3.11. Methods

Microsoft Excel and the statistical R software version 4.1.2 were used to analyse the data. A frequency table is used to present categorical variables. The format for continuous variables is Mean ± SD. The correlation between continuous variables was examined using Spearman's correlation. From creatinine, cystatin C, and eGFR from cystatin C, diagnostic values and area under the curve was estimated. $P<0.05$ was considered as statistically significant.

4. Results

In this study the mean age of participants was 57.07±13.07 years. Out of total participants, 27.04% were 51-60 years old, 25.16% were 61-70 years old, 18.24% were 41-50 years old and 15.72% were 71-80 years old. Only 13.84% were illiterate. 13 participants were educated up to the college level and only one participant was educated up to the post graduate level.31.45% of them belonged to Class 5 socioeconomic status. 64 participants were housewives and one fifth of the participants were unemployed (**Table 1**). 30 participants were involved in a job in the private sector, 18 participants had a government job and 14 participants owned a business. Almost three fourths of the participants were Hindu by religion and one fourth were Muslims. Three participants were Christians. Majority of the participants were married, 12 were widows and only five were unmarried. Almost half the participants, 78 had a positive family history and 81 didn't have any family history of diabetes in the family. Majority of the participants had a mixed diet while 32 were Vegetarians. Majority of the participants, didn't have any habits whereas six had the habit of tobacco chewing, two consumed alcohol and two smoked cigarettes.

Out of the total 63 participants had a fasting blood sugar >125mg/dl with a mean blood sugar of 205mg/dl. 46 participants were not taking their medications regularly and 27 were not on any medication. Only half of the participants exercised regularly for more than five days per week. 31 participants did not exercise at all, 29 exercised irregularly and 11 exercised occasionally.(**Table 2**)

The mean BMI of study participants was 26.22 ± 4.11. The mean waist circumference was 95.39±10.96 cms. The mean waist hip ratio was 0.95±0.04. As per the BMI measurement which is the primary metric, 18.86% of participants were obese in this study.(**Table 3**)

42.14% had an elevated serum creatinine level with a mean of 1.42 ± 0.81mg/dl. Half of participants had an elevated cystatin C with mean of 1.56±0.65mg/L. 77.9% of participants had reduced eGFR from Serum Creatinine with mean of 61.24±18.49mg/dl. 69.81% of participants had reduced eGFR from Serum Cystatin C with mean of 59.17±19.8 mg/L.(**Table 4**)

In the present study the sensitivity of serum creatinine was 52% and specificity was 91%. The sensitivity of serum Cystatin C was 72% and specificity was 100%.(**Table 5** and **Table 6**)

In the present study it was found that the area under curve is 65% (0.5966, 0.7064) for Serum Creatinine and 80% (0.7496, 0.858) for Serum Cystatin C (**Figure 1**).

Using Spearman's correlation, when compared to Serum Creatinine and eGFR (rho = -0.6931), there was a significant negative correlation (rho = -0.9794) between serum cystatin C and eGFR. This demonstrates that a decline in eGFR can be detected by a significant increase in Serum Cystatin C levels (**Figure 2**A,B).

Table 1: Sociodemographic characteristics of study participants

Sociodemographic variables		Number	Percentage
Age (in years)	≤ 40	20	12.58%
	41-50	29	18.24%
	51-60	43	27.04%
	61-70	40	25.16%
	71-80	25	15.72%
	≥ 81	2	1.26%

Table 1 Continued….

