ORIGINAL ARTICLE

# Diagnostic Accuracy of Serum Cystatin C for Early Detection of Kidney Damage in Patients with Type II Diabetes Mellitus: Bahawalpur, Pakistan

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**ABSTRACT**

**Objective:** To determine the diagnostic accuracy of serum Cystatin C for early detection of kidney damage taking albumin Creatinine Ratio as a reference standard among people of Bahawalpur having diabetes mellitus type 2.

**Study Design:** A cross-sectional study.

**Place and Duration of Study:** The Study was conducted at the Department of Pathology in collaboration with Kidney Center, Bahawalpur Victoria Hospital, Bahawalpur, Pakistan from 27th August 2021 to 26th February 2022.

**Methods:** There was a total of 200 patients having diabetes mellitus type 2 with GFR between 60-90ml/min with an age range from 40-60 years were selected. Study participants with a history of steroid intake, hypothyroidism, chronic liver disease, AIDS, and hypertension were not included in the study. For assessment of diagnostic accuracy of Cystatin C to evaluate the renal damages in early stages, microalbuminuria was evaluated. As per the guidelines provided by the respective manufacturers, individuals exhibiting two albumin creatinine ratio (ACR) levels exceeding 30 mg/g were classified as having diabetic nephropathy (DN).

**Results:** The overall sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of serum Cystatin C for the early detection of kidney damage, with albumin-to-creatinine ratio used as the reference standard among individuals with type 2 diabetes mellitus, were 90.98%, 78.21%, 86.72%, 84.72%, and 86.0%, respectively.

**Conclusion:** The findings of this investigation indicate that serum cystatin C (CysC) exhibits a considerable level of diagnostic accuracy in the early detection of kidney damage among individuals with type 2 diabetes mellitus, utilizing the albumin-to-creatinine ratio as a reference standard.

**Keywords:** *Creatinine, kidney diseases, Serum Cystatin, Sensitivity, Type 2 Diabetes Mellitus.*

***How to cite this:*** *Sajid I, Saeed R, Goheer A, Asghar S, Saqib S, Bashir F. Diagnostic Accuracy of Serum Cystatin C for Early Detection of Kidney Damage in Patients with Type II Diabetes Mellitus: Bahawalpur, Pakistan. Life and Science. 2024; 5(1): 41-47. doi: http://doi.org/10.37185/LnS.1.1.559*



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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Oct 18, 2023; Revised: Dec 22, 2023*
*Accepted: Dec 26, 2023*

## Introduction

Diabetes mellitus prevalence is surging worldwide, with diabetic nephropathy (DN) emerging as a formidable problem of diabetes mellitus type 2 (T2DM). In developed nations, DN assumes a prominent role as a main reason for end-stage renal failure, with Pakistan reporting a staggering 17.9% prevalence.[1] Despite numerous assays aimed at the early detection of renal deterioration in diabetic individuals, the search for precise markers to flag early renal impairment remains contentious.[2] The quantification of glomerular filtration rate (GFR) reigns as the most frequently employed method for assessing the function of the kidney, while serum

creatinine serves as the ubiquitous tool for swift GFR estimation.[3] Conversely, the measurement of the excretion rate of urinary albumin emerges as a crucial standard for detecting early-stage DN and vigilantly progression monitoring, taking unanimous acceptance as an initial and good indicator of renal damage.[4-6]

Human cystatin C (Cys C) is an unglycosylated, low molecular weight basic protein, and it is classified within the superfamily of cysteine proteinase inhibitors.[7] It is widely distributed across diverse tissues in the body and exists in relatively elevated concentrations in bodily fluids. Cys C is filtered from the bloodstream, completely reabsorbed, and catabolized within proximal tubules.[8] Cys C's exceptional uniqueness elevates it to the status of an endogenous benchmark for GFR assessment, unaffected by age, gender, or muscle mass.[9-11] This marker demonstrates a robust correlation with GFR measurements using intravenous iothalamate infusion.[12,13]

Prompt identification of renal damage is crucial in mitigating the dire consequences of DN. Thus, the demand for potent markers to stage and monitor DN remains pressing.[13,14] Cys C emerges as a pivotal, albeit controversial, diagnostic tool for kidney disease. It boasts a diagnostic sensitivity and specificity of 74% and 79% at a cutoff of 0.6 mg/L, yet its adoption as a routine clinical diagnostic instrument remains disputed.[15] Furthermore, no studies have explored its role within the South Punjab population. Indicators of renal impairment include urinary albumin excretion exceeding 30mg/g of creatinine and GFR dipping below 60 ml/min. Additionally, hemoglobin A1c (HbA1c) currently reigns supreme as the most commonly used marker to gauge glycemic status and guide diabetes therapy. Consequently, we conducted measurements of urinary albumin, GFR, HbA1c, and Cys C to investigate potential correlations among these markers. For the initial time, we evaluated the diagnostic precision of cystatin C (Cys C) in the early detection of diabetic nephropathy (DN) among patients with type 2 diabetes mellitus (T2DM) in the South Punjab region.

