ORIGINAL RESEARCH

Serum Cystatin C in the Diagnosis of Early DN and Its Comparison with UACR: A Cross-sectional Observational Study

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Abstract

Introduction: In India, diabetic nephropathy (DN) constitutes approximately 46% of chronic renal diseases in the elderly population, becoming the primary cause of end-stage renal disease (ESRD). Early detection of DN enables timely intervention and prevents progression to ESRD.

Aim of study: To study serum cystatin C as a biomarker of early DN in comparison with urinary albumin-to-creatinine ratio (UACR).

Materials and methods: This cross-sectional observational study was conducted in the Department of General Medicine of Employees' State Insurance Corporation Medical College and Hospital, Faridabad, from 2021 to 2024 after obtaining clearance from our institutional ethical committee. The duration for the sample collection was 1 year. A total of 50 consenting diabetic patients who fulfilled the inclusion and exclusion criteria within the study period were included in the study. In diabetic patients with a glomerular filtration rate (GFR) of ≥30 mL/min/1.73 m^2, indicating early DN (stages I, II, and III according to Mogensen's classification), serum cystatin C and UACR were assessed. We conducted a comparative analysis of serum cystatin C and the UACR as biomarkers for early DN.

Results: Among 50 patients, 68% of the patients were aged between 41 and 50 years. The mean age was 43.06 ± 6.9 years. Among total subjects, 52% were males and 48% were females. The most common complaints were polyuria (56%), fatigue (56%), polydipsia (38%), and weight loss (34%). Mean values of diabetic parameters for fasting blood sugar (FBS), postprandial blood sugar (PBS), random blood sugar, and HbA1c were 224.82 ± 79.02 mg/dL, 341 ± 90.1 mg/dL, 331.56 ± 76.98 mg/dL, and 9.85 ± 2.37%, respectively. Mean duration of diabetes was 4.4 ± 3.48 years. About 30% of the cases were classified as stage I, 22% as stage II, and 48% as stage III. Serum cystatin C levels were elevated in 78% of cases. The mean serum cystatin C levels was 1.19 ± 0.39 mg/L, UACR was 95.22 ± 102.15 mg/gm, and GFR was 102.62 ± 28.54 mL/min/1.73 m^2. Serum cystatin C levels showed a significant positive correlation with UACR with an *r*-value of 0.530 and significant *p*-value of < 0.001.

Conclusion: This study suggests that serum cystatin C is a valuable biomarker for early detection of DN and its rise before the onset of microalbuminuria highlights its utility in clinical practice.

Keywords: Cystatin C, Diabetes mellitus, Diabetic nephropathy, Urinary albumin-to-creatinine ratio.

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Introduction

American Diabetes Association (ADA; 2014) defines diabetes mellitus (DM) as a collection of metabolic disorders characterized by elevated blood glucose levels due to abnormalities in insulin secretion, insulin action, or a combination of both.[1]

Diabetes represents a major challenge for global healthcare systems, with its prevalence rising rapidly, particularly in developing countries like India. This increase is mainly due to growing rates of overweight/obesity and unhealthy lifestyles.[2]

Diabetes can lead to a variety of complications affecting different organs, which are typically divided into 2 main categories: Microvascular complications (including nephropathy, retinopathy, and neuropathy) and macrovascular complications (including cerebrovascular disease, peripheral vascular disease, and ischemic heart disease).[3] Among these, nephropathy is a significant contributor to both disease burden and mortality.

Diabetic nephropathy (DN) is a chronic, progressive kidney disease linked to diabetes, caused by structural and functional alterations in the glomeruli and tubules due to impaired glucose regulation. In India, DN accounts for about 46% of chronic kidney diseases in the elderly. The occurrence of DN has significantly increased among diabetics, establishing it as the primary cause of end-stage renal disease (ESRD).[4]

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The primary goal in managing diabetes is to identify an effective early marker for the detection of early-stage DN, preventing its progression to ESRD. Therefore, early identification of nephropathy in diabetic patients is crucial for timely intervention.