Gender	Male	74	46.54%
	Female	85	53.46%
Education status	Illiterate	22	13.84%
	Primary	55	34.59%
	High School	27	16.98%
	Collegiate	13	8.18%
	Graduation	41	25.79%
	Post- Graduation	1	0.63%
Socioeconomic status	I	8	5.03%
	II	20	12.58%
	III	37	23.27%
	IV	43	27.04%
	V	50	31.45%

Table 2: Characteristics related to diabetes

Characteristics		**Number**	**Percentage**
Fasting Blood sugar	>125 mg/dl	63	39.62%
	<110 mg/dl	96	60.38%
Duration of Diabetes	≤ 5 years	77	48.43%
	> 5 years	82	51.57%
Regular on treatment	No Medications	27	16.98%
	Yes	86	54.09%
	No	46	28.93%
Physical exercise	Regular	88	55.35%
	Irregular	29	18.24%
	Occasional	11	6.92%
	Not at all	31	19.5%

Table 3: Anthropometric measurements of study participants

Anthropometric measurements		**Number**	**Percentage**
BMI (Kg/m^2)	Under weight (<18.5)	4	2.52%
	Normal (18.5-24.99)	63	39.62%
	Pre-obese (25-29.99)	62	38.99%
	Obese Class I (30-34.99)	24	15.09%
	Obese Class II (35-39.99)	4	2.52%
	Obese Class III (>40)	2	1.26%
Waist Circumference (cm)	Normal (<102)	81	50.94%
	Obese (≥102)	78	49.06%
Waist Hip Ratio	Normal	67	42.14%
	Obese	92	57.86%

Table 4: Renal profile of study participants

Renal parameters			**Number**	**Percentage**	**Mean± SD**
Serum Creatinine	Male	Elevated (>1.3)	23	31.08%	1.97±0.92
		Normal (0.6-1.3)	51	68.92%	0.96±0.15
	Female	Elevated (>0.9)	44	51.76%	1.13±0.57
		Normal (0.5-0.9)	41	48.24%	0.81±0.10
	Total	Elevated	67	42.14%	1.42±0.81
		Normal	92	57.86%	0.89±0.15
Serum Cystatin C	Male	Elevated (>1)	47	63.51%	1.64±0.70
		Normal (0.6-1.0)	27	36.49%	0.804±0.129
	Female	Elevated (>1)	33	38.82%	1.46±0.58
		Normal (0.6-1.0)	52	61.18%	0.798±0.127
	Total	Elevated (>1)	80	50.31%	1.56±0.65
		Normal (0.6-1.0)	79	49.69%	0.8±0.13

Table 4 Continued…..				
eGFR from Serum Creatinine	Reduced (≤ 90)	124	77.99%	61.24±18.49
	Normal (> 90)	35	22.01%	107.73±13.5
eGFR from Serum Cystatin C	Reduced (≤ 90)	111	69.81%	59.17±19.8
	Normal (> 90)	48	30.19%	113.24±22.8

*Serum Creatinine- mg/dl; Serum Cystatin C- mg/L; eGFR- ml/min/1.73m^2

Table 5: Confusion matrix between serum creatinine and eGFR from creatinine, Serum Cystatin C and eGFR from Cystatin C

		eGFR from Creatinine		**Total**
		Reduced (≤ 90)	Normal (> 90)	
Serum Creatinine	Elevated	64	3	67
	Normal	60	32	92
Total		124	35	159
		eGFR from Cystatin C		
		Reduced (≤ 90)	Normal (> 90)	
Serum Cystatin C	Elevated	80	0	80
	Normal	31	48	79
Total		111	48	159

*eGFR- ml/min/1.73m^2

Table 6: Diagnostic accuracy of serum Creatinine and Cystatin C

Diagnostic accuracy	**Serum Creatinine with eGFR from Creatinine**	**Serum Cystatin C with eGFR from Cystatin C**
Sensitivity	52% (42%, 61%)	72% (63%, 80%)
Specificity	91% (77%, 98%)	100% (93%, 100%)
Positive Predictive Value (PPV)	96% (87%, 99%)	100% (95%, 100%)
Negative Predictive Value (NPV)	35% (25%, 45%)	61% (49%, 72%)
Accuracy	60.38%	80.50%