## Methods

A descriptive cross-sectional study was carried out in collaboration between the Pathology Department and the Kidney Center at Bahawalpur Victoria Hospital, Bahawalpur. The study conducted from August 27, 2021, to February 26, 2022, following approval from the Institutional Review Board vide letter no: 2285/DME/QAMC Bahawalpur held on: 7th August 2021. The study sample comprised 200 patients, determined through calculations using OpenEpi software, considering a prevalence rate of 17.9% and sensitivity and specificity of 74% and 79%, respectively, with a cutoff value of 0.6 mg/L.[15] The margin of error for sensitivity and specificity was set at 10% and 4.3%, respectively. The sampling method used was non-random consecutive sampling, and we secured written informed consent from all participants. Our criteria for inclusion consisted of both male and female individuals with type II diabetes, aged between 40 and 60 years, while those with pre-existing hepatic, cardiovascular, or various medical conditions with chronic history were not included. The assessment for diabetic nephropathy (DN) initially involved two continuous readings of micro-albumin-to-creatinine ratio (ACR), with individuals showing two ACR levels exceeding 30mg/g considered to have DN.

We took fasting blood samples to evaluate levels of HbA1c, cystatin C, and serum creatinine. Additionally, we obtained early-morning urine samples to assess urinary albumin levels. Serum cystatin C levels were determined using a standard sandwich enzyme-linked immunosorbent assay conducted with the Getin FIA instrument. Immunoturbidimetric methodology was employed for the measurement of urinary albumin levels, and urine creatinine levels were quantified using the Jaffe method. The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease (MDRD) equation, which incorporates serum creatinine levels, with specific cutoff values set at 0.6 mg/L for cystatin C and 1.5 mg/dl for creatinine.

Statistical analysis was performed using SPSS version 25. Descriptive statistics, including age, weight, height, and BMI, were computed. Gender distribution, serum cystatin C levels, and outcomes based on the gold standard were expressed in terms of frequency and percentage. To assess sensitivity,

specificity, positive predictive value, negative predictive value, and diagnostic accuracy, 2 x 2 tables were employed. The presentation of data was facilitated through tables and figures.

## Results

In the present study, the age of the participants spanned from 40 to 60 years, with a mean age of 51.27 ± 5.42 years. The preponderance of patients, comprising 117 individuals (58.50%), belonged to the age group of 51 to 60 years. Additionally, there was an almost equal distribution of male and female patients, with 49% being male and 51% female. The mean height observed was 159.87 ± 23.45 cm, while the mean weight was recorded at 82.33 ± 11.35 kg. The mean Body Mass Index (BMI) calculated was 29.60 ± 4.15 kg/m². Furthermore, the mean Cystatin C (Cys C) levels measured were 0.73 ± 0.32 mg/L, and the mean albumin creatinine ratio was 34.20 ± 15.43 mg/g. (Table 1).

In patients who tested positive for Cystatin C (Cys C), 111 individuals were correctly identified as True Positives, while 17 were incorrectly identified as False Positives. Among the 72 patients who tested negative for Cys C, 11 were incorrectly classified as False Negatives, while 61 were accurately identified as True Negatives. The statistical analysis revealed a significant result ($p$<0.001). In the broader context,