Several risk factors contribute to the onset and progression of DN. Some, like hypertension and hyperglycemia, can be managed

through treatment. Others, such as smoking and dietary habits, can be modified through lifestyle changes. While genetic factors remain constant, altering epigenetic influences on gene expression could potentially reduce the risk of DN, even in individuals with a genetic predisposition.[5]

Thus, the study aimed to compare serum cystatin C and the urinary albumin-to-creatinine ratio (UACR) as biomarkers for the early detection of DN.

Materials and Methods

This cross-sectional observational study was conducted at the Department of General Medicine, ESIC Medical College and Hospital, Faridabad, from 2021 to 2024, after receiving approval from the institutional ethics committee. Sample collection took place over a period of 1 year. The study included diabetic patients who met the criteria and provided written informed consent during the study period.

Inclusion Criteria

- Patients between ages 18 and 50 years.
- Patients with a glomerular filtration rate (GFR) ≥ 30 mL/min/1.73 m^2 (stages I, II, and III of Mogensen classification).

Exclusion Criteria

- Conditions that may cause false-positive cystatin C results:
  - Obesity [body mass index (BMI) > 30 kg/m^2].
  - Thyroid disorders.
  - Acute febrile illness.
- Conditions associated with proteinuria:
  - Active urinary tract infection.
  - Autoimmune diseases.
  - Nondiabetic kidney disorders.
- Other factors:
  - Pregnant women.
  - Use of medications that affect proteinuria, such as angiotensin-converting enzyme inhibitors or angiotensin receptor blockers.
  - Liver diseases.

Sample Size

A study by Jeon et al. observed a positive correlation between serum cystatin C levels and albuminuria, with a correlation coefficient (*r*) of 0.555.[6] Based on these findings, the minimum sample size required for a study with 90% power and a 99% confidence level was calculated to be 42 participants. To reduce the margin of error, the final sample size was increased to 50 participants.

Methodology

A comprehensive history and clinical examination were performed on all participants included in the study.

Clinical Examination

Standardized procedures were followed to obtain anthropometric measurements, including height and weight.

Body mass index was calculated by dividing weight (in kilograms) by height (in meters squared). Resting systolic and diastolic blood pressures were measured twice using an automated sphygmomanometer after a 5-minute rest period, and the average of the 2 readings was used to determine the mean blood pressure. Careful examination for signs of pallor, icterus, cyanosis clubbing, lymphadenopathy, pedal edema, and xanthelasma was done and systemic examination was done meticulously.

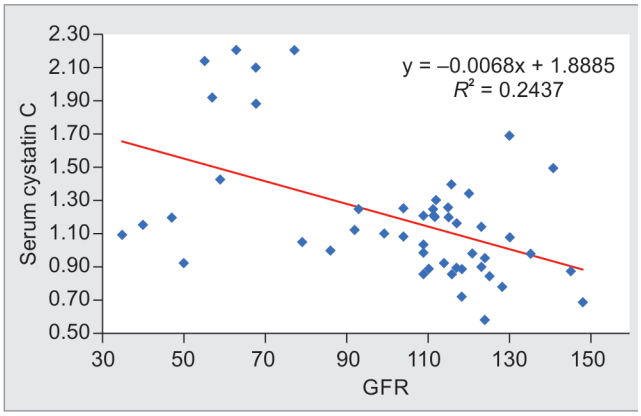

Fig. 1: Inverse correlation between serum cystatin C levels and GFR, exhibiting an *r*-value of –0.494, with a significant *p*-value of < 0.001

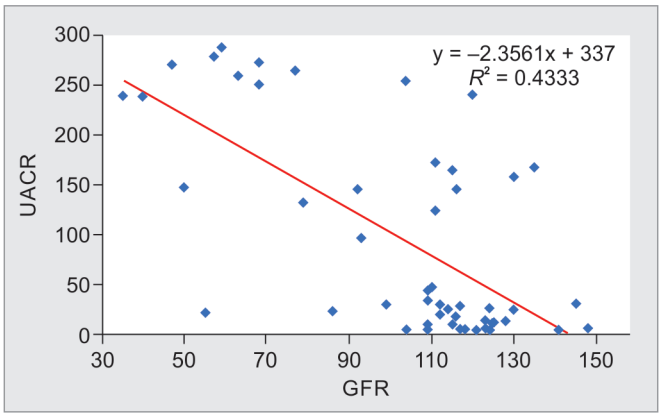

Fig. 2: Even stronger negative correlation between UACR and GFR, marked by an *r*-value of –0.658, also with a significant *p*-value of < 0.001