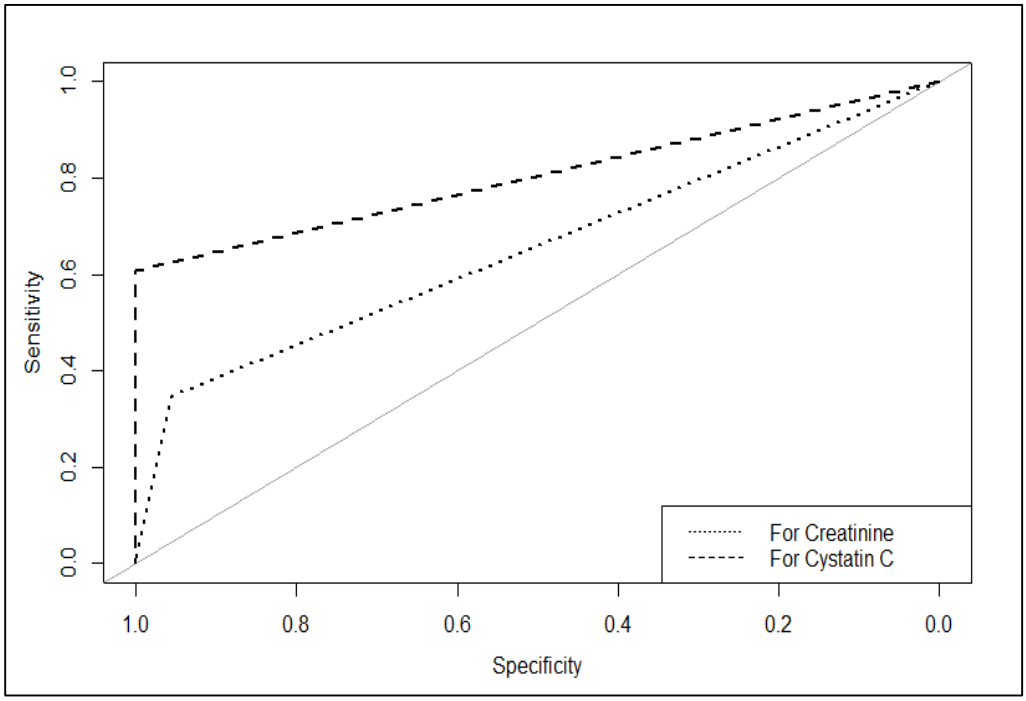

Figure 1: ROC curve analysis for Cystatin C and creatinine

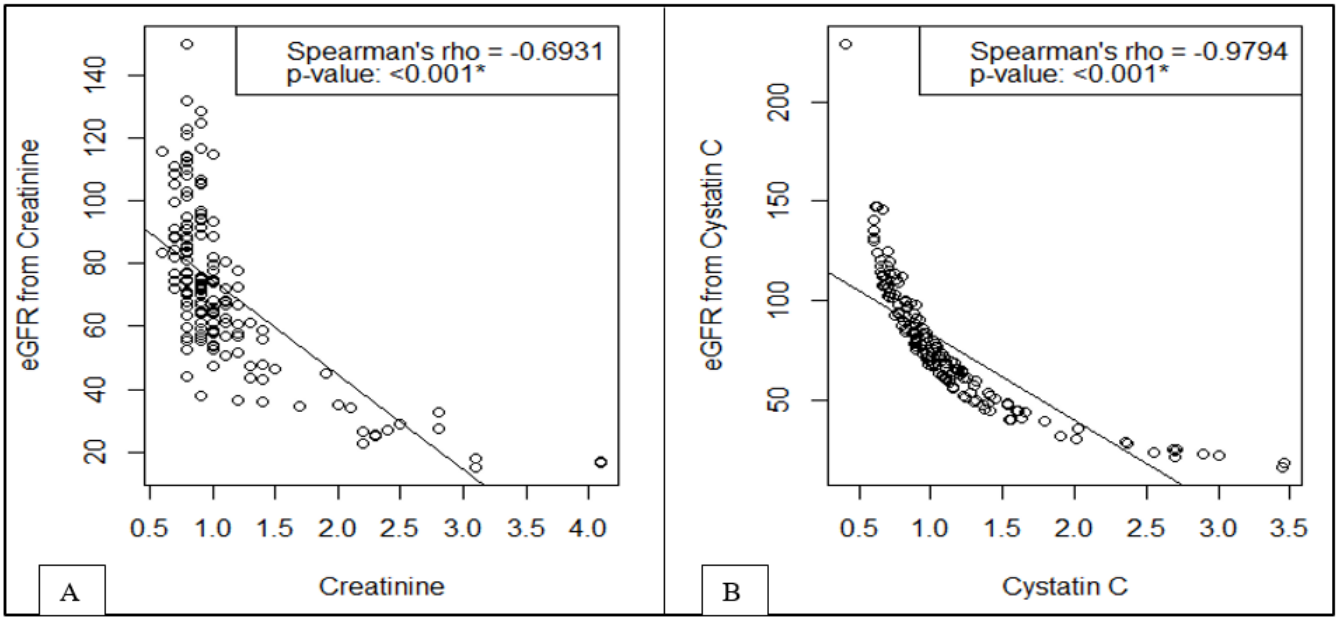

Figure 2: A): Correlation between Serum creatinine and eGFR from creatinine; **B):** Correlation between Serum Cystatin C and eGFR from Cystatin C

5. Discussion

Based on our findings, Cystatin C detected kidney disease in diabetic patients earlier than creatinine.[1,4,7] The current study's results are consistent with several studies that reported an early rise in Serum Cystatin C with a decline in kidney function, when compared to Creatinine.[7] Other studies, however, contradicted this, suggesting that Serum Creatinine is a more accurate marker than Serum Cystatin C in calculating eGFR.[12]

The average age of the participants in our study is 57.07 ± 13.07 years and three-fifths of the participants were unemployed or housewives. This socio-demographic profile is similar to other studies[4,6] with a mean age between 50-60 years. However, other studies had a predominantly male population[1,4] and 36% were housewives by occupation. In this study, 35% of the participants were educated up to the primary level whereas in a similar study conducted in Ethiopia around 50% of participants were educated at the college level and above.[10]

Half of the participants reported a positive family history of diabetes, whereas in the study done in Nepal, only 14% of participants had a positive family history. Three-fifths of the participants had a low socio-economic status, which as reported by several studies is a significant risk factor for CKD.[4,6] Most of the participants (79%) in this study were non-vegetarians, similar to the other studies. Habits including alcohol consumption and smoking, were low in this study which was similar to a study conducted in Nepal, where only 20% of participants consumed alcohol and 8% smoked cigarettes.(**Table 1**)