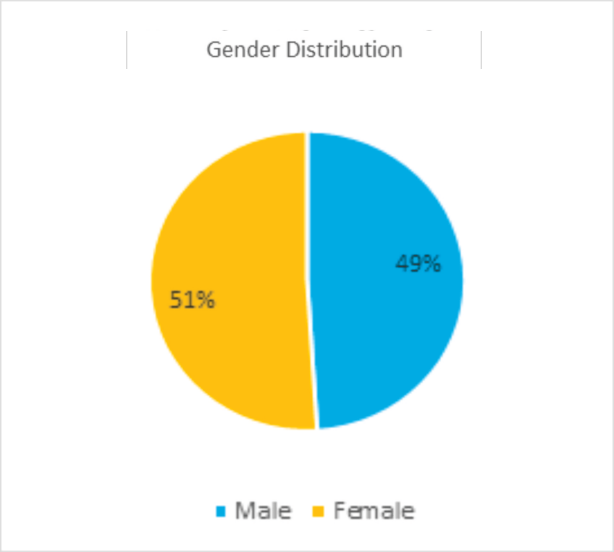


**Fig 1: Gender Distribution**

the comprehensive performance metrics of serum cystatin C (Cys C) for the early detection of kidney damage, using the albumin-to-creatinine ratio as a reference standard among individuals with type 2 diabetes mellitus, were as follows: sensitivity was 90.98%, specificity was 78.21%, positive predictive value was 86.72%, negative predictive value was 84.72%, and diagnostic accuracy was 86.0%. (Table 2).

## Discussion

Chronic kidney disease has become a significant

**Table 1: Stratification of diagnostic accuracy**

| Variables | Serum Cys tatin C Result | Albumin Creatinine Ratio Result | | *P*-value |
|---|---|---|---|---|
| | | Positive | Negative | |
| Diagnostic accuracy results of serum CysC | Positive | 111(TP) | 17(FP) | 0.0001 |
| | Negative | 11(FN) | 61(TN) | |
| Diagnostic precision age 40-50 years (n=83) | Positive | 44(TP) | 05(FP) | 0.001 |
| | Negative | 04(FN) | 30(TN) | |
| Diagnostic accuracy concerning the age bracket of 51 to 60 years (n=117) | Positive | 67(TP) | 12(FP) | 0.001 |
| | Negative | 07(FN) | 31(TN) | |
| Diagnostic accuracy Male gender (n=98) | Positive | 50(TP) | 06(FP) | 0.001 |
| | Negative | 06(FN) | 36(TN) | |
| Diagnostic accuracy Female gender (n=139) | Positive | 61(TP) | 11 (FP) | 0.001 |
| | Negative | 05(FN) | 25(TN) | |
| Diagnostic accuracy BMI of 30 kg/m² or less (n=103) | Positive | 50(TP) | 11(FP) | 0.001 |
| | Negative | 05(FN) | 37(TN) | |
| Diagnostic accuracy BMI >30 kg/m $^{2}$ (n=97) | Positive | 61(TP) | 06(FP) | 0.001 |
| | Negative | 06(FN) | 24(TN) | |

**Table 2: Serum CysC for early detection of kidney damage taking albumin Creatinine Ratio as a reference standard**

| Variable | Sensitivity | Specificity | PPV | NPV | Diagnostic Accuracy |
|---|---|---|---|---|---|
| Diagnostic precision of serum cystatin C (CysC) | 90.98% | 78.21% | 86.72% | 84.72% | 86.0% |
| Diagnostic accuracy Age 40 - 50 years (n=83) | 91.67% | 85.71% | 89.80% | 88.24% | 89.16% |
| Diagnostic precision Age 51 - 60 years (n=117) | 90.54% | 72.09% | 84.81% | 81.58% | 83.76% |
| Diagnostic precision Male gender (n=98) | 89.29% | 85.71% | 89.29% | 85.71% | 87.76% |
| Diagnostic precision female gender (n=139) | 92.42% | 69.44% | 84.72% | 83.33% | 84.31% |
| Diagnostic precision BMI (Body Mass Index) ≤30 kg/m² (n=103) | 90.91% | 77.08% | 81.97% | 88.09% | 84.47% |
| Diagnostic precision BMI (Bo dy Mass Index) >30 kg/m² (n=97) | 91.04% | 80.0% | 91.04% | 80.0% | 87.63% |

public health concern. However, the search for precise markers to detect early changes in renal function remains a subject of debate. Evaluating kidney function still primarily relies on measuring the glomerular filtration rate (GFR) using serum creatinine levels. Concurrently, the evaluation of urine albumin excretion rate remains a valuable conventional method for identifying the initial phases of diabetic nephropathy (DN) and closely tracking its advancement. This method has gained widespread acknowledgment as one of the earliest and most sensitive indicators of kidney damage. In this intricate scenario, human cystatin C (Cys C), a non-glycosylated, low-molecular-weight protein of essential significance, comes into consideration. It exhibits steady expression across most bodily tissues and boasts relatively high concentrations in bodily fluids. Notably, Cys C undergoes removal from the bloodstream via renal filtration and undergoes complete reabsorption and catabolism within proximal tubules.[16]