Fasting blood sugar (FBS), 2-hour postprandial blood sugar (PPBS), and random blood sugar (RBS) were assayed in all patients by colorimetric method. The HbA1c was measured using an enzymatic method. Serum electrolytes were done using the potentiometry method. The GFR was calculated using the CKD-EPI equation. Serum cystatin C levels were measured using a rapid particle-enhanced nephelometric immunoassay (Fig. 1). In this method, polystyrene beads coated with rabbit antibodies to cystatin C aggregate when exposed to samples containing cystatin C. The scattered light, measured by a nephelometer, correlates with the concentration of cystatin C (antigen) in the sample, which is determined by comparing the intensity of the scattered light to that of calibrator dilutions. The normal reference range for adults (18–50 years, male or female) is 0.56–0.89 mg/L.

Urinary albumin was quantified using the immunoturbidimetric method, while urinary creatinine was measured using Jaffe's method. The UACR was then calculated, with a normal reference range of <30 mg/gm (Figs 2 and 3).

Statistical Analysis

Quantitative data were expressed as mean ± standard deviation (SD), while categorical variables were reported as frequencies and percentages. Appropriate statistical tests, such as analysis

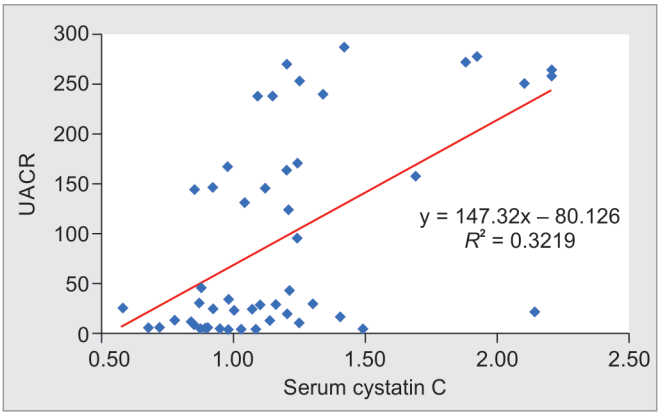

Fig. 3: Positive correlation between serum cystatin C and UACR, characterized by an *r*-value of 0.530 and a significant *p*-value of < 0.001

Table 1: Distribution of participants according to variables

Variables	*Number of patients (%)*
Age (years)	
21–30	3 (6)
31–40	13 (26)
41–50	34 (68)
Gender	
Male	26 (52)
Female	24 (48)
Chief complaints	
Polyuria	28 (56)
Polydipsia	19 (38)
Weight loss	17 (34)
Blurring of vision	16 (32)
Fatigue	28 (56)
Stages of DN (according to Mogensen classification)	
Stage I (hyperfiltration)	15 (30)
Stage II (silent)	11 (22)
Stage III (incipient nephropathy)	24 (48)

of variance for differences, Chi-square for associations, and rank correlation coefficient, were applied where necessary. Data analysis was performed using Statistical Package for the Social Sciences IBM version 25.0, with a *p*-value of < 0.05 deemed statistically significant.

Results

Table 1 illustrates that out of 50 patients, the majority (34 or 68%) were aged between 41 and 50 years, followed by 13 patients (26%) in the 31–40 years age-group, and 3 patients (6%) in the 21–30 years age-group. The mean age of the participants was 43.06 ± 6.9 years. Regarding gender, 52% of the participants were male and 48% were female. The most common symptoms reported were polyuria and fatigue, each affecting 56% of patients, followed by polydipsia in 38%, and weight loss in 34%. In terms of disease stage, 15 patients (30%) were classified as stage I (hyperfiltration), 11 patients (22%) as stage II (silent), and 24 patients (48%) as stage III (incipient nephropathy).