The mean duration of diabetes was more than five years, similar to other studies. 55% of the participants adhered to their medication regimes, which is higher than other studies findings.[4] Half of the participants exercised regularly, while one-fifth did not exercise at all (**Table 2**). The mean BMI of our study participants was 26.22 ± 4.11, with 60% categorized as overweight or obese. In comparison, the mean waist-to-hip ratio was slightly lower than that observed in a study conducted in Nepal.[4]

In the present study, the mean FBS is 205.95 ± 69.71 mg/dl in patients with uncontrolled diabetes, which is similar to other studies.[1] In patients with abnormal values, the average serum Creatinine is 1.42 ± 0.81 mg/dl and eGFR from serum Creatinine is 61.24 ± 18.49mg/dl. The average serum Cystatin C is 1.56 ± 0.65 mg/L and eGFR from serum Cystatin C is 59.17 ± 19.8mg/l. These findings were similar to the study done in CKD patients in Pakistan.[13] In patients with controlled FBS, the average serum Creatinine is 0.89 ± 0.15 mg/L and the eGFR from serum Creatinine is 107.73 ± 13.5mg/dl. The average serum Cystatin C is 0.8 ± 0.13 mg/L and the eGFR from serum Cystatin C is 113.24 ± 22.8mg/l, which is similar to other studies.[6,10] However, these were significantly higher than other studies[11,13] Serum Creatinine is routinely used in clinical practice to monitor kidney functions, it overestimates the GFR giving a false assurance and delaying treatment of kidney diseases. So, Serum Cystatin C can pick up changes in GFR more accurately which will help in early initiation of treatment and hence prevent further deterioration of kidney functions.