The unparalleled uniqueness of Cys C elevates it to the position of an endogenous benchmark for evaluating GFR. Remarkably, Cys C production remains impervious to the influences of age, gender, and muscle mass.This marker demonstrates a potent correlation with GFR measurements derived from intravenous iothalamate infusion.[17,18] In pursuit of shedding light on the diagnostic precision of serum Cys C in the early detection of kidney damage, this study hinges on albumin Creatinine Ratio as a reference standard among individuals residing in Bahawalpur afflicted with type 2 diabetes mellitus. Among patients testing positive for Cys C, 111 proved to be True Positives, while[17] were identified as False Positives (Perkins et al., 2005). Among the 72 individuals testing negative for Cys C, 11 turned out to be False Negatives, while 61 were indeed True Negatives ($p$=0.001).

Overall, the diagnostic performance of serum cystatin C (Cys C) for early detection of kidney damage in patients with type 2 diabetes mellitus, utilizing albumin-to-creatinine ratio as a reference standard, exhibited sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy at 90.98%, 78.21% (with a cutoff of 0.6 mg/L), 86.72%, 84.72%, and 86.0%, respectively.[18] Additionally, individuals with a glomerular filtration rate (GFR) below 60 ml/min displayed significantly elevated serum Cys C levels (993.25 ng/ml) compared to those with normal kidney function and healthy subjects. A marginally significant correlation was observed between Cys C and estimated GFR, measuring 86.72%, 84.72%, and 86.0%, respectively. Notably, Cys C demonstrated a sensitivity and specificity of approximately 74% and 79%, with a correlation of −0.16 ($p$ = 0.05) with microalbumin, which exhibited a strong correlation

(rs = 0.22, $p$ = 0.014). Serum Cys C displayed sensitivity and negative predictive values of 100% and 4%, respectively.[19]

A 2019 meta-analysis by Ceo et al. emphasized the superiority and cost-effectiveness of serum cystatin C as a biomarker for the early detection of diabetic nephropathy, playing a crucial role in assessing kidney function, progression, and predicting adverse outcomes in individuals with type 2 diabetes.[20]

Numerous studies affirm that serum cystatin C levels remain unaffected by factors such as age, gender, body mass, and inflammatory conditions.[21,22] The progression of diabetic nephropathy, marked by a decline in GFR among diabetic patients, significantly increases the risk of mortality and cardiovascular-related deaths when GFR falls below 60 ml/min/1.73 $m^2$.[23]

Patients with diabetic nephropathy exhibited significantly higher levels of serum cystatin C and microalbumin compared to those with type 2 diabetes mellitus and control group subjects.[24] Serum cystatin C demonstrated an area under curve of 0.994, while microalbumin had an area under curve of 0.944. Using a cutof point of 1.34 mg/L for serum cystatin C, sensitivity reached 96.7%, and specificity reached 91.7%. For microalbumin, a cutoff point of 138.5 mg/L resulted in a sensitivity and specificity of 90% and 83.3%, respectively.[25]

Rigalleau et al. argued that Cys C is a more efficient marker than serum creatinine for diagnosing early kidney damage among diabetic patients.[26] Similarly, Willems demonstrated Cys C's superior effectiveness compared to serum creatinine as a diagnostic marker for early diabetic nephropathy.[27] Studies have suggested that serum cystatin C levels remain independent of various risk factors for diabetes.[28] However, the incidence of type 2 diabetes mellitus is closely linked to Cys C, which, in turn, correlates closely with both diabetes and diabetic nephropathy. A clinical investigation established that urinary cystatin C concentration serves as an indicator of renal tubular dysfunction.[29]

## Conclusion

The results of this investigation demonstrate that serum cystatin C (Cys C) displays a notably elevated level of diagnostic precision for early detection of kidney damage when utilizing albumin-to-creatinine ratio as a reference standard among individuals with type 2 diabetes mellitus. Such inclusion will assist in the identification of suitable treatment strategies and the formulation of effective management plans.

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**Authors Contribution**

**IS:** Idea conception, data collection, data analysis, results and interpretation, manuscript writing and proof reading
**RS:** Study designing, data collection, data analysis, results and interpretation
**AG:** Data analysis, results and interpretation
**SA:** Data collection, manuscript writing and proof reading
**SS:** Data analysis, results and interpretation
**FB:** Data collection, manuscript writing and proof reading