Table 2: Diabetes parameters

Diabetes parameters	*Mean ± SD*
Fasting blood sugar (mg/dL)	224.82 ± 79.02
Postprandial blood sugar (mg/dL)	341 ± 90.1
Random blood sugar (mg/dL)	331.56 ± 76.98
HbA1c (%)	9.85 ± 2.37

Table 3: Association of variables and differences in parameters according to Mogensen classification

		Stages of diabetic nephropathy			
Variables	*Total (n = 50)*	*Stage I (n =15)*	*Stage II (n = 11)*	*Stage III (n = 24)*	*p-value*
Duration of diabetes (years)					
0–5	31 (62%)	12	7	12	0.407 (NS)
5–10	15 (30%)	3	3	9	
10–15	4 (8%)	0	1	3	
Smoking					
Present	13 (26%)	13	9	15	0.238 (NS)
Absent	37 (74%)	2	2	9	
Serum cystatin C level (mg/L)					
Normal	11 (22%)	8	1	2	0.003 (S)
Raised	39 (78%)	7	10	22	

NS, not significant; S, significant

Table 2 shows diabetes parameters where mean values for FBS, PPBS, RBS, and HbA1c were 224.82 ± 79.02 mg/dL, 341 ± 90.1 mg/dL, 331.56 ± 76.98 mg/dL, and 9.85 ± 2.37%, respectively.

Table 3 presents the relationship between the duration of diabetes, smoking status, and serum cystatin C levels with the stages of DN. Among the patients, 31 (62%) had been diagnosed with diabetes for 0–5 years. Smoking was reported in 37 (74%) of the cases, and serum cystatin C levels were elevated in 39 (78%) of the patients. A significant association was observed between serum cystatin C levels and the stages of DN ($p < 0.05$), while no significant association was found between the duration of diabetes or smoking status and the stages of DN ($p > 0.05$).

Table 4 presents the differences in the duration of diabetes, smoking status, serum cystatin C levels, and UACR across the stages of DN. The average duration of diabetes was 4.4 ± 3.48 years. The mean serum cystatin C level was 1.19 ± 0.39 mg/L, while the average UACR was 95.22 ± 102.15 mg/gm, and the mean GFR was 102.62 ± 28.54 mL/min/1.73 m^2. Significant differences were observed in the mean duration of diabetes, serum cystatin C levels, GFR, and UACR across the stages of the Mogensen classification.

Discussion

In our study, the largest proportion of patients (68%) fell within the 41–50 years age-group, followed by 26% in the 31–40 years age-group. This age distribution is similar to that observed in the study by Ashok et al., where 39% of patients fell into the 41–50 years category.[7] The mean age in our study was 43.06 years, which is slightly lower than the mean age of 52.0 ± 11.0 years reported

Table 4: Test of significant difference in various parameters according to stages of DN

		Stages of diabetic nephropathy			
Parameters	*Total (n = 50) mean ± SD*	*Stage I (n =15) mean ± SD*	*Stage II (n =11) mean ± SD*	*Stage III (n = 24) mean ± SD*	*p-value*
Duration of diabetes (years)	4.4 ± 3.48	2.42 ± 2.37	4.7 ± 3.74	6.08 ± 3.92	0.015 (S)
Serum cystatin C level (mg/L)	1.19 ± 0.39	0.94 ± 0.2	1.2 ± 0.38	1.35 ± 0.42	0.005 (S)
GFR (mL/min/1.73 m^2)	102.62 ± 28.54	124.73 ± 11.4	105.64 ± 20.53	87.42 ± 30.14	<0.001 (S)
UACR (mg/gm)	95.22 ± 102.15	10.3 ± 9.24	17.79 ± 9.16	183.78 ± 79.91	<0.001 (S)

S, significant

by Sapkota et al. in their study of Type 2 DM (T2DM) patients.[8] Additionally, Chatterjee et al. found that the prevalence of T2DM increases with age, especially after 40, which aligns with the findings of our study.[9] Regarding gender distribution, our study population consisted of 52% males and 48% females, which mirrors the slightly higher global prevalence of T2DM in males as observed by Kautzky-Willer et al.[10] Other studies, however, have reported a higher proportion of males, such as 54 and 36.2%, respectively.[8,11] The most frequently reported symptoms in our study were polyuria and fatigue (both 56%), followed by polydipsia (38%) and weight loss (34%). These are consistent with findings from Chatterjee et al., who highlighted polyphagia, polydipsia, and polyuria as classic symptoms in T2DM patients with uncontrolled hyperglycemia.[9]