Our study found that Serum Creatinine had a sensitivity of 52% and a specificity of 91% when compared to serum cystatin C. For cystatin C, the sensitivity was higher at 72%, and specificity was 100%. These values differ as compared to a study done in Egypt which had a sensitivity of 81.4% and specificity of 82.4% for Cystatin C.[1] A higher sensitivity of

cystatin C in detecting mild GFR reduction when compared to creatinine in this study indicates that it can pick up most cases of early-stage CKD even if they are subtle or borderline.

The positive predictive value (PPV) for Creatinine and Cystatin C was found to be 96% and 100% which was significantly higher than the study done in Nepal which had a PPV of 44.44% and 62% for Creatinine and Cystatin C respectively.[4] The negative predictive value (NPV) for Creatinine and Cystatin C was found to be 35% and 61% which was lesser than the study done in Nepal, which had a NPV of 42.86% and 69.57% for Creatinine and Cystatin C respectively (**Table 6**). Studies done on Atherosclerosis risk communities also showed that eGFRcys either alone or combined with eGFRcr provides better prediction of adverse when compared to eGFRcr alone.[14,15] This study shows that Cystatin C as a screening tool has high predictive value which indicates that a positive test really picks up early kidney damage.

In this study certain factors such as lifestyle choices (including diet, smoking, and alcohol consumption), treatment adherence and socioeconomic status could act as potential confounders in interpreting serum cystatin C levels. However, their complex interactions with kidney function and biomarker levels were not assessed. Future research with stratified analyses or multivariate modelling could help clarify the independent contribution of cystatin C to early kidney damage detection while adjusting for these confounders.

Cystatin C usage in clinical practice is limited due to the high cost and lack of large population studies but a study done in Australia found that the high cost of the test can be reduced with the use of an auto analyser.[11] Most of the studies are done in the clinical setting with chronic kidney disease patients. But diabetic patients in the community have stable kidney function and detection of early kidney damage and risk stratification becomes important for favourable outcomes. So, study of this kind will help to identify hidden kidney dysfunction in community by early diagnosis and intervention through a good biomarker (Cystain C) as it can pick the damage when GFR is less 90 ml/ min when compared to creatinine which picks the damage much later.

6. Conclusion

Serum Cystatin C with high sensitivity and positive predictive value demonstrated superior performance as a screening tool when compared to creatinine to identify early kidney damage with mild reductions in GFR in the community setting. Though cost and accessibility of Serum Cystatin C continues to be challenge but the clinical use of using Cystatin C in routine practice at least in borderline or high-risk individuals helps in early diagnosis, medical dosing, risk stratification of kidney disease among general population.

7. Recommendations

1. Health care providers should use cystatin C in kidney function screening protocols especially among high-risk individuals and borderline creatinine based GFR (60-89 ml/min/1.73m^2)
2. Cystatin C can be used as second marker in cases where laboratory findings of creatinine and clinical findings are not consistent especially where early kidney damage is suspected.
3. Large scale community-based studies have to be done to validate cost-effectiveness, predictive value of Cystatin C in routine screening programmes.

8. Source of Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

9. Ethical Clearance

Ethical clearance was obtained from Institutional Ethics Committee letter no EC/24-25/D-740.

10. Author Contributions

All authors were involved in Formal analysis, investigation, methodology, project administration, resources, supervision, validation, visualization, writing-original draft, writing - review and editing of manuscript.

11. Acknowledgement

The authors wish to express their gratitude to the patients who enrolled and gave up their valuable time for the study.

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Cite this article: Baliga SS, Singh A, Walvekar P, Handigund RS, Karishetti MS. Serum cystatin C as a biomarker for early kidney disease detection among type 2 diabetes patients: A cross-sectional study. *Indian J Forensic Community Med*. 2025;12(4):333–340.