The majority of our patients (62%) had been diagnosed with diabetes for 0–5 years, similar to the median duration of 5 years reported by Sapkota S et al.[8] However, Chatterjee et al. noted that the duration of diabetes can vary significantly among individuals, influenced by factors such as the age at diagnosis, lifestyle, and treatment adherence.[9] Regarding smoking, 74% of our participants reported no history of smoking, while 26% were smokers. Studies by Willi et al. and Pan et al. found that smoking contributes to insulin resistance and impaired glucose metabolism, thereby increasing the risk of developing T2DM.[12,13]

In our study, the mean GFR was 102.62 ± 28.54 mL/min/1.73 m^2, which falls within the normal range. However, the mean FBS was 224.82 ± 79.02 mg/dL, PPBS was 341 ± 90.1 mg/dL, RBS was 331.56 ± 76.98 mg/dL, and HbA1c was 9.85 ± 2.37%, all of which indicate poor glycemic control, aligning with the diagnostic criteria for DM (ADA, 2023).[14]

Our study observed that the mean duration of diabetes was 2.42 ± 2.37 years for stage I participants, 4.7 ± 3.74 years for stage II, and 6.08 ± 3.92 years for stage III, suggesting that the severity of DN increases with the duration of the disease.

We found elevated serum cystatin C levels in 78% of patients, with a mean level of 1.19 ± 0.39 mg/L. Increased serum cystatin C levels in DN patients have been previously reported by Jeon et al., Cho et al., Alicic et al., and Mussap et al., who observed this increase particularly in patients with T2DM and DN.[6,15–17] Serum cystatin C levels rise as GFR decreases, leading to its accumulation in the bloodstream.[6]

The mean UACR in our study was 95.22 ± 102.15 mg/gm, indicating a significant presence of microalbuminuria (ACR between 30 and 300 mg/gm) in many of our participants. Microalbuminuria is an early indicator of DN, and as DN progresses, overt proteinuria and a decline in estimated GFR (eGFR) typically follow. Studies by Cho et al. and Alicic et al. found DN in 20–40% of T2DM patients.[15,16]

Our findings showed that 30% of patients were classified as stage I (hyperfiltration), 22% as stage II (silent), and 48% as stage III (incipient nephropathy). Patients in stage I exhibited higher mean GFR values than those in stage II, and those in stage II had higher GFR than patients in stage III. This progression of decreasing GFR is typical in DN, supporting its natural course.

Of the study subjects, 52% showed normoalbuminuria, while 48% had microalbuminuria. The mean UACR values were 10.3 in stage I, 17.7 in stage II, and 183.78 in stage III, highlighting the presence of microalbuminuria in the more advanced stages of DN.

We also observed a progressive increase in mean serum cystatin C levels with advancing stages of DN. Elevated cystatin C levels in normoalbuminuric patients in stages I and 2 emphasize its potential as an early diagnostic marker for DN, detectable even before the onset of microalbuminuria. Therefore, it offers an early detection tool, aiding in the implementation of preventative and treatment strategies to reduce the risk of developing ESRD. A similar study by Gupta et al. found elevated cystatin C levels in patients who had not yet developed microalbuminuria, supporting the potential of cystatin C as an earlier marker of DN.[18]

In this study, we found a moderately positive correlation (correlation coefficient 0.53) between serum cystatin C levels and UACR across all study participants. Moreover, there was a strong positive correlation (correlation coefficient 0.647) between serum cystatin C levels and UACR in stage III patients, indicating a stronger correlation as DN progresses. This finding is consistent with studies by Sapkota et al., Jeon et al., and Shohaib et al., who also observed a positive correlation between serum cystatin C and albuminuria in T2DM patients.[6,8,19] The correlation between serum cystatin C and UACR suggests that both biomarkers reflect the underlying pathophysiological process of renal dysfunction in T2DM patients.[6,19]

Conclusion

The study emphasizes the promising role of cystatin C as a biomarker for early DN. Elevated levels of serum cystatin C were detected in stages I and II of DN, even before microalbuminuria sets in. This highlights its potential to detect DN at an earlier, more manageable stage, making cystatin C a key tool in proactive diagnosis and intervention. Its significant association with both the Mogensen classification and UACR further supports its clinical relevance. These findings suggest that combining serum cystatin C with other biomarkers could improve diagnostic accuracy in diabetic patients.

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References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37(1):S81–S90. DOI: 10.2337/dc14-S081.
2. International Diabetes Federation. IDF diabetes atlas. 7th ed. Brussels (Belgium): International Diabetes Federation; 2015. p. 33.
3. UK Prospective Diabetes Study (UKPDS). VIII. Study design, progress and performance. Diabetologia 1991;34:877–890. PMID: 1778353.
4. Sharma RK. API textbook of medicine. 10th ed. Diabetes and kidney disease; vol. 1. New Delhi: Jaypee Brothers Medical Publisher; 2015. pp. 528–533.
5. Fornoni A, Nelson RG, Najafian B, et al. Epidemiology of diabetic kidney disease. In: Yu ASL, Chertow GM, Luyckx VA, et al., editors. Brenner & Rector's The kidney. 11th ed. Canada: Elsevier; 2020. pp. 1332–1337.
6. Jeon YK, Kim MR, Huh JE, et al. Cystatin C as an early biomarker of nephropathy in patients with type 2 diabetes. J Korean Med Sci 2011;26(2):258–263. DOI: 10.3346/jkms.2011.26.2.258.
7. Ashok ML, Prashanth VN, Dudhewala A. Study of serum cystatin-C and its correlation with microalbuminuria as marker for diabetic nephropathy in a tertiary care hospital. Int J Basic Med Sci 2015;6(1):1–11.
8. Sapkota S, Khatiwada S, Shrestha S, et al. Diagnostic accuracy of serum cystatin C for early recognition of nephropathy in type 2 diabetes mellitus. Int J Nephrol 2021;2021:8884126. DOI: 10.1155/2021/8884126.
9. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet 2017; 389(10085):2239–2251. DOI: 10.1016/S0140-6736(17)30058-2.
10. Kautzky-Willer A, Harreiter J, Pacini G. Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. Endocrine Rev 2016;37(3):278–316. DOI: 10.1210/er.2015-1137.
11. Mojiminiyi OA, Abdella N. Evaluation of cystatin C and β-2 microglobulin as markers of renal function in patients with type 2 diabetes mellitus. J Diabetes Complications 2003;17(3):160–168. DOI: 10.1016/s1056-8727(02)00177-0.
12. Willi C, Bodenmann P, Ghali WA, et al. Active smoking and the risk of type 2 diabetes: A systematic review and meta-analysis. JAMA 2007;298(22):2654–2664. DOI: 10.1001/jama.298.22.2654.
13. Pan A, Wang Y, Talaei M, et al. Relation of active, passive, and quitting smoking with incident type 2 diabetes: A systematic review and meta-analysis. Lancet Diabetes Endocrinol 2015;3(12):958–967. DOI: 10.1016/S2213-8587(15)00316-2.
14. American Diabetes Association. 11. Microvascular complications and foot care: Standards of medical care in diabetes – 2019. Diabetes Care 2019;42(Suppl 1): S124–S138. DOI: 10.2337/dc19-S011.
15. Cho Y, Park HS, Huh BW, et al. Prevalence and risk of diabetic complications in young-onset versus late-onset type 2 diabetes mellitus. Diabetes Metab 2022;48(6):101389. DOI: 10.1016/j.diabet.2022.101389.
16. Alicic RZ, Rooney MT, Tuttle KR. Diabetic kidney disease: Challenges, progress, and possibilities. Clin J Am Soc Nephrol 2017;12(12):2032–2045. DOI: 10.2215/CJN.11491116.
17. Mussap M, Vestra MD, Fioretto P, et al. Cystatin C is a more sensitive marker than creatinine for the estimation of GFR in type 2 diabetic patients. Kidney Int 2002;61(4):1453–1461. DOI: 10.1046/j.1523-1755.2002.00253.x.
18. Gupta K, Nayyar SB, Sachdeva J, et al. Cystatin C in the early diagnosis of diabetic nephropathy and its correlation with albuminuria. Int J Adv Med 2017;4(1):56–59. DOI: 10.18203/2349-3933.ijam20170020.
19. Shohaib AA, Seleem AS, Barbary HE, et al. Evaluation of serum cystatin C as an indicator of early renal function decline in type 2 diabetes. Menoufia Med J 2014;27(1):60–65. DOI: 10.4103/1110-2098.132748.